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Convergiendo en la epigenética. Ensayo clínico aleatorizado de Vorinostat en Deterioro Cognitivo Leve tipo Amnésico y Enfermedad de Alzheimer temprana

Almudena Boix Lago

TRABAJO DE FIN DE GRADO

Convergiendo en la epigenética

Ensayo clínico aleatorizado de Vorinostat en Deterioro Cognitivo Leve tipo Amnésico y Enfermedad de Alzheimer temprana

Grado en Medicina

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Study protocol

Putting the focus on epigenetics

Phase III randomized, multicenter, double-blind, controlled with placebo, tow-parallel-groups clinical trial to evaluate the efficacy and security of Vorinostat in patients with amnestic Mild Cognitive Impairment and mild Alzheimer's disease

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Project duration: 2.5 years (30 months)

Resumen

Introducción: La Enfermedad de Alzheimer (EA) es considerada hoy en día la epidemia del siglo XXI, afectando al 17% de las personas mayores de 65 años y al 50% de más de 85 años, convirtiéndose así en un problema creciente en salud pública. Se ha logrado un progreso sustancial en la comprensión de la fisiopatología de la enfermedad, pero en los últimos 15 años ningún nuevo medicamento ha demostrado ser eficaz frente a la misma. Sabemos que la expresión génica puede ser regulada por fármacos epigenéticos, como Vorinostat, un modificador de histonas inhibidor del enzima HDAC (Histona deacetilasa), que ha demostrado mejorar la memoria y el aprendizaje y tener un efecto neuroprotector en modelos preclínicos neurodegenerativos.

Objetivos: Demostrar si la administración de Vorinostat puede atenuar la transición natural desde fases tempranas de EA y/o Deterioro Cognitivo Leve tipo amnésico (DCLa) a enfermedad avanzada, en comparación con placebo. Se evaluará el cambio desde el inicio de tratamiento en la Subescala Cognitiva Modificada de la Enfermedad de Alzheimer (por sus siglas en inglés, ADAS-Cog modificado) después de 12 meses de tratamiento.

Diseño, espacio y participantes: En este ensayo clínico fase 3, doble ciego, multicéntrico, aleatorizado, controlado con placebo, se evaluará la eficacia de la administración de Vorinostat en pacientes con DCLa y pacientes diagnosticados con probable EA temprana. Los pacientes serán aleatorizados para recibir por vía oral diariamente Vorinostat 400 mg o placebo todos los días durante 12 meses.

Principales variables y medidas: El principal resultado de interés será el cambio desde el inicio hasta los 12 meses en la escala modificada de ADAS-Cog. Los resultados secundarios serán los cambios desde el inicio hasta los 6 y 12 meses de seguimiento, en la escala Montreal Cognitive Assessment (MoCA), el Inventario de Actividades de la Vida Diaria (por sus siglas en inglés, ADCS-ADL), el Inventario Neuropsiquiátrico (NPI-Q), en volúmenes cerebrales por RM, niveles de β-amiloide en líquido cefalorraquídeo y actividad de HDAC en hipocampo por PET, tanto en el

grupo de DCLa como en EA temprana. Como otro resultado secundario, se evaluará la posible progresión en el estadiaje de la enfermedad a los 12 meses. La seguridad se evaluará en todos los pacientes desde la primera administración hasta 6 meses después de finalizar el ensayo clínico.

Resultados previstos: Vorinostat administrado diariamente durante 12 meses, modificaría de forma significativa la progresión de la EA y el declive cognitivo propio de la enfermedad, en comparación con placebo.

Relevancia: Existe una urgente necesidad de nuevos tratamientos modificadores de la EA. Tras la falta de resultados en la investigación de otras vías fisiopatológicas, la epigenética podría ofrecer una nueva perspectiva en el tratamiento de la enfermedad y contribuir a una mejor calidad de vida.

Palabras clave: Epigenética, Cognición, Enfermedad de Alzheimer temprana, Deterioro Cognitivo Leve tipo amnésico, Modificaciones de histonas.

Resum

Introducció: La Malaltia d'Alzheimer (MA) és considerada avui dia l'epidèmia del segle XXI, afectant al 17% de les persones majors de 65 anys i al 50% dels majors de 85 anys, convertint-se així, en un problema creixent en salut pública. S'ha produït un progrés substancial en la comprensió de la fisiopatologia de la malaltia, però en els últims 15 anys cap nou medicament ha demostrat ser eficaç enfront de la mateixa. Sabem que l'expressió gènica pot ser regulada per fàrmacs epigenètics, com Vorinostat, un modificador d'histones inhibidor de l'enzim HDAC (Histona deacetilasa), que ha demostrat millorar memòria i aprenentatge i tenir un efecte neuroprotector en models preclínics neurodegeneratius.

Objectius: Demostrar si l'administració de Vorinostat pot atenuar la transició natural des de fases primerenques de MA i/o Deteriorament Cognitiu Lleu tipus amnèsic (DCLa) a malaltia avançada, en comparació amb placebo. S'avaluarà el canvi des de l'inici de tractament en la Subescala Cognitiva Modificada de la

Malaltia d'Alzheimer (per les seves sigles en anglès, ADAS-Cog modificat) després de 12 mesos de tractament.

Disseny, espai i participants: En aquest assaig clínic fase 3, doble cec, multicèntric, aleatoritzat controlat amb placebo, s'avaluarà l'eficàcia de l'administració de Vorinostat en pacients amb DCLa i pacients diagnosticats amb probable MA primerenca. Els pacients seran aleatoritzats per rebre diariament Vorinostat 400 mg per vía oral o placebo durant 12 mesos.

Principals variables i mesures: El principal resultat d'interès serà el canvi des de l'inici fins als 12 mesos en l'escala modificada de ADAS-Cog. Els resultats secundaris seran els canvis des de l'inici fins als 6 i 12 mesos de seguiment, en la escala Montreal Cognitive Assessment (MoCA), en l'Inventari d'Activitats de la Vida Diària (per les sigles en anglès, ADCS-ADL), en l'Inventari Neuropsiquiàtric (NPI-Q), en volums cerebrals per RM, nivells de β-amiloide en líquid cefaloraquidi i activitat de HDAC a l'hipocamp per PET, tant en el grup de DCLa com a MA primerenca., Com a altre resultat secundari, s'evaluarà la possible progressió en l'estadiatge de la enfermetat als 12 mesos. La seguretat s'avaluarà en tots els pacients des de la primera administració fins a 6 mesos després de finalitzar l'assaig clínic.

Resultats previstos: Vorinostat administrat diàriament durant 12 mesos, modificaria de manera significativa la progressió de la MA i el declivi cognitiu propi de la malaltia, en comparació amb placebo.

Rellevància: Hi ha una necessitat urgent de nous tractaments modificadors de la MA. Després de la manca de resultats en la recerca d'altres vies fisiopatològiques, l'epigenètica podria oferir una nova perspectiva en el tractament de la malaltia i contribuir a una millor qualitat de vida.

Paraules clau: Epigenètica, Cognició, Malaltia d'Alzheimer primerenca, Deteriorament Cognitiu Lleu tipus amnèsic, Modificacions d'histones.

Abstract

Introduction: Alzheimer Disease (AD) is nowadays considered the epidemic of the 21st century, affecting 17% of people older than 65 years and 50% older than 85 years, thus becoming a growing problem in public health. Substantial progress has been made in understanding the pathophysiology of the disease but no new drugs have shown efficacy in the past 15 years. We know that gene expression can be regulated by epigenetic drugs, such as Vorinostat, a histone modifier inhibitor of the HDAC (Histone deacetylase) enzyme, which has shown to improve memory and learning and to have a neuroprotective effect in neurodegenerative preclinical models.

Objective: To demonstrate that the administration of Vorinostat could attenuate the natural transition from mild stages of AD and/or Amnestic Mild Cognitive Impairment (aMCI) to an advanced symptomatic AD compared with placebo, measured by change from baseline on the modified Cognitive Subscale of the Alzheimer's Disease Assessment Scale (ADAS-Cog modified) after 12 months of treatment.

Design, setting and participants: In this phase 3, double-blind, multicenter, randomized, placebo-controlled trial consisting of 2 parallel-groups; we will assess the efficacy of Vorinostat in patients with aMCI and mild AD. Patients will be randomized to receive daily Vorinostat 400 mg orally or placebo every day for 12 months.

Main outcomes and measures: The primary outcome will be change from baseline to 12 months in modified ADAS-Cog scale. The secondary outcomes will be the change from baseline to 6 months and 12 months follow-up in Montreal Cognitive Assessment (MoCA), Alzheimer's Disease Cooperative Study Activities of Daily Living Inventory (ADCS-ADL), Neuropsychiatric Inventory Questionnaire (NPI-Q), brain volumes in MRI, levels of cerebrospinal fluid β-amyloid and hippocampal HDAC activity in PET, in both aMCI and mild AD. As another secondary outcome, the possible progression in the staging of the disease at 12

months will be evaluated. Safety will be assessed in patients from the first administration until 6 months after terminating the clinical trial.

Expected results: Vorinostat administered daily for 12 months, would significantly modify the progression of AD and the cognitive decline characteristic of the disease, compared with placebo.

Relevance: There is an urgent need for new modifying disease treatments in AD. After the lack of results in the research of other physiopathological pathways, epigenetics could offer a new perspective in the management of the disease and contribute to a better quality of life.

Key words: Epigenetics, Cognition, Mild Alzheimer Disease, Amnestic Mild Cognitive Impairment, Histone modifications.

Abbreviations

EPAD: European Prevention of Alzheimer's Dementia Consortium

aMCI: amnestic Mild Cognitive Impairment

AD: Alzheimer's Disease

HDAC: Histone deacetylase

MoCA: Montreal Cognitive Assessment

ADAS-Cog: Cognitive Subscale of the Alzheimer's Disease Assessment Scale

ADCS-ADL: Alzheimer's Disease Cooperative Study Activities of Daily Living Inventory

MRI: Magnetic Resonance Imaging

PET: Positron Emission Tomography

CSF: Cerebrospinal fluid

Aβ: Amyloid β

APOE: Apolipoprotein E

ECG: Electrocardiogram

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Introduction

Increasing life expectancy and global population growth have led to an increased in the incidence and prevalence of neurological diseases and consequently in its burden. According to the *Global Burden of Diseases, Injuries, and Risk Factors (GBD) Study*, the neurological disorders are one of the most important causes of disability and the second-leading cause group of deaths. (1) In particular, it's expected that dementia will affect 75 million people by 2030 and 132 million by 2050, being Alzheimer disease (AD) the most common cause. (2)

AD consists of a progressive loss of episodic or recent memory and a subsequent alteration of the rest of cortical cognitive functions (apraxia, agnosia, aphasia, executive alteration). It is a degenerative, cortical and diffuse dementia. The most important risk factor for AD is age, doubling the risk every 5 years after 65 years old. Other risk factors for AD are low educational level, traumatic brain injury, genetics (Apolipoprotein E4 (APOE4), Amyloid Precursor Protein (APP), Presenilin - PSN1, PSN2), Down syndrome and cerebrovascular disease (which include arterial hypertension and secondary vascular complications, cardiomyopathy, physical inactivity, smoking, obesity and diabetes mellitus). (2)(3) It is important to highlight that 95% of AD cases are explained by a multifactorial model due to interactions between genetics and environment, and only 5% are due to mendelian, monogenic inheritance. This explains why most studies recommend a multimodal intervention that simultaneously targets several factors that contribute to the pathogenesis of AD as the most effective strategy in the management of the disease. (4) Specifically, the *National Academy of Medicine Report* found evidence that cognitive training, blood pressure management in people with hypertension, and increased physical activity, are useful interventions for prevention of dementia (5) and a recent longitudinal study, found that high cardiovascular fitness in midlife was associated with an 88% decreased risk of dementia in a population of women followed up for 44 years. (6)

Regarding the pathogenesis of the disease, although there has been marked scientific progress in this field, numerous molecular mechanisms have been implicated and more research is needed. The most widely accepted theory is that

an accumulation of misfolded β-amyloid proteins (amyloid plaques) and aggregates of hyperphosphorylated tau protein (neurofibrillary tangles) in an aging brain would result in oxidative and inflammatory damage, which would cause synaptic dysfunction (synaptophysin, glutamate, and cholinergic neurons reduction). (3) This heterogeneity in the pathogenesis that initiates and drives AD represents a challenge for the treatment and management in clinical practice. Clinical progression follows a predictable pattern in congruence with the neuropathological changes and it is well accepted that underlying pathological changes begin 10 to 15 years prior to clinical AD diagnosis.
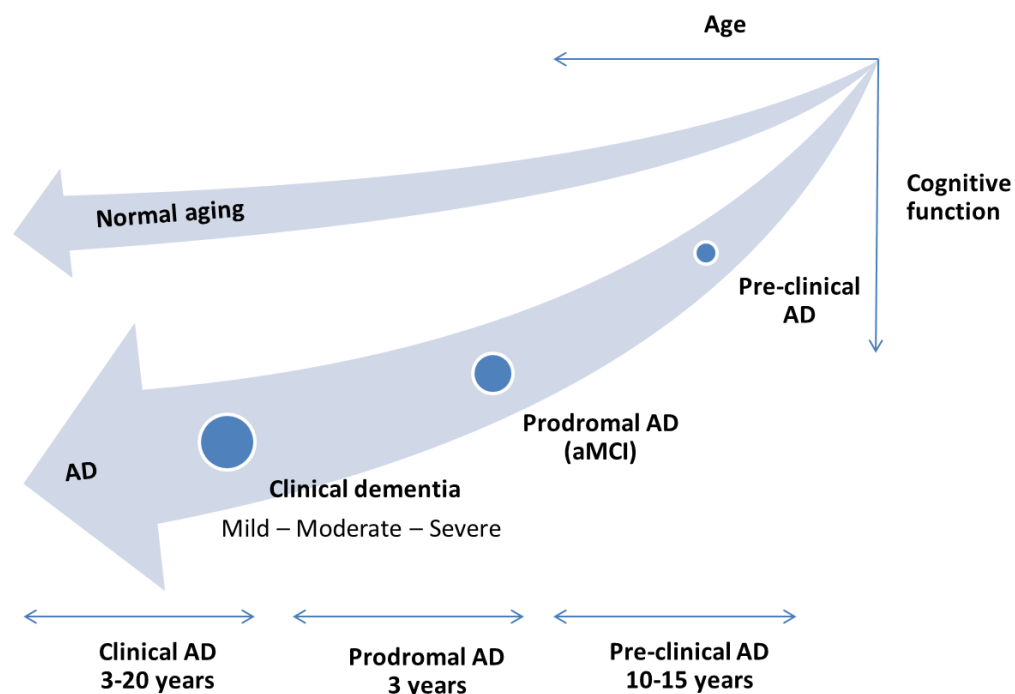
Figure 1. **The natural progression of AD.** *Cognitive decline from a pre-clinical stage (asymptomatic patient with positive biomarkers amyloidosis, neurodegeneration), to a prodromal stage with memory impairment and mild cognitive decline, also called Mild Cognitive Impairment (MCI). The final progression to a clinical dementia is divided into three stages: mild, moderate and severe and the evolution is highly variable in time, from 3 to 20 years. Current diagnosis and treatment occurs in the dementia stage. AD= Alzheimer's Disease; aMCI= Amnestic Mild Cognitive Impairment*

The NIH defines amnestic MCI (aMCI) due to AD as a symptomatic individual non-demented, characterized by memory impairment and decline in cognitive function with intact general daily functioning, that cannot be explained by vascular or other medical causes. (7) It is considered an intermediate clinical state between normal cognitive aging and dementia. aMCI patients are at higher risk of progressing to AD than age-matched controls (10-15% progress to dementia annually) (8), although the neuronal damage is less evident and the reversibility is more probable in aMCI compared to AD. This explains why recent studies focus on early forms of AD or aMCI, since starting the drug therapy too late in disease development could be one of the theories explaining the lack of satisfactory results.

AD is now considered the epidemic of the 21st century and the greatest global challenge for health and social care. Moreover, the actual treatment of AD has only a symptomatic effect with poor cost-effectiveness, circumscribed to acetylcholinesterase inhibitors (Donepezil, Galantamine and Rivastigmine) and Memantine, an NMDA-antagonist, and there is no valid treatment that changes the progression of the disease or slows aMCI. Future research should focus on early stages of AD where there are only subtle or non-cognitive deficits. In the absence of satisfactory results in the management of aMCI and AD, it is necessary to consider new ways of treatment. Could epigenetics offer a new perspective on the natural history of the disease?

Since the sequencing of the human genome in 2001 (9), interest has grown in genetics and its possible use in preventive medicine, diagnosis and treatment of multiple diseases, although it does not seem that its use has been standardized in clinical practice. Genomics, the study of the function and interaction of these genes within the genome, is of special interest in those diseases with a non-mendelian or polygenic inheritance, and includes the so-called epigenetics.

The term epigenetic was coined by Conrad Waddington in 1939 as the molecular mechanisms that convert the genetic information through environmental interactions, into observable traits or phenotypes. (10) Epigenetics consists of three basic mechanisms: DNA methylation by DNMT enzymes of CpG islands, gene bodies or repetitive sequences; histone modifications by histone acetyltransferases

(HATs) and histone deacetylases (HDACs) enzymes (acetylation, methylation and phosphorylation) and nucleosome positioning (chromatin remodeling) of certain portions of the genome, allowing genetic activation or suppression depending on environmental changes. For this reason it is considered that epigenetics is the link between our genetics and the environment. (11) Moreover, recent studies have identified histone variants as additional epigenetic mechanisms: microRNAs and long non-coding RNAs. (12) Epigenetics alters or modifies the functioning of genes and by changing the software of cells, changes the expression of these. Moreover, these epigenetic marks can be transmitted to offspring, but not by modifying the DNA sequence, making for example, monozygotic twins identical in DNA sequence but different in DNA methylation. (13) Epigenetic changes have been described in cancer, neurological and autoimmune disorders, among others. For instance, DNA methylation patterns appear to be distorted (CpG island hypermethylation of neprilysin, S100A2 and SORBS3 promoters) and reduction in histone acetylation and phosphorylation alterations have been found in AD, as well as abnormal levels of HDACs isoforms (low levels of HDAC1, 2, 4 and high levels of HDAC5, 6) (14), these changes would lead to synapsis impairment and release of β-amyloid. (15) The epigenetic changes described in AD are summarized in appendix 4.

Development (X chr. inactivation)
Environment (stress, toxins)
Disease (Prader-Willi, Angelman)
Response to drugs

MicroRNAs and non-coding RNAs

DNA methylation by DNMT

DNA hypomethylation by methycytosine dioxigenases

Histone changes by HATs and HDACs enzymes

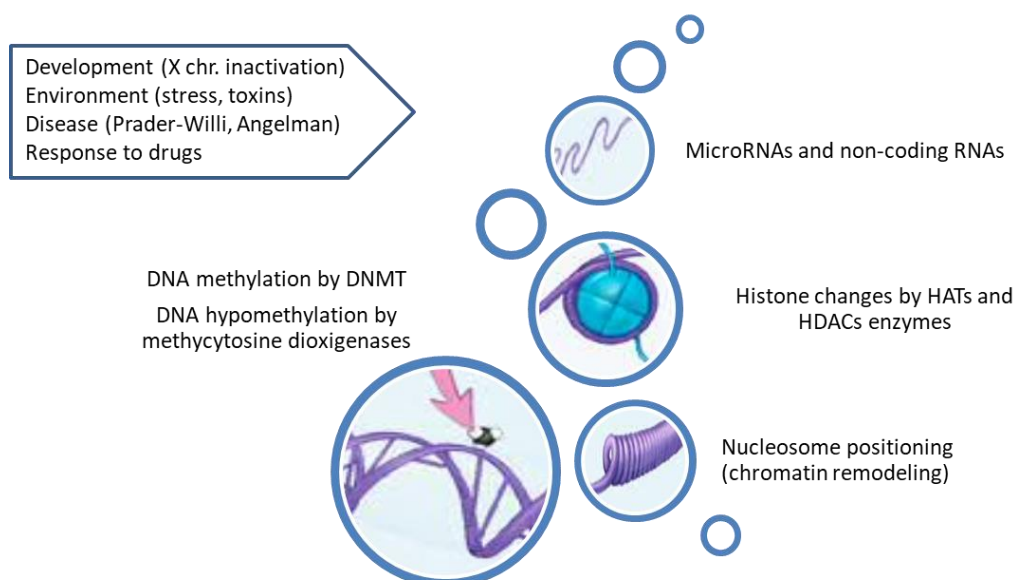Nucleosome positioning (chromatin remodeling)

Figure 2. **Epigenetic changes: altering gene expression without changing DNA sequence.** *We can describe four main epigenetics mechanisms: nucleosome*

positioning, DNA methylation, histone changes and micro-RNAs. DNA methylation consists of a covalent modification of cytosine at 5' position and is generally associated with gene silencing. Post-translational modifications of histones consist of different enzymatic processes (acetylation, phosphorylation, methylation and ubiquitylation) and can lead to gene silencing or activation by modifying the double-helix structure. Nucleosome remodeling changes the density of nucleosomes leading to facilitation or repression of transcription. Finally, RNA interference by non-coding RNA and micro-RNAs has been described. Epigenetic information can be modulated by environment (diet, nicotine and other toxins, post-traumatic stress disorder, exercise etc.) and by drugs (Valproic acid, Curcumin derivates, Hydroxamic acids...) and is directly responsible for development features (such as chromosome X inactivation) and diseases (such as Prader-Willi or Angelman).

The research landscape in AD is contradictory: on one side, there have been great advances in finding the complex pathophysiological processes that underlie the disease progression, but on the other side the research of possible candidate drugs as a treatment for the disease have been disappointing with a lack of efficacy or toxicity problems. If we compare with oncology research: for every dollar invested in dementia, 25 are invested in cancer; and there is a great difference between the two fields: while the general aim in cancer is to destroy harmful cells, in neurodegenerative disorders the aim is to save neurons. In view of the devastating effects of this disease on patients and their families, along with its growing prevalence and the lack of valid treatment in slowing the progression, innovative therapies are necessary.

In the last decade, no drugs have been successfully applied for the treatment of patients with AD (no new treatments have been approved for AD since 2003). An example of this would be the phase 3 trials of anti-amyloid approaches (Semagacestat, Bapineuzumab, Solanezumab, Gantenerumab) (16) (17) and the latest clinical trial with Idalopirdine (a selective serotonin antagonist) with disappointing results. (18) Several reasons could explain the lack of satisfactory results in the trials of disease-modifying drugs for AD: including starting the drug therapy too late in disease development, incorrect posology, wrong treatment

target, and an inadequate understanding of the biology of AD. (19) Since none of phase 3 clinical trials conducted to date that targeted β-amyloid demonstrated a significant positive effect in patients with AD; it would be interesting to focus on different targets. Currently, there are 27 phase III and 8 phase II clinical trials waiting for results.
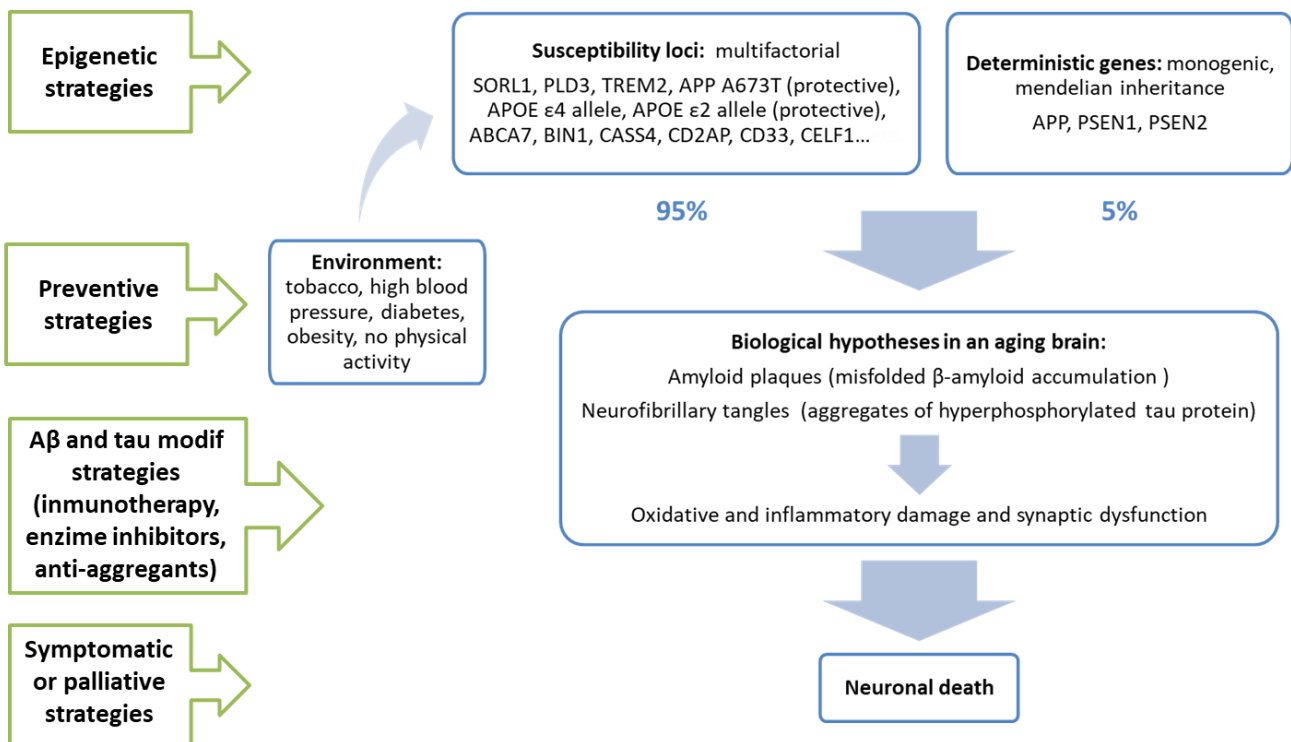

<u>Figure 3</u>. **Pathways to Alzheimer's Disease.** *The most widely accepted theory is that an accumulation of misfolded β-amyloid proteins and aggregates of hyperphosphorylated tau protein in an aging brain would result in oxidative and inflammatory damage, which would cause a synaptic dysfunction (synaptophysin, glutamate, and cholinergic neurons reduction) and ultimately lead to neuronal death, cognitive impairment and dementia. Epigenetic and preventive strategies would act on a pre-damaged brain in susceptible individuals in order to prevent or delay the onset of the disease. (20)*

There are five drugs approved as epigenetic therapy in leukemias and lymphomas with possible applicability in AD: DNA-demethylating drugs (5-aza-2'-Deoxycytidine, Decitabine) and HDAC inhibitors (Suberoylanilide hydroxamic acid, Romidepsin, Phenylbutyrate trichostatin A), and another twenty in

experimentation. Suberoylanilide hydroxamic acid (Vorinostat) has proven to promote synapsis and memory facilitation, and Phenylbutyrate trichostatin A promotes learning behavior and re-establish long-term memories in mouse models. (14) (15)

The weight of genetics, the description of epigenetic patterns and the influence of environmental factors have been proven in AD; moreover, epigenetic modifications are reversible and can be potentially targeted in the treatment of aMCI and AD. The vast majority of research on the use of epigenetic drugs in neurodegenerative diseases has been conducted in preclinical models (mouse or human cells). Some human trials have been performed, especially with HDAC inhibitors (Sodium butyrate, Valproic acid, Trichostatin A, Vorinostat) in SNC neurodegenerative diseases (AD, Huntington Disease and Parkinson) and in motor neuron neurodegenerative diseases (amyotrophic lateral sclerosis, spinal muscular atrophy) with promising but variable results.

The aim of this clinical trial is to test the cognitive and molecular effect of an HDAC inhibitor, Vorinostat (Suberoylanilide hydroxamic acid), in aMCI and mild AD patients. This drug was approved by the FDA in 2006 for the treatment of recurrent cutaneous T-cell lymphoma and it could be a promising therapy for AD patients. Vorinostat inhibits the enzymatic activity of class I histone deacetylases (HDAC isoforms 1 to 3) and class II (HDAC 6 isoform). (21) As explained before, these enzymes catalyze the elimination of acetyl groups from lysine residues of proteins, including histones and transcription factors. Some studies have demonstrated the overexpression of HDACs and the histone hypoacetylation in AD (which would lead in a condensed chromatin and repression of gene transcription) and increments in histone acetylation have consistently been shown to favor learning and memory. Vorinostat has proven a neuroprotective effect in neurological preclinical models (acute and chronic brain injury, neurodegeneration, ischemia, Huntington, Parkinson and Alzheimer) inducing synaptic plasticity, decreasing β-amyloid production and Tau hyperphosphorylation and reinstating learning and memory. (22)

Hypothesis

Null hypothesis (Ho): Obtaining outcomes with no statistically significant changes in the modified ADAS-Cog from baseline to 12 months in the two intervention groups (aMCI and mild AD) compared with placebo.

Alternative hypothesis (H1): Obtaining outcomes with statistically significant changes in the modified ADAS-Cog from baseline to 12 months in the two intervention groups compared to placebo; showing that Vorinostat is superior to placebo and demonstrating a positive treatment effect.

Secondary hypothesis:

- Vorinostat administered during 1 year would show a statistically significant improvement in MoCA, ADCS-ADL, NPI-Q scales compared with placebo.
- Vorinostat administred during 1 year would show a statistically significant decrease in progression in the staging of the disease.
- Vorinostat administered during 1 year would show a statistically significant increase of cerebrospinal fluid (CSF) β-amyloid levels compared to placebo.
- Vorinostat administered during 1 year would show a statistically significant decrease of annual rate of hippocampal atrophy measured in Magnetic Resonance Imaging (MRI) compared to placebo.
- Vorinostat administered during 1 year would show a statistically significant decrease of hippocampal HDAC activity compared to placebo.
- Vorinostat would show a safe administration with none or mild side effects compared to placebo.

Objectives

- Efficacy
  - The primary objective of this study is to test the hypothesis that the oral administration of Vorinostat could attenuate the natural transition from mild stages of AD and/or aMCI to clinical dementia compared with placebo, measured by change from baseline on the modified ADAS-Cog after 12 months of treatment.

- o The secondary objectives of the study are to assess the global benefit of Vorinostat treatment as demonstrated through changes from baseline to 6 and 12 months follow-up in both aMCI and mild AD in clinical scores: Alzheimer's Disease Cooperative Study – Activities of Daily Living Inventory (ADCS-ADL), Montreal Cognitive Assessment (MoCA) and Neuropsychiatric Inventory (NPI).

- Biomarkers
  - o The secondary objective of this study is to evaluate the effect of Vorinostat compared with placebo in aMCI and mild-AD in:
    - CSF β-amyloid levels
    - Hippocampal HDAC activity measured by Positron Emission Tomography (PET).
    - MRI brain volumes

- Safety
  - o The secondary objective of this study is to evaluate the safety of Vorinostat compared with placebo in aMCI and mild-AD: frequency, severity and chronology of side effects; vital signs, physical exploration, blood analysis and ECG.

Methods

Study design

A phase 3 double-blind, multicenter, randomized placebo-controlled trial consisting of 2 parallel-groups will be performed. The study will be conducted at 10 sites (hospitals, clinics, health centers) in Europe between January 2022 and July 2024 during 2.5 years (enrollment period: 12 months, intervention period: 12 months, safety follow-up: 6 months).

An expected number of 2500 participants will be assigned to 2 different groups according to previous diagnosis: group A (amnestic MCI; n=1250) and group B (mild AD; n=1250). On each group, participants will be randomized to either receive a placebo treatment (low-dose albumin once a day) or to the experimental group (administration of Vorinostat - Suberoylanilide hydroxamic acid) during 12

months. The drug will be administered 400 mg orally once a day, this dose has proven to be well tolerated, safe and with minimal side effects in healthy and oncologic patients.

Randomization will be performed by the generation of random numbers on Microsoft Office Excel program by pairing participants to numbers and sorting them into two groups, intervention and control group, in a 1:1 proportion.

The clinical trial will be conducted as a double-blind study, where participants, clinicians and study team will remain blinded to treatment assignments to reduce possible bias from researchers and patients. The personnel in charge of the statistical analyzes will know the assignment of the treatment.

The experimental medication and placebo will be administered to individuals taking the current approved AD or MCI medications (cholinesterase inhibitors for mild AD). We expect a 20% sample loss during the intervention period, obtaining a final sample of 2000 participants. All trials will be conducted in accordance with the principles of good clinical practice and the Declaration of Helsinki. A registration will be made on ClinicalTrials.gov.

This study protocol was elaborated as an efficacy, explanatory trial, so we will use strict inclusion and exclusion criteria to produce a relatively homogeneous sample of participants in settings as controlled as possible, in order to obtain a high internal validity and an adequate external validity.

Study participants and setting

Participants will be recruited from the European platform "EPAD: European Prevention of Alzheimer's Dementia Consortium" (http://ep-ad.org/), an international cohort of volunteers dedicated to the prevention and research of new pharmacological therapies in prodromal stages of the disease; and via 10 hospital and clinics affiliated with the project. Detailed information about centers can be found in appendix 3. Advertising will be made through community centers, hospitals and newspaper, and information will be provided via webpage or

telephone. An external investigator will have access to database from affiliated hospitals in order to contact eligible participants and give information via telephone. A first approach with the participant will be completed over the telephone in order to determine eligibility, and a second approach will be made via an interview with the participant. The volunteers, aged between 65 and 85 years old, medically stable, will be either diagnosed in a probable mild stage of AD or amnestic MCI by an expert and external neurologist, according the National Institute of Health (NIH) criteria (23) and the National Institute of Neurologic and Communicative Disorders and Stroke—Alzheimer's Disease and Related Disorders Association. (24) Besides, a biochemical evaluation for CSF β-amyloid will be performed as biomarker of the disease. We expect the duration of the enrollment period to be approximately of 12 months. Participants meeting diagnostic, inclusion and exclusion criteria will be selected. Participants will agree to the administration of Vorinostat as well as to detailed clinical and cognitive evaluations, brain imaging (MRI and PET) and lumbar puncture every 6 months, and blood analysis and ECG every 2 weeks during the first 2 months and monthly thereafter. Written informed consent will be obtained from each participant. Information about the project, safety of the drug intervention and general information about the disease will be included on a paper form and given to each participant (see detailed information in appendix 2).
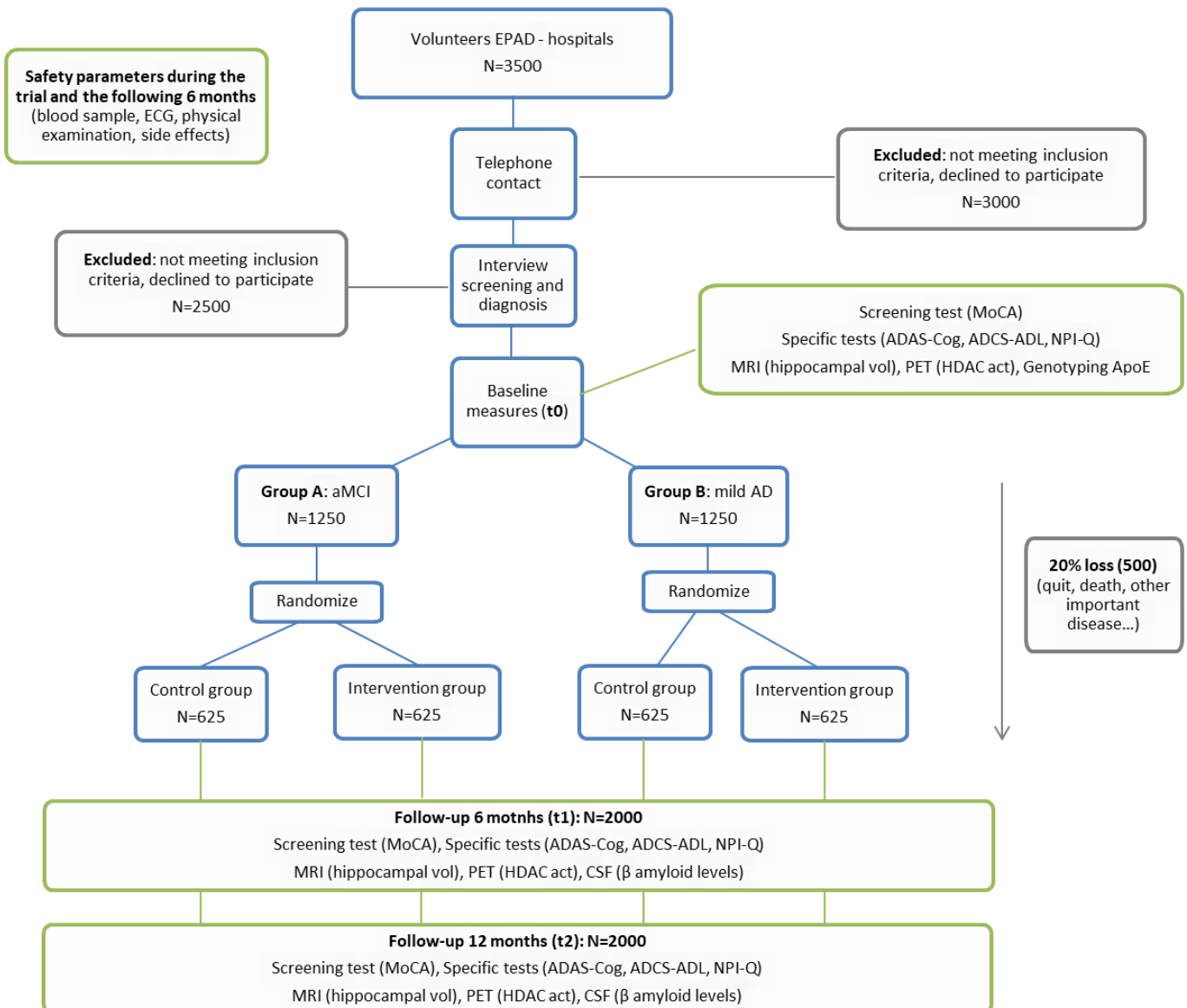
Figure 3. **Schematic study design.** *The expected recruitment and intervention duration is 12 months each. Safety parameters will be assessed throughout the clinical trial and 6 months thereafter. In blue: participants' distribution, green: variables assessment, grey: sample losses. EPAD = European Prevention of Alzheimer's Dementia Consortium; AD = Alzheimer's disease; aMCI = amnestic mild cognitive impairment; ADAS-Cog = Alzheimer's Disease Assessment Scale; ECG = electrocardiogram; MoCA = Montreal Cognitive Assessment; ADCS-ADL = Alzheimer's Disease Cooperative Study Activities of Daily Living Inventory; NPI-Q = Neuropsychiatric Inventory Questionnaire; MRI = Magnetic Resonance Imaging; PET = Positron Emission Tomography, CSF = cerebrospinal fluid.*

Inclusion criteria

Participants meeting the following criteria will be eligible to participate in the clinical trial:

- Males and females aged between 65-85 years old.
- Adequate visual and auditory function with regular non affected reading and writing skills (necessary for cognitive assessment). Proficiency of the language from the country where the study is carried out.
- Preserved mental and physical skills in order to understand and sign the informed consent and carry out the techniques specified in this study (lumbar puncture, genotyping, MRI, PET)
- Participant's commitment to not maintain sexual relations or to use contraception during treatment and for at least 8 weeks after the last dose of the drug.
- Competent caregiver/informant who is willing to monitor compliance of the drug administration and answer a questionnaire follow-up.
- Concomitant treatment with acetylcholinesterase inhibitors will be allowed if the dosage has remained stable for the last 3 months.
- Evidence of amyloid pathology determined by lumbar puncture with CSF Aβ1–42 ≤ 600 ng/L as biomarker for detection of the disease.
- Diagnosis of aMCI or mild-AD by an expert neurologist following the previously cited criteria:
  - *aMCI*: subjective memory decline for at least one year corroborated by an informant, normal general cognitive function (Clinical Dementia Rating (CDR) scale of 0.5), Mini-Mental State Examination (MMSE) score above 24, preserved activities of daily living and no clinics of dementia.
  - *Probable mild AD*: MMSE between 20 and 24, CDR scale of 0.5-1, neuropsychological tests: deficits in two or more areas of cognition, progressive worsening of memory and other cognitive functions, no disturbance of consciousness, onset between ages 40 and 90 (most often after 65 years) and absence of systemic disorders or other

brain diseases that in and of themselves could account for the progressive deficits in memory and cognition.

Inclusion criteria will be revised during the study, any participant already selected who fails to meet the criteria during the intervention period will be discarded.

Exclusion criteria

Participants will be excluded from the study if they meet any of the following criteria:

- Diagnosis of any other condition that could affect cognition: moderate or advanced AD, diagnosis of other type of dementia, Parkinson's disease, Creutzfeldt-Jakob, corticobasal degeneration, progressive supranuclear palsy, frontotemporal degeneration, Huntington disease, normotensive hydrocephaly, epileptic seizures.
- ≥ 4 in Hachinski ischemia score.
- History of familiar early-onset dementia.
- Diagnostic of any major psychiatric syndrome: major depression (≤ 6 on the Geriatric Depression Scale), schizophrenia, bipolar disorder, alcoholism, drug addiction, suicide risk.
- History of stroke during the previous 2 years or transient ischemic attack during the previous 6 months, relevant vascular disease (carotid stenosis, aortic aneurism, arteriovenous malformation), severe trauma (cerebral contusion), intracranial tumors or autoimmune disorders that could have neurological involvement (multiple sclerosis, lupus erythematosus, antiphospholipid syndrome, Behçet disease).
- B12 and folate deficiency, hypo or hyperthyroidism, syphilis, Lyme titers, HIV, meningitis/encephalitis, diagnosis of cancer in the previous 5 years.
- Use of other HDAC inhibitor (e.g., Valproic acid), Benzodiazepines, Antidepressants, Opioids, Barbiturates, Psychostimulants (Amphetamine, Methylphenidate), Antiepileptic or Antipsychotic drugs that could affect the cognition of the participant during the previous year.

- Any brain damage demonstrated by RM: stroke, hydrocephaly, space-occupying brain lesions, aneurisms, >3 microhemorrhages, >2 lacunar infarcts.
- Hepatic insufficiency, hepatitis B or C not adequately treated and systemic immunosuppression.
- Abnormal clinically relevant results in blood and urine analysis.
- Uncontrolled hypertension or hyperglycemia, unstable cardiac, renal, hepatic or pulmonary disease, as well as any unstable general medical condition.
- Enrollment in any other experimental drug study.
- Contraindications for RM (pacemaker, artificial heart valves, metallic objects).

Exclusion criteria will be revised during the study, any participant already selected who meets the criteria during the intervention period will be discarded.

Data collection

A telephone contact will be performed to every volunteer in order to collect demographic characteristics (gender, age, caregiver information, place of residence, concomitant acetylcholinesterase inhibitors, use of other medication and educational level) and supervise inclusion and exclusion criteria. A second contact with the participant will be made via a face-to-face interview to re-monitor inclusion/exclusion criteria, perform an exhaustive medical history and diagnose the participant. Data collection will be obtained through personal interviews at the baseline (t0) and at each follow-up: t1 (6 months) and t2 (12 months) using standardized cognitive test, magnetic resonance imaging, PET and lumbar puncture. Official standardized translations for each cognitive test will be used in the different countries. In addition, genotyping of the Apolipoprotein E (APOE) will be tested at baseline (t0) via venous blood samples by Sequenom Massarray Analyzer 4. The genetic analyses will be used and analyzed as a depending factor for cognitive impairment in participants. Blood analysis will be performed every 2 weeks during the first 2 months and monthly thereafter for safety reasons. All data will be managed using anonymous codes to protect participant confidentiality.

Safety will be assessed by checking for adverse events, ECG, exploring history, examinations, laboratory tests and brain MRI during the study and in the following 6 months.

Variables

Cognitive assessment will be performed to all participants every 6 months from the baseline until a one-year follow-up via a face-to-face interview with an expert and trained neurologist. Neuropsychological assessments will be conducted by the same evaluator in each hospital and in the same order to minimize variability in participant and/or caregiver responses. The cognitive evaluation will include different recognized tests: a screening test such as Montreal Cognitive Assessment (MoCA) and detailed specific scales such as modified Alzheimer's Disease Assessment Scale-cognitive subscale (ADAS-Cog modified), Alzheimer's Disease Cooperative Study Activities of Daily Living Inventory (ADCS-ADL) and Neuropsychiatric Inventory Questionnaire (NPI-Q). The expected total duration of the cognitive evaluation will be less than 1.5 hours.

MoCA

As cognitive screening measure, the MoCA has proven to be more sensible but slightly less specific to detect mild or prodromal forms of AD than the Mini-Mental State Examination (MMSE) (7) (25) (26), even if the MMSE is probably more often used in regular clinical practice. The MoCA is a standardized screening scale for neurologic impairment used in clinical practice that evaluates 8 different cognitive domains and provides a total score between 0 and 30 points (>26: normal; <26: MCI; <20: AD) with a sensitivity of 90% and a specificity of 87% for MCI and a sensitivity of 100% and a specificity of 87% for mild-AD. Moreover, positive and negative predictive values for the MoCA are 89% and 91%, respectively for MCI and 89% and 100%, respectively for AD. The expected duration of the test is 10 minutes. (26)

ADAS-Cog modified

The ADAS-Cog modified is the most widely used cognitive measure in clinical trials of AD, it assesses 13 cognitive domains including memory, language, praxis, orientation and executive functioning and it has proven to be more sensitive than

the first version designed by Rosen et al. to study MCI. (27) It assesses the severity of the dysfunction in the cognitive and non-cognitive behaviors characteristic of persons with AD and it will be used as a primary efficacy measure. The scale ranges from 0 to 85, higher scores indicating greater cognitive impairment. The expected duration of the test is 30 minutes. (28)

ADCS-ADL

The ADCS-ADL is a standardized 18-item questionnaire to assess behavior and daily functioning via the patient's caregiver. For each of the specific items (e.g. reading books or magazines, pastime activities, household chores...), the caregiver is asked if the patient did the activity during the past 6 months and he is asked to rate the patient's performance level based on a set of performance descriptions. The range for the total test score is 0 to 53, higher scores indicating less functional impairment. The expected duration of the test is 30 minutes. (29)

NPI-Q

The NPI-Q (30) consists on the assessment of neuropsychiatric symptomatology (delusions, anxiety, irritability...). It provides symptom severity and caregiver distress ratings for each symptom reported, and a total score from 0 to 36 and from 0 to 60 in severity and distress respectively. We consider a score of NPI<10, participants without clinically meaningful behavioral or psychiatric disturbances. The expected duration of the tests is 5 minutes or less.

Brain volumes in MRI

All participants will receive 1.5 T structural Magnetic Resonance Imaging (MRI) on the enrollment period and intervention period: baseline (0 months), t1 (6 months) and t2 (12 months). MRI will be performed in every center with the same acquisition method.

In every scan, we will acquire T1-weighted gradient echo, axial 5mm T2*-weighted gradient echo, T2-weighted spin-echo and diffusion-weighted. Total volumes of the brain, the left and right inferior and lateral ventricles, left and right hippocampal and parahippocampal regions and entorhinal cortex will be measured. The aim of using MRI was for subject screening on the enrollment period, safety monitoring and to determine possible changes after treatment. This technique offers a double

function, on one hand it may rule out potential previous unknown causes for cognitive decline (subdural hematoma, hydrocephaly, stroke) and on the other hand and more importantly, it will help in defining anatomopathological changes in aMCI and mild AD (cerebral atrophy, microinfarcts, atherosclerosis, hippocampal sclerosis). Images will be quality-controlled and examined by a neuroradiologist, who will not meet or have any direct contact with the patients and will receive the images with an identification code. The criteria for MRI quality control included visualization of image artifacts (head movements, ringing...) and appearance of gray/white matter contrast. The protocol form from *Alzheimer's Disease Neuroimaging Initiative* (http://www.adni-info.org/Scientists/ADNIStudyProcedures.html) will be followed.

Images will be processed using Freesurfer software. Rate measurements will be expressed as the annualized percentage change (APC) from baseline volumes.

Hippocampal HDAC activity

Positron Emission Tomography (PET) will be performed at baseline, 6 months and 12 months follow-up. PET will be performed in every center with the same acquisition method.

Radiotracer-specific PET imaging will be performed as a reliable in vivo technique to determine HDAC expression. A small (<400 Da) and lipophilic radiotracer will be chosen to be able to cross the blood-brain barrier. We will detect HDAC activity with [11C] Martinostat, an imaging radiotracer selective for class I HDACs isoforms 1 to 3; isoforms that have proven to be implicated in regulating neuroplasticity and cognitive function. Previous studies have found that HDAC expression is lower in hippocampus and amygdala in AD compared with control patients. (31) An intravenous line will be placed in the antecubital vein and a licensed nuclear medicine technologist will administer [11C] Martinostat. The expected duration of the PET scan is 60 minutes. The standard uptake values (SUVs) in the hippocampus will be the image-based endpoints assessed.

β-amyloid levels

According to the hypothetical model of dynamic biomarkers of the AD pathological cascade (32), the biomarker abnormality could be detected in the following order:

CSF Aβ1-42, amyloid PET, CSF tau, MRI and finally cognitive impairment. That is the reason why we will select CSF Aβ1-42 as inclusion criteria and as one of the outcomes in this study, instead of CSF tau or amyloid PET imaging. Aβ1-42 has proven to be the most sensitive biomarker for AD (sensitivity 96%, specificity 77%). (33) Levels of Aβ42 have been found to be low in aMCI and mild AD due to cortical amyloid deposition (34) and this descent would precede AD dementia by approximately 10 years. Accordingly, an increased from baseline in levels of CSF Aβ in intervention group compared with placebo participants may be indicative of a reduction of cortical amyloid deposition.

A lumbar puncture in L3/L4 or L4/L5 intervertebral spaces will be performed in the diagnostic procedure, t1 (6 months) and t2 (12 months) to analyze levels of Aβ-42. The protocol form from *Alzheimer's Disease Neuroimaging Initiative* (http://www.adni-info.org/Scientists/ADNIStudyProcedures.html) will be followed, assuring that the same technique and preservation will be performed in every lumbar puncture. The protocol will be approved by an ethical board and all patients will sign a written consent.

Safety parameters

Vorinostat drug was manufactured by Merck & Co in accordance with Merck standards and European regulations.

The administration of Vorinostat will be 400 mg orally once a day accompanied by food. If a patient is intolerant or in cases of mild-moderate hepatic impairment, the dose can be reduced to 300 mg orally once a day. Drug storage: 20-25°C, excursions permitted between 15-30°C.

Vorinostat does not have any known contraindications. All participants (control and intervention group) will be controlled for any signs or symptoms of pulmonary embolism and deep vein thrombosis, gastrointestinal toxicity (nausea, vomiting and diarrhea), thrombocytopenia, anemia and hyperglycemia. Electrolytes, creatinine, magnesium and calcium levels will be assessed (blood analysis every 2 weeks during the first 2 months and monthly thereafter). Some studies report ECG changes (QT prolongation, cardiac ischemia). In case of

concomitant treatment with coumarin-derivative anticoagulants, it is necessary to monitor the INR. The most common adverse reactions (incidence ≥20%) are in order of frequency: fatigue, diarrhea, nausea, dysgeusia, thrombocytopenia and anorexia. (35)

Every participant should be instructed for adverse events (signs and symptoms) and a detailed form will be handled in the first interview (see appendix).

Physical examination, vital signs, body weight and height, ECG (heart rate, PR, QRS, QT, QTc, and ST – *see abnormal criteria in appendix 8*) and specific examination of possible side effects will be assessed in every interview and 6 months after finishing the clinical trial. Blood analysis and ECG will be performed every 2 weeks during the first 2 months and monthly thereafter for safety reasons. All adverse events will be registered in every center and monthly centralized.

Variable categorization and assessment

Independent variable: Qualitative dichotomous Vorinostat administration variable (Yes: oral Vorinostat 400 mg administration or No: orally placebo administration)

Principal dependent variable: Quantitative discrete modified ADAS-Cog variable with a score between 0 and 85, higher scores indicating greater cognitive impairment.

Secondary dependent variables:

- Quantitative discrete variables: MoCA (score from 0 to 30), ADCS-ADL (score from 0 to 53), NPI-Q (score from 0 to 36).
- Quantitative continuous variables: MRI volumes (% APC), HDAC activity (hippocampal standard uptake values (SUVs), β-amyloid (ng/L)
- Qualitative dichotomous: progression to clinical dementia (Yes: meet criteria diagnosis of probable AD; No: do not meet criteria).

Additional variables of interest:

- To be measured solely at the initial visit:
    - <u>Age</u>: quantitative continuous variable (65-85)
    - <u>Gender</u>: nominal dichotomous variable (Female/Male)
    - <u>Years of education</u>: quantitative discrete (from 0 to 25 years)
    - <u>Place of residence</u>: qualitative ordinal (Denmark, Benelux, Switzerland, Spain, Italy, United Kingdom)
    - <u>APOE allele</u>: qualitative ordinal (Genotyping ApoE4, ApoE2, others)
    - <u>Family history of AD</u>: qualitative dichotomous (clinical history: Yes: first relative with diagnosed AD / No: no-first relative with AD).
    - <u>Diagnosis</u>: qualitative dichotomous (aMCI or mild-AD)
- To be measured in every visit:
    - <u>Adverse events</u>: qualitative ordinal. We will consider common side effects (fatigue, diarrhea, nausea, dysgeusia, thrombocytopenia or anorexia), rare side effects (pulmonary embolism, deep vein thrombosis, QT prolongation or cardiac ischemia), none side effects.
    - <u>Participant's loss</u>: qualitative dichotomous (Yes: no treatment adherence, voluntary abandonment of the study, subsequent emergence of exclusion criteria / No: patient that realizes the study)
    - <u>Acetylcholinesterase inhibitors</u>: qualitative dichotomous (Yes: concomitant treatment / No: no concomitant treatment)
    - <u>At risk alcohol consumption</u>: qualitative dichotomous (Yes: >60 gr/day in men or >40gr/day in women / No: <60 gr/day in men or <40gr/day in women)

Variable name	Type	Definition
Independent variable (exposure)		
Vorinostat administration	Qualitative dichotomous	Yes: oral Vorinostat 400 mg No: oral placebo
Dependent variables (outcomes)		
<u>**Primary**</u> **mADAS-Cog**	Quantitative discrete	[0-85]
<u>**Secondary**</u> **MoCA**	Quantitative discrete	Score [0-30]
ADCS-ADL	Quantitative discrete	Score [0-53]
NPI-Q	Quantitative discrete	Score [0-36]
vMRI	Quantitative continuous	%APC
HDAC activity	Quantitative continuous	Standard uptake values in the hippocampus (SUVs)
β-amyloid levels	Quantitative continuous	CSF Aβ (ng/L)
Progression to dementia	Qualitative dichotomous	Yes: meet criteria diagnosis of probable AD No: do not meet criteria
Control variables		
Adverse events	Qualitative ordinal	- Common side effects - Rare side effects - None side effects
Participant's loss	Qualitative dichotomous	**Yes**: participant's loss **No**: non loss
Years of education	Quantitative discrete	Years [0-25]
Place of residence	Qualitative ordinal	Denmark, Benelux, Switzerland, Spain, Italy, United Kingdom
Acetylcholin. Inhib.	Qualitative dichotomous	**Yes**: concomitant treatment **No**: no concomitant treatment
APOE allele	Qualitative ordinal	ApoE4, ApoE2, others
Family history of AD	Qualitative dichotomous	**Yes**: first relative with AD **No**: no-first relative with AD
At risk alcohol consumption	Qualitative dichotomous	**Yes**: >60 gr/day in men or >40gr/day in women **No**: <60 gr/day in men or <40gr/day in women
Stratification variables		
Diagnosis	Qualitative dichotomous	aMCI and mild AD
Age	Quantitative continuous	Years, months, days [65-85]
Gender	Qualitative dichotomous	Female / Male

<u>Table 1</u>. Study variables characteristics

Primary outcome assessment

The primary outcome will be the change from baseline to 12 months in the quantitative discrete variable modified ADAS-Cog scale. According to the current research, we estimate an expected decline of 0.4 points/month for placebo and 0.3 points/month for Vorinostat (improvement of 25% over placebo), meaning a difference of 1.2 points over 12 months. We will consider a 1.2-point change in the modified ADAS-Cog as clinically significant.

Secondary outcomes assessment

ADCS-ADL

Regarding the quantitative discrete variable, ADCS-ADL scale and according to previous studies, we estimate an expected decline of 0.82 points/month for placebo and 0.6 points/month for Vorinostat (improvement of 25% over placebo), meaning a difference of 2.64 points over 12 months. We will consider a 2.64-point change in the ADCS-ADL as clinically significant.

MoCA

For the screening scale MoCA, the following reference values will be accepted, scores >26: normal; <26: MCI; <20: AD. A change in 2 points or more and a category change will be considered clinically relevant for the study purpose. The cutting-point for MCI used in MoCA will be a score under 26 but above 20 (MMSE>24 but<28 inclusive) and a neuropsychological test defined as amnestic MCI with objective memory deficits and intact functional activities. A MoCA score under 20 (MMSE > 17 but <24) and an ADAS-Cog > 31 is required in mild AD. Another secondary outcome will be the time to progression to clinical diagnosis of probable mild AD up to 12 months in aMCI or to moderate AD in mild AD.

NPI-Q

We will compare the change from baseline to 6 and 12 months in NPI-Q. We consider a score of NPI<10, participants without clinically meaningful behavioral or psychiatric disturbances. We consider a change in 1 point of the scale as clinically meaningful and study relevant.

Brain volumes in MRI

The total volumes of the brain, the left and right inferior and lateral ventricles, left and right hippocampal and parahippocampal regions and entorhinal cortex will be expressed as the annualized percentage change (APC) from baseline volumes. APC is defined as follows: APC = (change from baseline/value at baseline) x (365/MRI delay) x 100. The primary MRI outcome will be the APC of total hippocampal volume and secondary outcomes will include total volumes of the brain, left and right inferior and lateral ventricles, parahippocampal regions and entorhinal cortex. There is progressive increased hippocampal atrophy as patients evolve from aMCI to clinical mild, moderate and severe AD. According to previous studies, we hypothesized that Vorinostat will decrease annual rate of hippocampal atrophy, we estimate a decrease of 3.3% and 2.3% of the hippocampal volume in subjects treated with placebo and Vorinostat respectively, meaning a difference of 1% over 12 months.

β-amyloid levels

Cerebrospinal fluid sample will be analyzed using the standardized multiplex xMAP Luminex platform with Innogenetics (INNO-BIA AlzBio3) immunoassay kit– based reagents with antibodies targeting the first and last aminoacid of Aβ42 (Aβ1-42). Evidence of amyloid pathology will be determined by a CSF Aβ1–42 level ≤ 600 ng/L according to ROC curves from previous studies. (34) (17)

Hippocampal HDAC activity

Regarding HDAC activity in PET, the standard uptake values in the hippocampus (SUVs) will be the image-based endpoints assessed. Previous studies found SUV in healthy subjects in the hippocampus of 2.4 ± 0.3; we expect a significant lower value in our sample. (31)

Sample size

Power and sample size calculations will be based on the expected results of the primary objective. According to previous studies, to calculate power we will use an expected decline for placebo in modified ADAS-Cog of 0.4 points/month and an improvement of 25% over placebo in Vorinostat group, meaning a decline of 0.3 points/month, with a common standard deviation of 9. This is equivalent to a difference of 1.2 points over 12 months, with an effect size of 0.2.

The sample size estimation will be performed by the statistical software program "G-power". We estimate that 500 patients will be needed on each experimental group (2000 in total) to find possible statistical differences given an alpha level of 5%, β value of 20% (80% power) and an effect size of 0,2 according to previous studies. We will have 80% power to detect a difference of 1.2 points change in modified ADAS-Cog (effect size = 0.2) between the experimental groups after 12 months of treatment.

A total of 2500 participants will be recruited, after applying inclusion/exclusion criteria, based on a possible loss of 20% (quit, death, other important diseases…) at the 12-months follow-up (500 participants), ending up on a final sample of 2000 participants divided into four groups of 500 patients.

Statistical Analysis

In this analysis per-protocol, we will only include patients who have received the interventions assigned in the randomization and have completed the study. We will discard patients non-controlled. We hypothesized a maximum patient loss of 20% from the original sample (n=500), a greater loss would introduce a selection bias.

The primary objective of this study protocol is to test whether treatment with Vorinostat will attenuate cognitive decline in aMCI and mild AD patients as compared with placebo. This will be assessed using the modified ADAS-Cog scale, with the hypothesis that the decline from baseline until 12 months for Vorinostat will be significantly less than for placebo.

The primary null hypothesis will be obtaining outcomes with no statistically significant changes in the modified ADAS-Cog from baseline to 12 months in the two intervention groups (aMCI and mild AD). A rejection of the null hypothesis in favor of the alternative, showing that Vorinostat is superior to placebo, will demonstrate a positive treatment effect.

We will consider the two groups (group A: aMCI and group B: mild AD) in an independent way to analyze the results, but ultimately, the percentage of change will be compared between intervention groups in order to assess the differential response to Vorinostat in aMCI and mild AD.

We will use SPSS (IBM Corp. Armonk, NY, USA) for Windows (version 22.0) for data analysis and a 0.05 significance level with a 95% confidence interval to accept statistically significant differences.

- A univariate, descriptive analysis will be done resulting in a table with demographic description and baseline characteristics according to the assigned treatment group: age, gender, years of education, place of residency, concomitant acetylcholinesterase inhibitors, family history of AD, APOE allele, group diagnosis, mADAS-Cog, MoCA, ADCS-ADL, NPI-Q, β-amyloid levels, HDAC activity and MRI volumes. Specifically, we will use the mean, standard deviation, median, minimum and maximum value for quantitative variables. Categorical variables will be expressed as a frequency and percentage of patients.
- A bivariate analysis will be performed to analyze the influence of the independent variable, with each of the dependent variables at baseline and during the follow-up (6 and 12 months).
  - We will use t-student for independent data to analyze and compare the mean between groups for quantitative discrete (years of education, MoCA, mADAS-Cog, ADCS-ADL, NPI-Q) and quantitative continuous variables (age, MRI volumes, β-amyloid levels and HDAC activity).
  - Chi-squared test will be performed to compare qualitative variables (gender, place of residence, concomitant acetylcholinesterase

inhibitors, family history of AD, APOE allele, progression to dementia) between groups. No differences between the treatment and respective control for each group should be found in demographic and baseline characteristics. We expect to find significant differences baseline characteristics between aMCI and mild AD, due to the differential stage of the disease.

- In order to estimate the size of the effect, lineal regression models will be used to correlate quantitative outcomes of the 2 intervention groups (aMCI and mild AD) and the control group, and logistic regression models will be used to correlate categorical outcomes. If necessary, we will adjust for baseline differences by including the variables in the regression model, controlling for age, gender, years of education, place of residency, concomitant acetylcholinesterase inhibitors, APOE allele and family history of AD.

Description of primary endpoint:

- Change from baseline in modified ADAS-Cog using t-student for independent data to compare control and intervention group. The decline from baseline at the end of the treatment period should be significantly less for Vorinostat than for placebo.

Description of secondary endpoints:

- Change from baseline until 6 and 12 months in clinical scores (ADCS-ADL, MoCA, NPI-Q) using t-test for comparison between groups.
- Change from baseline until 6 and 12 months in biomarkers (vMRI, CSF Aβ levels, HDAC expression) using t-test for comparison between groups.
- Association of baseline volumes of whole brain, the ventricles, hippocampus and entorhinal cortex with baseline cognition (mADAS-Cog, ADCS-ADL, MoCA, NPI-Q) using linear regression models.
- Association of CSF Aβ levels with cognition (mADAS-Cog, ADCS-ADL, MoCA, NPI-Q) using linear regression models.

- Evaluation of differences in HDAC expression levels, as well as differences in nuclear density, size, and total area between brain volumes with an unpaired t-test. Differences in HDAC expression levels between the mentioned brain regions will be evaluated with an ordinary one-way ANOVA. Association of HDAC activity with cognition (mADAS-Cog, ADCS-ADL, MoCA, NPI-Q) using linear regression models.

- For the NPI, we will separate patients depending on the threshold described above (NPI = 10). For patients without meaningful psychiatric disturbances (NPI<10), time (days) until worsening symptoms will be analyzed (NPI>10). For patients NPI>10, a decrease in the total score will be studied and compared between groups using chi-squared test.

- To assess differences in response between aMCI and mild AD, changes from baseline in cognitive assessments and vMRI, CSF Aβ levels and HDAC activity, will be compared using linear regression models. Progression to dementia will be compared between groups using logistic regression models.

Subgroup analyses:

- To analyze the effect of baseline and demographic characteristics we will study the age, gender, family history of AD, concomitant acetylcholinesterase inhibitors, APOE allele, place of residence, years of education and at risk alcohol consumption and create different subgroups. The aim of the subgroup analysis is to compare and recognize the possible effect of these characteristics in the cognitive outcome.

- To assess the consistency and reliability of the primary outcome, a subgroup analysis for the 10 centers will be performed to compare the change from baseline in ADAS-Cog using ANOVA analysis.

The analysis of safety data will be assessed by summarizing adverse events, laboratory analyses, vital signs, ECGs, and brain MRIs and collecting the information in a table with the frequency and percentages of patient affected in each group. Comparisons between intervention and control group will be

performed. The safety qualitative variables (adverse effects and patient's loss) will be analyzed and compare between groups using Chi-squared test.

If we determine the existence of statistical differences between the groups, an analysis post-hoc will be performed to identify the subsets that do not differ between them.

Chronogram

Authorization from the EMA (European Medicines Agency) will be acquired, as well as from the ethical committee from each participant center.

The project will start in January 2022 and the predicted endpoint will be in July 2024. The enrollment period will be approximately of 12 months. We expect a final sample of 2000 participants with demographic and neuropathological characteristics comparable across the samples. Treatment with placebo or Vorinostat 400 mg orally will be administered every day for a year. General medical and demographic information will be recollected in visit 0. Participants will perform cognitive test and brain imaging (MRI and PET) at visit 0 (0 months) and visit 1 and 2 (6 and 12 months) and lumbar puncture at visit 1 and 2. Additionally, MRI and lumbar puncture will be performed during the verification procedure of the inclusion and exclusion criteria. A third visit will take place 6 months after finishing the study in order to assess safety measures (ECG, blood sample, physical examination, side effects). Blood analysis and ECG will be performed every 2 weeks during the first 2 months and monthly thereafter. Specific side effects will be summarized throughout the clinical trial by instructing the participants (they will have access to a contact number exclusively intended for the prevention and treatment of adverse effects) and will be evaluated at each visit. The total duration of the study will be 2.5 years (30 months), including a follow-up visit 6 months after finishing the study. Detailed information about study schedule can be found in appendix 4.

Informed consent, ethical review and other considerations

The research team is responsible for ensuring that the participant receives and understands all information regarding the risks and benefits of the study through written information, questions to the researcher and the informed consent (see appendix). The informed consent delivered before the patient enters the study, will explain the risks and benefits of the study in a simple and basic language. Likewise, it will remain as proof that the patient voluntarily participates in the study.

Informed consent must be compliant with the International Conference on Harmonisation guideline for good clinical practice (guideline available on http://www.ich.org/products/guidelines.html). Authorization from the EMA (European Medicines Agency) will be acquired, as well as from the ethical committee from each participant center. All trials will be conducted in accordance with the principles of good clinical practice and the Declaration of Helsinki.

Limitations

Being an efficacy, explanatory clinical trial, we will prioritize the internal validity over the external validity. The analysis will be made by protocol, with the risk of a selection bias by excluding lost and non-compliant participants and thereby overestimate results and underestimate toxicity problems.

The "EPAD: European Prevention of Alzheimer's Dementia Consortium" is a voluntary cohort, and the participants will generally have higher education levels, healthier behaviors and less comorbidity than the general population, this could establish a selection bias, since it's well known that a larger cognitive reserve and less cardiovascular risk factors have a protective effect on the progression from mild to advanced forms of the disease. To avoid this bias, half of the participants will be selected from hospital databases. There could be a change in the behavior of participants knowing that they are part of a clinical trial, known as Hawthorne effect. The process of randomization, stratification and blinding can prevent these selection and information biases.

Possible confounding variables could damage the internal validity of the clinical trial; we expect to reduce this probability by randomization and controlling for educational level, age, gender, concomitant treatment, family history of AD, place of residency and APOE allele for the statistical analysis.

We decided to program 2 parallel groups (aMCI & mild AD) in order to be able to compare the effects of the drug in two different stages and to highlight and determine the differential cognitive and epigenetic changes, even if consequently we will need a larger sample.

Currently, there is an active phase 1 clinical trial evaluating Vorinostat toxicity and its effects on the genome-wide transcriptome profile and in memory performance in patients with AD (NCT03056495). The safety parameters of Vorinostat have been evaluated in patients with cutaneous T-cell lymphoma aged between 37-83 years old, on therapy between 2 to 480 days. Even though, no significant differences in safety or effectiveness were observed between geriatric subjects and younger subjects. The chosen dose in this protocol study (Vorinostat 400 mg orally) is based on the results obtained in clinical trials involving patients with cutaneous T-cell lymphoma. It would be necessary to corroborate the appropriate dose and the specific effectiveness determined by specific phase 1 and 2 studies since no phase 2 clinical trial has been yet performed to evaluate specific effectiveness in patients with prodromal or mild AD.

Intervention period could be too short to detect significant variability between placebo and Vorinostat group. Depending on the results obtained, a replication trial with a longer intervention time could be considered.

We expect that the study will have a high economic cost, due to the sample needed, the multicentricity and the quantity and quality of the outcomes to be studied. We hope to alleviate this deficit through fellowships from the European Union and/or associated centers and financing from the pharmacological industry, as well as from the EPAD platform.

Expected results and relevance

The current protocol presents a phase 3 double-blind, randomized placebo-controlled trial consisting of 2 parallel-groups. The aim of this clinical trial is to test the cognitive and molecular effect of an HDAC inhibitor, Vorinostat, in aMCI and mild AD patients during a one-year follow-up. Previous studies focused on the pharmacological treatment of AD have failed to prove a positive or protective effect; consequently, there is an urgent need to find new strategies in the management of the disease. Unlike these studies, which focused on β-amyloid protein, in this clinical trial we focused on another pathophysiological aspect of AD: epigenetics. We hypothesized that the administration of Vorinostat could attenuate the natural transition from early stages of AD or aMCI to the symptomatic disease. Regardless of the possible result, it is important to promote research in this field and not give up in the absence of results.

The management of AD should include risk-reduction strategies and targeted therapies to make an impact in the natural evolution of the disease. The objective would be to consider AD as a cardiovascular disease or other chronic diseases, with both prevention and treatment options. Epigenetic and preventive strategies would act on a pre-damaged brain in susceptible individuals in order to prevent or delay the onset of the disease.

In order to establish a demonstrated positive effect of Vorinostat on mild AD and aMCI, it is necessary to replicate the possible findings in other clinical studies and to consider effectiveness, intention to treat trial.

Declaration of interest

References

1. Feigin VL, Abajobir AA, Abate KH, Abd-Allah F, Abdulle AM, Abera SF, et al. Global, regional, and national burden of neurological disorders during 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet Neurol. 2017;16(11):877–97.

2. Livingston G, Sommerlad A, Orgeta V, Costafreda SG, Huntley J, Ames D, et al. Dementia prevention, intervention, and care. Lancet. 2017;6736(17).

3. Querfurth HW, Laferla FM. Alzheimer's Disease. new Engl J Med Rev. 2010;9:329–44.

4. Miia Kivipelto. World Wide Fingers. Homepage. http://wwfingers .com/. Published 2017. Accessed January 6, 2018.

5. Decline PC, Forward DAW. Preventing Cognitive Decline and Dementia [Internet]. 2017. Available from: https://www.nap.edu/catalog/24782

6. Hörder H, Johansson L, Guo X, Grimby G, Kern S, Östling S, et al. Midlife cardiovascular fitness and dementia. Neurology [Internet]. 2018;0:10.1212/WNL.0000000000005290. Available from: http://www.neurology.org/lookup/doi/10.1212/WNL.0000000000005290

7. Langa K, Levine D. The Diagnosis and Management of Mild Cognitive Impairment: A Clinical Review. J Am Med Assoc. 2014;312(23):2551–61.

8. Petersen RC, Lopez O, Armstrong MJ, Getchius TSD, Ganguli M, Gloss D, et al. Practice guideline update summary: Mild cognitive impairment. Neurology [Internet]. 2018;90(3):126–35. Available from: http://www.neurology.org/lookup/doi/10.1212/WNL.0000000000004826

9. Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, et al. Initial sequencing and analysis of the human genome. Nature [Internet]. 2001;409(6822):860–921. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11237011%5Cnhttp://www.nature.com/nature/journal/v409/n6822/pdf/409860a0.pdf

10. Waddington CH. An introduction to modern genetics. Eugen Rev. 1940;31(4):218.

11. Portela A, Esteller M. Epigenetic modifications and human disease. Nat Biotechnol [Internet]. 2010;28(10):1057–68. Available from: http://dx.doi.org/10.1038/nbt.1685

12. Hwang JY, Aromolaran KA, Zukin RS. The emerging field of epigenetics in neurodegeneration and neuroprotection. Nat Rev Neurosci [Internet]. 2017;18(6):347–61. Available from: http://dx.doi.org/10.1038/nrn.2017.46

13. Kaminsky ZA, Tang T, Wang SC, Ptak C, Oh GHT, Wong AHC, et al. DNA methylation profiles in monozygotic and dizygotic twins. Nat Genet. 2009;41(2):240–5.

14. Dragunow PN and M. Neuroepigenomics in Aging and Disease, Advances in Experimental Medicine and Biology. In: Springer International Publishing AG [Internet]. 2017. Available from: http://link.springer.com/10.1007/978-3-319-53889-1

15. Urdinguio RG, Sanchez-Mut J V., Esteller M. Epigenetic mechanisms in neurological diseases: genes, syndromes, and therapies. Lancet Neurol [Internet]. 2009;8(11):1056–72. Available from: http://dx.doi.org/10.1016/S1474-4422(09)70262-5

16. Brody, Marka | Liu, Enchib; 1 | Di, Jianingb | Lu, Mingc | Margolin, Richard A.d; 2 | Werth, John L.e | Booth, Kevine | Shadman, Annaf; 3 | Brashear, H. Robertf | Novak G*. A Phase II, Randomized, Double-Blind, Placebo-Controlled Study of Safety, Pharmacokinetics, and Biomarker Results of Subcutaneous Bapineuzumab in Patients with mild to moderate Alzheimer's disease. J Alzheimer's Dis vol 54, no 4, pp 1509-1519, 2016.

17. Ostrowitzki S, Lasser RA, Dorflinger E, Scheltens P, Barkhof F, Nikolcheva T, et al. A phase III randomized trial of gantenerumab in prodromal Alzheimer's disease. Alzheimers Res Ther [Internet]. 2017;9(1):95. Available from: https://alzres.biomedcentral.com/articles/10.1186/s13195-017-0318-y

18. Atri A, Frölich L, Ballard C, Tariot PN, Molinuevo JL, Boneva N, et al. Effect of Idalopirdine as Adjunct to Cholinesterase Inhibitors on Change in Cognition in Patients With Alzheimer Disease. Jama [Internet]. 2018;319(2):130. Available from: http://jama.jamanetwork.com/article.aspx?doi=10.1001/jama.2017.20373

19. Gauthier S, Albert M, Fox N, Goedert M, Kivipelto M, Mestre-Ferrandiz J, et al. Why has therapy development for dementia failed in the last two decades? Alzheimer's Dement [Internet]. 2016;12(1):60–4. Available from: http://dx.doi.org/10.1016/j.jalz.2015.12.003

20. Winblad B, Amouyel P, Andrieu S, Ballard C, Brayne C, Brodaty H, et al. Defeating Alzheimer's disease and other dementias: A priority for European science and society. Lancet Neurol. 2016;15(5):455–532.

21. Kumar-Sinha C, Chinnaiyan AM. Precision oncology in the age of integrative genomics. Nat Biotechnol [Internet]. 2018;36(1):46–60. Available from: http://www.nature.com/doifinder/10.1038/nbt.4017

22. Jakovcevski M, Akbarian S. Epigenetic mechanisms in neurological disease. Nat Med [Internet]. 2012;18(8):1194–204. Available from: http://dx.doi.org/10.1038/nm.2828

23. Albert MS, DeKosky ST, Dickson D, Dubois B, Feldman HH, Fox NC, et al. The diagnosis of mild cognitive impairment due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimer's Dement [Internet]. 2011;7(3):270–9. Available from:

http://dx.doi.org/10.1016/j.jalz.2011.03.008

24. Mckhann G, Drachman D, Folstein M. Clinical diagnosis of Alzheimer 's disease. Neurology. 1984;34(7):939--944.

25. Folstein MF, Folstein SE MP. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res. 1975;12(3):189-.

26. Nasreddine ZS, Phillips NA, Bédirian V, Charbonneau S, Whitehead V, Collin I, et al. The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. J Am Geriatr Soc [Internet]. 2005;53(4):695–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15817019

27. Rosen WG, Mohs RC DK. A new rating scale for Alzheimer's disease. Am J Psychiatry 141, 1356–1364. 1984;

28. Mohs RC, Knopman D PR et al. Development of cognitive instruments for use in clinical trials of antidementia drugs: additions to the Alzheimer's Disease Assessment Scale that broaden its scope: the Alzheimer's Disease Cooperative Study. Alzheimer Dis Assoc Disord 11 Suppl 2 S13-S21. 1997;

29. Galasko D, Bennett D, Sano M, Ernesto C, Thomas R, Grundman M FS. An inventory to assess activities of daily living for clinical trials in Alzheimer's disease. The Alzheimer's Disease Cooperative Study. Alzheimer Dis Assoc Disord. 1997;

30. de Jonghe JFM, Kat MG, Kalisvaart CJ, Boelaarts L. [Neuropsychiatric inventory questionnaire (NPI-Q): A validity study of the Dutch form]. Tijdschr Gerontol Geriatr [Internet]. 2003;34(2):74–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17052006

31. Samson M, Porter N, Orekoya O, Hebert JR, Adams SA, Bennett CL, et al. Insights into neuroepigenetics through human histone deacetylase PET imaging. Sci Transl Med. 2017;155(1):3–12.

32. Jack CR, Knopman DS, Jagust WJ, Shaw LM, Aisen PS, Weiner MW, et al. Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. Lancet Neurol. 2010;9(1):119–28.

33. Shaw LM, Vanderstichele H, Knapik-Czajka M, Clark CM, Aisen PS, Petersen RC, et al. Cerebrospinal fluid biomarker signature in alzheimer's disease neuroimaging initiative subjects. Ann Neurol. 2009;65(4):403–13.

34. Olsson B, Lautner R, Andreasson U, Öhrfelt A, Portelius E, Bjerke M, et al. CSF and blood biomarkers for the diagnosis of Alzheimer's disease: A systematic review and meta-analysis. Lancet Neurol. 2016;673–84.

35. MERCK. INDICATIONS AND USAGE ZOLINZA [Internet]. 2006. Available from: https://www.merck.com/product/usa/pi_circulars/z/zolinza/zolinza_pi.pdf

36. Relkin NR, Thomas RG, Rissman RA, Brewer JB, Rafii MS, Van Dyck CH, et al. A phase 3 trial of IV immunoglobulin for Alzheimer disease. Neurology. 2017;88(18):1768–75.

37. Honig. Trial of Solanezumab for Mild Dementia Due to Alzheimer's Disease. N Engl J Med [Internet]. 2018;3784378:321–30. Available from: http://www.nejm.org/doi/pdf/10.1056/NEJMoa1705971

38. Sevigny J, Chiao P, Bussière T, Weinreb PH, Williams L, Maier M, et al. The antibody aducanumab reduces Aβ plaques in Alzheimer's disease. Nature [Internet]. 2016;537(7618):50–6. Available from: http://dx.doi.org/10.1038/nature19323

Appendix

Table 1 Clinical protocol synopsis

Name of investigational product: Vorinostat (Suberoylanilide hydroxamic acid)	
Title of study: Phase III randomized clinical trial of Vorinostat in Amnestic Mild Cognitive Impairment and Mild Alzheimer's Disease	

Number of planned participants:
    Entered: 3500
    Enrolled/randomized: 2500
    Completed: 2000
	Phase development: 3

Length of study: Approximately 1,5 years (18 months)	
Objectives: The primary objective of this study is to test the hypothesis that the oral administration of Vorinostat could attenuate the natural transition from mild stages of AD and/or Amnestic Mild Cognitive Impairment (aMCI) to an advanced symptomatic AD compared with placebo, measured by change from baseline on the modified Cognitive Subscale of the Alzheimer's Disease Assessment Scale (ADAS-Cog modified) after 12 months of treatment. The hypothesis is that the change at 12 months of treatment for Vorinostat treatment will be significantly less than for placebo.	

The secondary objectives of the study are: to assess the global benefit of Vorinostat treatment as demonstrated through changes from baseline to 6 and 12 months follow-up in both aMCI and mild AD in clinical scores, brain volumes and biomarkers: Alzheimer's Disease Cooperative Study – Activities of Daily Living Inventory (ADCS-ADL), Montreal Cognitive Assessment (MoCA), Neuropsychiatric Inventory (NPI), changes in cerebrospinal fluid (CSF) β amyloid levels, volumetric magnetic resonance imaging (vMRI) and HDAC activity measured by Positron Emission Tomography (PET).

Study design: Multicenter, phase 3 double-blind, randomized placebo-controlled trial consisting of 2 parallel-group, we will assess the efficacy of Vorinostat over 1 year in patients with aMCI and mild AD. Patients will be randomized to receive orally Vorinostat 400 mg or placebo every day for 12 months. Concomitant treatment with acetylcholinesterase inhibitors at stable doses will be permitted.

Diagnosis and main criteria for inclusion and exclusion: Participants (males and females) aged between 65-85 years old with adequate auditory and visual function diagnosed in a probable mild stage of AD or amnestic MCI by an expert and external neurologist, according the National Institute of Health (NIH) criteria and the National Institute of Neurologic and Communicative Disorders and Stroke—Alzheimer's Disease and Related Disorders Association. Participants will be excluded if they are diagnosed of other type of dementia, any major psychiatric syndrome, use of other HDAC inhibitor (e.g., valproic acid), as well as any unstable general medical condition.

Test product, dosage and mode of administration: Vorinostat 400 mg will be administered orally once a day.

Planned duration of treatment: 12 months with safety evaluation 6 months after last dose

Placebo, dosage and mode of administration: Placebo (low dose albumin) will be administered orally once a day.

Criteria for evaluation:
Clinical: modified ADAS-Cog, MoCA, ADCS-ADL, NPI-Q
Imaging: vMRI, HDAC activity PET
Bioanalytical: CSF Aβ
Safety: adverse events, vital signs, laboratory evaluations, ECGs and MRI
Participant's loss: treatment adherence, voluntary abandonment of the study, subsequent emergence of exclusion criteria
Others: age, gender, years of education, place of residence, concomitant acetylcholinesterase inhibitors, APOE allele and family history of AD.

Statistical methods: All analyses will follow the per-protocol principle. The change from baseline will be assessed if patients have a baseline and a post-baseline measure available. All efficacy hypothesis tests will use a 0.05 significance level.

Sample size calculation: A sample size of 500 patients per group (2000 in total) will have 80% power to detect a difference of 1.2 points over 12 months, with an effect size of 0.2 between the treatment groups.

Primary analysis: Modified ADAS-Cog in both groups (mild AD and aMCI) will be analyzed. The change from baseline score at 12 months will be the dependent variable. We will control for age, gender, years of education, concomitant acetylcholinesterase inhibitors and APOE allele.

Secondary endpoints: Change from baseline to endpoint will be assessed using ADCS-ADL,

MoCA, NPI-Q, vMRI, HDAC activity PET and CSF Aβ.

<u>Analysis of safety data</u>: Safety will be assessed by summarizing adverse effects, laboratory results, vital signs, ECGs and brain MRIs.

Table 2 List of participating centers

South East Aigaion S.A. *New Ring Road Ornos Post Code 84600 PO Box 617 Copenhague, Denmark* Phone : +30 6944345234 Email : info@seamedical.gr Website: http://seamedical. gr	**Double Check** *Zollikerstrasse 60, 8702 Zollikon, Switzerland* Phone : +41 43 541 11 52 Email: h.szabo@kusnachtpr actice.ch Web: http://www.doublec heck.ch/	**International Health Plus** *IHP Boston Business Centre 69-75 Boston Manor Road - Brentford, Middx London. United Kingdom* Phone : +440-208-231-8855 Email: info@ihp.london Web: http://www.ihp.londo n/
Salus Gate *Via Ampola 11 Milan, Italy 20139* Phone : 39-3423-981363 Email: deborah.duncan@sal usgate.com Website: www.salusgate.co m	**The Kusnacht Practice** *Zollikerstrasse 60, Zollikon, Switzerland* Phone : +41 43 541 11 52 Email: h.szabo@kusnachtpr actice.ch Web: https://kusnachtprac tice.com/en	**Hospital Ramón y Cajal** *Ctra. Colmenar Viejo, Km. 9,1. Madrid, Spain. 28034* Phone : (34) 913368000 Email:mgarciamolero@hrc.c om Web: http://www.ramonycajal.es
Poliklinika Bagatin *Green Gold Tower Grada Vukovara 269a/10, 10000 Amsterdam, The Netherlands* Phone : 385 15574 624 Email: info@poliklinikabaga tin.hr Web: http://www.poliklinik abagatin.hr/eng/#￼	**Erasme** *1070 Brussels Brussels-Capital (Region Brussels-Capital)* Phone: 02/434.79.11 Email:jeangasn@erasme.co m Web:http://www.erasme.u lb.ac.be	**Clcc Institut Curie** *26 Rue D Ulm - 1074 Brussels* Phone : 0144324000 Email:marie.lepin@clcccurie .com Web: http://www.clcccurie.fr
Hospital universitario y politécnico la fe *Avda. Campanar, 21. Valencia, Spain. 46009* Phone : (34) 963862700 Email:lluisboix@huplf.com Web: ttp://www.hlafe.es		

Table 3 National leads EPAD project

Participant	Name	Country/region
UEDIN	Craig Ritchie	United Kingdom
KI	Miia Kivipelto	Denmark
BBRC	José Luis Molinuevo	Spain
VU-VUmc	Philip Scheltens	Benelux
UNIGE	Giovanni Frisoni	Switzerland/Italy

Latest phase III clinical trials in AD		
A phase 3 trial of IV immunoglobulin for AD (36)	Ab IgG anti-Aβ ev in mild to moderate AD (18 months). Not beneficial effects on cognition or function compared to participants that received placebo.	Neurology 2017 88:1768–1775
Trial of Solanezumab for Mild Dementia Due to AD (37)	Monoclonal Ab IgG anti-Aβ 400mg ev /4 weeks for 76 weeks. Not significantly effect in cognitive decline and cerebral amyloid.	New Engl J Med 2018 378:321-30
Effect of Idalopirdine as Adjunct to Cholinesterase Inhibitors on Change in Cognition in Patients With AD (18)	Selective 5-hydroxytryptamine-6 receptor antag (serotonin, related with memory and learning) In patients with mild to moderate AD, the use of idalopirdine compared with placebo did not improve cognition over 24 weeks of treatment.	JAMA 2018 319(2):130-142.
A Phase III, Randomized, Double-Blind, Placebo-Controlled Study of Safety, Pharmacokinetics, and Biomarker Results of Sc Bapineuzumab in Patients with mild to moderate AD (16)	Humanized monoclonal Ab anti-amyloid-β (Aβ1-40/1 -42) - neurotoxic amyloid proteins. Bapineuzumab SC once-monthly did not demonstrate significant treatment difference over placebo on cerebral amyloid signal and cognitive function at one year but was well-tolerated.	J Alzheimers Dis. 2016 18;54(4):1509-1519.
A phase III randomized trial of Gantenerumab in prodromal AD (17)	Humanized monoclonal Ab anti-amyloid-β No efficacy in any dose.	Alzheimer's Research & Therapy (2017) 9:95

Table 4 Latest phase 3 clinical trials in AD

Promising in-course clinical trials in AD		
Phase III Aducanumab	Monoclonal Ab anti amyloid-β Reduces Aβ plaques in phase II (38)	ENGAGE (NCT02477800) EMERGE (NCT02484547)
Phase III Verubecestat in aMCI	Inhibits BACE activity (β-secretase inhibitors)	NCT01953601
Phase III TRx0237 in mild AD	Reduce tau aggregation	NCT01689233
Semagacestat (LY450139) in mild-moderate AD	Functional γ-secretase inhibitor (to lower β-amyloid in CSF)	NCT00762411

Table 5 Promising in-course clinical trials in AD

Epigenetic changes in AD		
Epigenetic change	**Characteristics**	**Changes in AD**
DNA methylation / demethylation	DNA methylation (DNMTs) inhibits transcription and its involved in memory processes	Genome-wide decrease in DNA methylation Hypermethylation: MTHFR, Neprilysin, MAPT, APOE, SORB3, ANK1 (ankyrin 1) Hypomethylation: APP, BACE, PSEN1, PP2A, S100A2, CREB5)
Histone modifications	Histone acetylation (HATs): activation Histone deacetylation (HDACs): inactivation	Histone acetylation is globally reduced HDAC6 (tau-interacting protein and a potential modulator of tau phosphorylation and accumulation), is increased in cortical and hippocampal regions in AD Inhibition of HDAC induces dendritic sprouting, increases synaptic number and improves long-term memory
Chromatin remodeling	Controls accessibility of transcription factors (activation or repression)	More research is needed
Non-coding RNAs	Interacts with proteins or genomic DNA	Several lncRNAs are dysregulated in AD (Sox2OT, 1810014B01Rik, BC200, BACE1-AS, NAT-Rad18, 17A, GDNFOS) and other neurological diseases Several miRNAs are directly linked to AD pathogenesis

Table 6 Epigenetic changes in AD (15)

Criteria for abnormal ECG parameters	
Parameter	**Criteria**
Heart rate	≤ 40 bpm and/or ≥ 120 bpm
PR interval	≥ 200 msec
QRS duration	≥ 100 msec
QT interval	≥ 450 msec

Criteria for prolonged QTc
1. In adult males, QTc ≥ 450 msec; in adult females, QTc ≥ 470 msec
2. QTc ≥ 500 msec
3. Increase of > 0 msec and ≤ 30 msec relative to baseline
4. Increase of > 30 msec and ≤ 60 msec relative to baseline
5. Increase of > 60 msec relative to baseline

Table 7 Criteria for abnormal ECG parameters (38)

Adapted from the template for Clinical Studies (WHO ERC)

Research Ethics Review Committee

(WHO ERC)

20, AVENUE APPIA – CH-1211 GENEVA 27 – SWITZERLAND –
HTTP://INTRANET.WHO.INT/HOMES/RPC/ERC – HTTP://WWW.WHO.INT/RPC/RESEARCH_ETHICS

Informed Consent Form for Clinical Studies

Universitat Internacional de Catalunya (UIC) Research Institute

Name of Principle Investigator: Almudena Boix Lago

Informed Consent form for men and women who volunteer for "EPAD: European Prevention of Alzheimer's Dementia Consortium" and recruited via hospital and clinics affiliated with the project, who are invited to participate in research on mild and preclinical forms of Alzheimer's Disease. The title of our research project is: "Clinical trial of Vorinostat in Amnestic Mild Cognitive Impairment and Mild Alzheimer's Disease"

Research information:
Name of Principal Investigator: Almudena Boix Lago
Name of Organization: Universitat Internacional de Catalunya (UIC) Research Institute
Name of Sponsor: Merck & Co

This Informed Consent Form has two parts:
- **Information Sheet (to share information about the research with you)**
- **Certificate of Consent (for signatures if you agree to take part)**

You will be given a copy of the full Informed Consent Form

PART I: Information Sheet

Introduction
I am Almudena Boix Lago, working for the Universitat Internacional de Catalunya (UIC) Research Institute. We are doing research on early stages of Alzheimer Disease, which is very common in this country. I am going to give you information and invite you to be part of this research. You do not have to decide today whether or not you will participate in the

research. Before you decide, you can talk to anyone you feel comfortable with about the research.

There may be some words that you do not understand. Please ask me to stop as we go through the information and I will take time to explain. If you have questions later, you can ask me, the study doctor or the staff.

Purpose of the research
Alzheimer Disease is the most common form of dementia and is very prevalent in our country. The drugs that are currently used to help people with Alzheimer are not as good as we would like them to be. In fact, none of them are able to slow down the progression of the disease. There is a new drug which may work better. The reason why we are doing this research is to find out if the new drug VORINOSTAT is better than placebo in people taking the regular used drugs.

Type of Research Intervention
This research will involve a single pill every day for a year, as well as three follow-up visits to the clinic where a detailed interview, brain scan, lumbar puncture and blood sample will be taken.

Participant selection
We are inviting all adults between 65 and 85 years old with a diagnosis of Amnestic Mild Cognitive Impairment or Mild Alzheimer Disease who attend the associated EPAD clinics to participate in the research on the new Alzheimer drug.

Voluntary Participation
Your participation in this research is entirely voluntary. It is your choice whether to participate or not. Whether you choose to participate or not, all the services you receive at your clinic will continue and nothing will change. If you choose not to participate in this research project, you will be offered the treatment that is routinely offered in your clinic/hospital for Alzheimer Disease, and we will tell you more about it later. You may change your mind later and stop participating even if you agreed earlier.

Information on the Trial Drug VORINOSTAT
The drug we are testing in this research is called Vorinostat. It has been tested before with people who do not have Alzheimer but who are exposed to the same risks factors and/or age. We now want to test the drug on people who have Alzheimer to see if the drug is effective. This second research is called a "phase 3" trial.

The drug Vorinostat is made by the Company Merck & Co. You should know that this drug is already used in the treatment of recurrent cutaneous T-cell lymphoma and it has a few side effects. One of the side effects, or problems, is that you may feel tired for the first day after being given the drug. Also, 20% of the people who tried the drug in previous research experienced fatigue, diarrhea, nausea, distortion of the sense of taste, low platelets and anorexia.

Some participants in the research will not be given the drug which we are testing. Instead, they will be given a placebo, an innocuous pill with no pharmacological effects. There is no risk associated with placebo and no known problems.

Procedures and Protocol

<u>A. Unfamiliar Procedures</u>

1) <u>Randomization and blinding</u>:

Because we do not know if the new Alzheimer drug is better than the currently available drug for treating Alzheimer and can change the progression of the disease, we need to examine the efficacy of the drug. To do this, we will put people taking part in this research into two groups. The groups are selected by chance, as if by tossing a coin.

Participants in one group will be given the test drug while participants in the other group will be given a placebo. It is important that neither you nor we, know which of the two drugs you are given. This information will be in our files, but we will not look at these files until after the research is finished. This is the best way we have for testing without being influenced by what we think or hope might happen. We will then compare which of the two has the best results.

The healthcare workers will be looking after you and the other participants very carefully during the study. If we are concerned about what the drug is doing, we will find out which drug you are getting and make changes. If there is anything you are concerned about or that is bothering you about the research please talk to me or one of the other researchers.

2) <u>Placebo</u>:

A placebo or inactive medicine looks like real medicine but it is not. It is a dummy or pretend medicine. It has no effect on a person because it has no real medicine in it. Sometimes when we want to know whether a new medicine is good, we give some people the new medicine and some people the pretend or dummy medicine. For the research to be good, it is important that you do not know whether you have been given the real medicine or the pretend or dummy medicine. This is one of the best ways we have for knowing what the medicine we are testing really does.

3) <u>Blood samples:</u>

We will take blood from your arm using a syringe and needle. Each time we will take about a small black coffee cup of blood. In total, we will take about a bottle of water in 12 months. At the end of the research, in 1 year, any left-over blood sample will be destroyed.

4) <u>Lumbar puncture</u>:

We will take cerebrospinal fluid from your back using a syringe and needle. Each time we will take about 4-5 drops. In total, we will take about a glass of water in 12 months. At the end of the research, in 1 year, any left-over blood sample will be destroyed.

5) <u>Brain imaging</u>:

We will take photographs of your brain. The procedure takes 30 minutes long and it can be a bit claustrophobic for some people. You can talk with one of the technicians during the throughout procedure and get out at any moment if you feel sick.

6) <u>Interviews</u>:

An interview with a neurologist will be made. The approximate duration of the interview is about 2 hours but you will only have to do it every 6 months, three times in total.

B. Description of the Process

During the research you will make five visits to the clinic.
- <u>Stage 0</u>: Once diagnosed, in the first visit, a small amount of blood, equal to about a teaspoon, will be taken from your arm with a syringe. This blood will be tested for the presence of substances for safety reasons and to test biomarkers. You will be performed a lumbar puncture and 4-5 drops will be taken. Brain imaging will be done during 30 minutes. We will also ask you a few questions about your daily life and ask you to perform some test during 3 hours.
- <u>Stage 1</u>: At the next visit, you will be given either the test drug or the placebo. As explained before, neither you nor we will know whether you have received the test or the dummy/pretend drug.
- <u>Stage 2</u>: After six months, you will come back to the clinic to perform the same tests as in the first visit (stage 0)
- <u>Stage 3</u>: After six months, you will come back to the clinic to perform the same tests as in the first visit (stage 0). The study will be completed at this point and you will stop taking the drug.
- <u>Stage 4</u>: After six months, you will come back to the clinic to check that everything is ok and that you do not have any adverse effect.

Duration
The research takes place over 12 months in total. During that time, it will be necessary for you to come to the clinic/hospital/health facility 5 days, for 4 hours each day. We would like to meet with you six months after your last clinic visit for a final check-up.

In total, you will be asked to come 5 times to the clinic in 18 months. At the end of 18 months, the research will be finished.

Side Effects
As already mentioned, this drug can have some unwanted effects. It can cause fatigue, diarrhea, nausea, distortion of the sense of taste, low platelets and anorexia. In some rare cases, it can cause pulmonary embolism and deep vein thrombosis, heart electrical changes. It is possible that it may also cause some problems that we are not aware of. However, we will follow you closely and keep track of any unwanted effects or any problems. We may use some other medicines to decrease the symptoms of the side effects or reactions. Or we may stop the use of one or more drugs. If this is necessary we will discuss it together with you and you will always be consulted before we move to the next step.

Risks
By participating in this research it is possible that you will be at greater risk than you would otherwise be. There is, for example, a risk that your disease will not get better and that the new medicine doesn't work even as well as the old one. If, however, the medicine is not working or you have any dangerous side effects we will stop the treatment.

Benefits
If you participate in this research, you will have the following benefits: any interim illnesses will be treated at no charge to you. If your child falls sick during this period he/she will be treated free of charge. There may not be any benefit for you but your participation is likely to help us find the answer to the research question. There may not be any benefit to the society at this stage of the research, but future generations are likely to benefit.

Reimbursements
We will give you a reasonable amount of money to pay for your travel to the clinic/parking and for lost work time. You will not be given any other money or gifts to take part in this research.

Confidentiality
With this research, something out of the ordinary is being done in your community. It is possible that if others in the community are aware that you are participating, they may ask you questions. We will not be sharing the identity of those participating in the research.

The information that we collect from this research project will be kept confidential. Information about you that will be collected during the research will be put away and no-one but the researchers will be able to see it. Any information about you will have a number on it instead of your name. Only the researchers will know what your number is and we will lock that information up with a lock and key. It will not be shared with or given to anyone except your assigned clinician and psychologist.

Sharing the Results
The knowledge that we get from doing this research will be shared with you through community meetings before it is made widely available to the public. Confidential information will not be shared. There will be small meetings in the community and these will be announced. After these meetings, we will publish the results in order that other interested people may learn from our research.

Right to Refuse or Withdraw
You do not have to take part in this research if you do not wish to do so and refusing to participate will not affect your treatment at this clinic in any way. You will still have all the benefits that you would otherwise have at this clinic. You may stop participating in the research at any time that you wish without losing any of your rights as a patient here. Your treatment at this clinic will not be affected in any way.

Alternatives to Participating
If you do not wish to take part in the research, you will be provided with the established standard treatment available at the centre/institute/hospital. People who have early forms of Alzheimer Disease are given acetylcholinesterase inhibitors (donepezil, galantamine and rivastigmine) and in people with Mild Cognitive Impairment, there is no recommended/demonstrated treatment.

Who to Contact
If you have any questions you may ask them now or later, even after the study has started. If you wish to ask questions later, you may contact the following person: Almudena Boix Lago, Universitat Internacional de Catalunya (UIC) Research Institute; C/ Josep Trueta s/n. Sant Cugat del Vallès. Tel. 0034-912443509. Email: aboixlago@uic.es

This proposal has been reviewed and approved by the local IRB, which is a committee whose task it is to make sure that research participants are protected from harm. If you wish to find about more about the IRB, contact Joshua Gerald, tel. 0041 654897234, email: j.geraldt@irb.ie. It has also been reviewed by the Ethics Review Committee of the World Health Organization (WHO), which is supporting the study.

You can ask me any more questions about any part of the research study, if you wish to.

PART II: Certificate of Consent

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate as a participant in this research.

Print Name of Participant_____

Signature of Participant _____

Date _____
        Day/month/year

If illiterate
A literate witness must sign (if possible, this person should be selected by the participant and should have no connection to the research team). Participants who are illiterate should include their thumb-print as well.

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of witness_____   AND   Thumb print of participant

Signature of witness _____

Date _____
        Day/month/year

Statement by the researcher/person taking consent
I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands that the following will be done:
1.
2.
3.
I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

 A copy of this ICF has been provided to the participant.

Print Name of Researcher/person taking the consent_____

Signature of Researcher /person taking the consent_____

Date _____
        Day/month/year

Protocol forms for lumbar puncture, extraction and conservation of amyloid β levels, as well as MRI and PET procedure, image criteria and analysis, are not included in this study protocol due to the length of them; but are available for free in Alzheimer's Disease Neuroimaging Initiative http://www.adni-info.org/Scientists/ADNIStudyProcedures.html.

9 Scales for diagnosis and inclusion/exclusion criteria

GERIATRIC DEPRESSION SCALE*

Patient: _____ *Examiner:* _____ *Date:* _____

Directions to Patient: Please choose the best answer for how you have felt over the past week.

Directions to Examiner: Present questions *verbally*. Circle answer given by patient. Do not show scale to patient.

1.	Are you basically satisfied with your life?	Yes	**No (1)**
2.	Have you dropped many of your activities and interests?	**Yes (1)**	No
3.	Do you feel that your life is empty?	**Yes (1)**	No
4.	Do you often get bored?	**Yes (1)**	No
5.	Are you hopeful about the future?	Yes	**No (1)**
6.	Are you bothered by thoughts you can't get out of your head?	**Yes (1)**	No
7.	Are you in good spirits most of the time?	Yes	**No (1)**
8.	Are you afraid that something bad is going to happen to you?	**Yes (1)**	No
9.	Do you feel happy most of the time?	Yes	**No (1)**
10.	Do you often feel helpless?	**Yes (1)**	No
11.	Do you often get restless and fidgety?	**Yes (1)**	No
12.	Do you prefer to stay at home rather than go out and do things?	**Yes (1)**	No
13.	Do you frequently worry about the future?	**Yes (1)**	No
14.	Do you feel you have more problems with memory than most?	**Yes (1)**	No
15.	Do you think it is wonderful to be alive now?	Yes	**No (1)**
16.	Do you feel downhearted and blue?	**Yes (1)**	No
17.	Do you feel pretty worthless the way you are now?	**Yes (1)**	No
18.	Do you worry a lot about the past?	**Yes (1)**	No
19.	Do you find life very exciting?	Yes	**No (1)**
20.	Is it hard for you to get started on new projects?	**Yes (1)**	No
21.	Do you feel full of energy?	Yes	**No (1)**
22.	Do you feel that your situation is hopeless?	**Yes (1)**	No
23.	Do you think that most people are better off than you are?	**Yes (1)**	No
24.	Do you frequently get upset over little things?	**Yes (1)**	No
25.	Do you frequently feel like crying?	**Yes (1)**	No
26.	Do you have trouble concentrating?	**Yes (1)**	No
27.	Do you enjoy getting up in the morning?	Yes	**No (1)**
28.	Do you prefer to avoid social occasions?	**Yes (1)**	No
29.	Is it easy for you to make decisions?	Yes	**No (1)**
30.	Is your mind as clear as it used to be?	Yes	**No (1)**

Total: Please sum all bolded answers (worth one point) for a total score. _____

Scores: 0 to 10 = Normal; 11 to 20 = Moderate Depression; 21 to 30 = Severe Depression.

* *Format modified slightly from original. Reprinted from Yesavage, J.A., Brink, T.L., Rose, T.L., Lum, O., Huang, V., Adey, M., & Leirer, V.O. (1983). Development and validation of a geriatric depression screening scale: A preliminary report.* Journal of Psychiatric Research, *17, 37-49. Reprinted with permission from Elsevier Science.*

HACHINSKI ISCHAEMIA SCORE

Patient Name: _____

Rater Name: _____

Date: _____

Feature	Score	Feature	Score
Abrupt onset	2	Emotional incontinence	1
Stepwise deterioration	1	History of hypertension	1
Fluctuating course	2	History of strokes	2
Nocturnal confusion	1	Evidence of associated atherosclerosis	1
Relative preservation of personality	1	Focal neurological symptoms	2
Depression	1	Focal neurological signs	2
Somatic complaints	1		

TOTAL SCORE _____

References

Hachinski VC, Iliff LD, Zilhka E, Du Boulay GH, McAllister VL, Marshall J, Russell RW, Symon L. "Cerebral blood flow in dementia."
Arch Neurol. 1975;32:632-7.

Molsa PK, Paljarvi L, Rinne JO, Rinne UK, Sako E. "Validity of clinical diagnosis in dementia: a prospective clinicopathological study."
J Neurol Neurosurg Psychiatry. 1985;48:1085-90.

A score < 4 suggest a degenerative disorder. Between 4 and 7 points: doubtful cases and mixed dementias and a score > 7 suggest a vascular dementia.

CDR™ Scoring Table

Subject Initials _____

CLINICAL DEMENTIA RATING (CDR™):	0	0.5	1	2	3

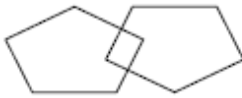|	Impairment				
	None 0	Questionable 0.5	Mild 1	Moderate 2	Severe 3
Memory	No memory loss or slight inconsistent forgetfulness	Consistent slight forgetfulness; partial recollection of events; "benign" forgetfulness	Moderate memory loss; more marked for recent events; defect interferes with everyday activities	Severe memory loss; only highly learned material retained; new material rapidly lost	Severe memory loss; only fragments remain
Orientation	Fully oriented	Fully oriented except for slight difficulty with time relationships	Moderate difficulty with time relationships; oriented for place at examination; may have geographic disorientation elsewhere	Severe difficulty with time relationships; usually disoriented to time, often to place	Oriented to person only
Judgment & Problem Solving	Solves everyday problems & handles business & financial affairs well; judgment good in relation to past performance	Slight impairment in solving problems, similarities, and differences	Moderate difficulty in handling problems, similarities, and differences; social judgment usually maintained	Severely impaired in handling problems, similarities, and differences; social judgment usually impaired	Unable to make judgments or solve problems
Community Affairs	Independent function at usual level in job, shopping, volunteer and social groups	Slight impairment in these activities	Unable to function independently at these activities although may still be engaged in some; appears normal to casual inspection	No pretense of independent function outside home Appears well enough to be taken to functions outside a family home	Appears too ill to be taken to functions outside a family home
Home and Hobbies	Life at home, hobbies, and intellectual interests well maintained	Life at home, hobbies, and intellectual interests slightly impaired	Mild but definite impairment of function at home; more difficult chores abandoned; more complicated hobbies and interests abandoned	Only simple chores preserved; very restricted interests, poorly maintained	No significant function in home
Personal Care	Fully capable of self-care		Needs prompting	Requires assistance in dressing, hygiene, keeping of personal effects	Requires much help with personal care; frequent incontinence

Score only as decline from previous usual level due to cognitive loss, not impairment due to other factors.

Total scores: 0 = Normal; 0.5 = Very Mild Dementia; 1 = Mild Dementia; 2 = Moderate Dementia; 3 = Severe Dementia

Mini-Mental State Examination (MMSE)

Patient's Name: _____   Date: _____

Instructions: Ask the questions in the order listed. Score one point for each correct response within each question or activity.

Maximum Score	Patient's Score	Questions
5		"What is the year? Season? Date? Day of the week? Month?"
5		"Where are we now: State? County? Town/city? Hospital? Floor?"
3		The examiner names three unrelated objects clearly and slowly, then asks the patient to name all three of them. The patient's response is used for scoring. The examiner repeats them until patient learns all of them, if possible. Number of trials: _____
5		"I would like you to count backward from 100 by sevens." (93, 86, 79, 72, 65, …) Stop after five answers. Alternative: "Spell WORLD backwards." (D-L-R-O-W)
3		"Earlier I told you the names of three things. Can you tell me what those were?"
2		Show the patient two simple objects, such as a wristwatch and a pencil, and ask the patient to name them.
1		"Repeat the phrase: 'No ifs, ands, or buts.'"
3		"Take the paper in your right hand, fold it in half, and put it on the floor." (The examiner gives the patient a piece of blank paper.)
1		"Please read this and do what it says." (Written instruction is "Close your eyes.")
1		"Make up and write a sentence about anything." (This sentence must contain a noun and a verb.)
1		"Please copy this picture." (The examiner gives the patient a blank piece of paper and asks him/her to draw the symbol below. All 10 angles must be present and two must intersect.)
30		TOTAL

(Adapted from Rovner & Folstein, 1987)

Instructions for administration and scoring of the MMSE

Orientation (10 points):
- Ask for the date. Then specifically ask for parts omitted (e.g., "Can you also tell me what season it is?"). One point for each correct answer.
- Ask in turn, "Can you tell me the name of this hospital (town, county, etc.)?" One point for each correct answer.

Registration (3 points):
- Say the names of three unrelated objects clearly and slowly, allowing approximately one second for each. After you have said all three, ask the patient to repeat them. The number of objects the patient names correctly upon the first repetition determines the score (0-3). If the patient does not repeat all three objects the first time, continue saying the names until the patient is able to repeat all three items, up to six trials. Record the number of trials it takes for the patient to learn the words. If the patient does not eventually learn all three, recall cannot be meaningfully tested.
- After completing this task, tell the patient, "Try to remember the words, as I will ask for them in a little while."

Attention and Calculation (5 points):
- Ask the patient to begin with 100 and count backward by sevens. Stop after five subtractions (93, 86, 79, 72, 65). Score the total number of correct answers.
- If the patient cannot or will not perform the subtraction task, ask the patient to spell the word "world" backwards. The score is the number of letters in correct order (e.g., dlrow=5, dlorw=3).

Recall (3 points):
- Ask the patient if he or she can recall the three words you previously asked him or her to remember. Score the total number of correct answers (0-3).

Language and Praxis (9 points):
- Naming: Show the patient a wrist watch and ask the patient what it is. Repeat with a pencil. Score one point for each correct naming (0-2).
- Repetition: Ask the patient to repeat the sentence after you ("No ifs, ands, or buts."). Allow only one trial. Score 0 or 1.
- 3-Stage Command: Give the patient a piece of blank paper and say, "Take this paper in your right hand, fold it in half, and put it on the floor." Score one point for each part of the command correctly executed.
- Reading: On a blank piece of paper print the sentence, "Close your eyes," in letters large enough for the patient to see clearly. Ask the patient to read the sentence and do what it says. Score one point only if the patient actually closes his or her eyes. This is not a test of memory, so you may prompt the patient to "do what it says" after the patient reads the sentence.
- Writing: Give the patient a blank piece of paper and ask him or her to write a sentence for you. Do not dictate a sentence; it should be written spontaneously. The sentence must contain a subject and a verb and make sense. Correct grammar and punctuation are not necessary.
- Copying: Show the patient the picture of two intersecting pentagons and ask the patient to copy the figure exactly as it is. All ten angles must be present and two must intersect to score one point. Ignore tremor and rotation.

(Folstein, Folstein & McHugh, 1975)

Interpretation of the MMSE

Method	Score	Interpretation
Single Cutoff	<24	Abnormal
Range	<21	Increased odds of dementia
	>25	Decreased odds of dementia
Education	21	Abnormal for 8th grade education
	<23	Abnormal for high school education
	<24	Abnormal for college education
Severity	24-30	No cognitive impairment
	18-23	Mild cognitive impairment
	0-17	Severe cognitive impairment

Sources:

- Crum RM, Anthony JC, Bassett SS, Folstein MF. Population-based norms for the mini-mental state examination by age and educational level. *JAMA*. 1993;269(18):2386-2391.
- Folstein MF, Folstein SE, McHugh PR. "Mini-mental state": a practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res*. 1975;12:189-198.
- Rovner BW, Folstein MF. Mini-mental state exam in clinical practice. *Hosp Pract*. 1987;22(1A):99, 103, 106, 110.
- Tombaugh TN, McIntyre NJ. The mini-mental state examination: a comprehensive review. *J Am Geriatr Soc*. 1992;40(9):922-935.

MONTREAL COGNITIVE ASSESSMENT (MOCA)

NAME :
Education :   Date of birth :
Sex :   DATE :

VISUOSPATIAL / EXECUTIVE		POINTS
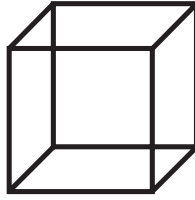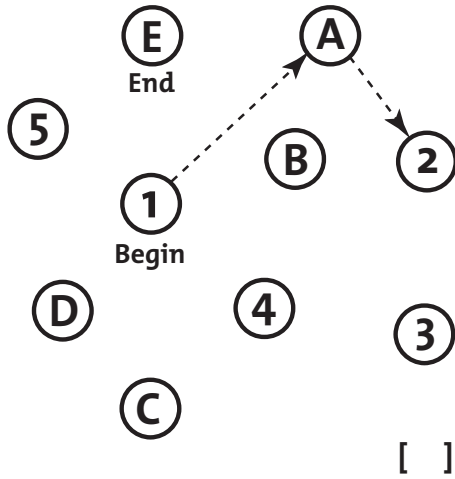
Copy cube

Draw CLOCK (Ten past eleven)
(3 points)

E End

A

5

B

2

1 Begin

D

4

3

C

[ ]   [ ]

[ ]   [ ]   [ ]   __/5
Contour   Numbers   Hands

NAMING
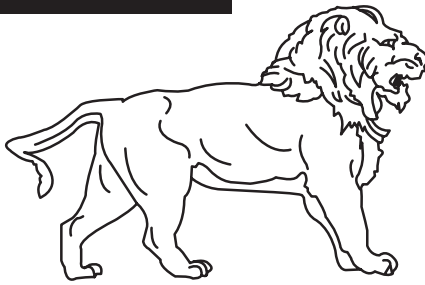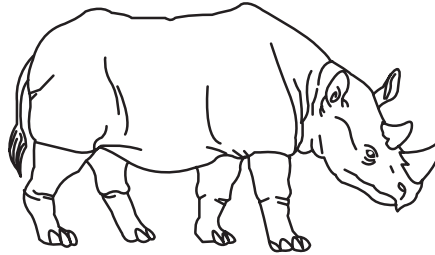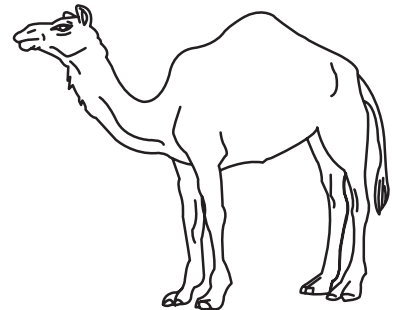
[ ]   [ ]   [ ]   __/3

MEMORY

Read list of words, subject must repeat them. Do 2 trials. Do a recall after 5 minutes.		FACE	VELVET	CHURCH	DAISY	RED	No points
	1st trial						
	2nd trial						

ATTENTION

| Read list of digits (1 digit/ sec.). | Subject has to repeat them in the forward order [ ] 2 1 8 5 4 | __/2 |
| | Subject has to repeat them in the backward order [ ] 7 4 2 | |

Read list of letters. The subject must tap with his hand at each letter A. No points if ≥ 2 errors
[ ] F B A C M N A A J K L B A F A K D E A A A J A M O F A A B   __/1

Serial 7 subtraction starting at 100   [ ] 93   [ ] 86   [ ] 79   [ ] 72   [ ] 65   __/3
4 or 5 correct subtractions: **3 pts**, 2 or 3 correct: **2 pts**, 1 correct: **1 pt**, 0 correct: **0 pt**

LANGUAGE

Repeat : I only know that John is the one to help today. [ ]   __/2
The cat always hid under the couch when dogs were in the room. [ ]

Fluency / Name maximum number of words in one minute that begin with the letter F   [ ] _____ (N ≥ 11 words)   __/1

ABSTRACTION

Similarity between e.g. banana - orange = fruit   [ ] train – bicycle   [ ] watch - ruler   __/2

DELAYED RECALL

Has to recall words WITH NO CUE	FACE [ ]	VELVET [ ]	CHURCH [ ]	DAISY [ ]	RED [ ]	Points for UNCUED recall only	__/5
Optional	Category cue						
	Multiple choice cue						

ORIENTATION

[ ]Date   [ ]Month   [ ]Year   [ ]Day   [ ]Place   [ ]City   __/6

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www.mocatest.org

Normal ≥ 26 / 30

TOTAL   __/30

Add 1 point if ≤ 12 yr edu

ALZHEIMER'S DISEASE ASSESSMENT SCALE ADAS - COG (Modified) (85 point):

1. WORD RECALL TASK

Word Recalled?	Trial 1 Yes (1)	No (2)	Word Recalled?	Trial 2 Yes (1)	No (2)	Word Recalled?	Trial 3 Yes (1)	No (2)
Butter (Word 1)	☐	☐	Pole (Word 11)	☐	☐	Shore (Word 21)	☐	☐
Arm (Word 2)	☐	☐	Letter (Word 12)	☐	☐	Letter (Word 22)	☐	☐
Shore (Word 3)	☐	☐	Butter (Word 13)	☐	☐	Arm (Word 23)	☐	☐
Letter (Word 4)	☐	☐	Queen (Word 14)	☐	☐	Cabin (Word 24)	☐	☐
Queen (Word 5)	☐	☐	Arm (Word 15)	☐	☐	Pole (Word 25)	☐	☐
Cabin (Word 6)	☐	☐	Shore (Word 16)	☐	☐	Ticket (Word 26)	☐	☐
Pole (Word 7)	☐	☐	Grass (Word 17)	☐	☐	Engine (Word 27)	☐	☐
Ticket (Word 8)	☐	☐	Cabin (Word 18)	☐	☐	Grass (Word 28)	☐	☐
Grass (Word 9)	☐	☐	Ticket (Word 19)	☐	☐	Butter (Word 29)	☐	☐
Engine (Word 10)	☐	☐	Engine (Word 20)	☐	☐	Queen (Word 30)	☐	☐

If any task not administered or not completed, check one:

☐ (1) Participant refused

☐ (2) Participant unable for physical reasons

☐ (3) Participant unable for cognitive reasons

☐ (4) Not done (for reasons other than physical/cognitive)

Time Ended: ⌊_⌊_⌋ : ⌊_⌊_⌋
(24 hour clock)

2. NAMING TASK

A. The subject is asked to name 12 randomly presented real objects.

Object <5>		Clues <5>	Response Correct? Yes (1)	No (2)
Flower	(Object 1)	grows in garden	☐	☐
Bed	(Object 2)	used for sleeping	☐	☐
Whistle	(Object 3)	makes a sound when you blow on it	☐	☐
Pencil	(Object 4)	used for writing	☐	☐
Rattle	(Object 5)	a baby's toy	☐	☐
Mask	(Object 6)	hides your face	☐	☐
Scissors	(Object 7)	cuts paper	☐	☐
Comb	(Object 8)	used on hair	☐	☐
Wallet	(Object 9)	holds your money	☐	☐
Harmonica	(Object 10)	a musical instrument	☐	☐
Stethoscope	(Object 11)	doctor uses it to listen to your heart	☐	☐
<6> Tongs	(Object 12)	picks up food	☐	☐

<6> Tweezers   (Object 12)   used for extracting or plucking things

B. The subject is asked to name the fingers of his/her **dominant** hand.

Response correct?

Thumb	☐ (1) Yes	☐ (2) No
Index/forefinger/pointer	☐ (1) Yes	☐ (2) No
Middle	☐ (1) Yes	☐ (2) No
Ring	☐ (1) Yes	☐ (2) No
Pinky or little finger	☐ (1) Yes	☐ (2) No

If any task not administered or not completed, check one:

☐ (1) Participant refused

☐ (2) Participant unable for physical reasons

☐ (3) Participant unable for cognitive reasons

☐ (4) Not done for reasons other than physical/cognitive

Used with permission from the NIA Alzheimer's Disease Cooperative Study (NIA Grant AG10483).

3.   COMMANDS   **Response correct?**

1.	"Make a <u>fist</u>."	☐ (1) Yes	☐ (2) No
2.	"Point to the <u>ceiling</u> and then to the <u>floor</u>."	☐ (1) Yes	☐ (2) No
3.	"Put the <u>pencil on top of the card</u>, then <u>put it back</u>."	☐ (1) Yes	☐ (2) No
4.	"Put the <u>watch</u> on the other <u>side of the pencil</u> and then <u>turn over the card</u>."	☐ (1) Yes	☐ (2) No
5.	"<u>Tap</u> <u>each shoulder</u> <u>twice</u> with <u>two fingers</u>, keeping your <u>eyes shut</u>."	☐ (1) Yes	☐ (2) No

If any task not administered or not completed, check one:

☐ (1) Participant refused

☐ (2) Participant unable for physical reasons

☐ (3) Participant unable for cognitive reasons

☐ (4) Not done for reasons other than physical/cognitive

<2> Time Began: ⊔⊔ : ⊔⊔
(24 hour clock)

4. DELAYED WORD-RECALL TASK

Word	Word Recalled?	
	Yes (1)	No (2)
Butter (Word 1)	☐	☐
Arm (Word 2)	☐	☐
Shore (Word 3)	☐	☐
Letter (Word 4)	☐	☐
Queen (Word 5)	☐	☐
Cabin (Word 6)	☐	☐
Pole (Word 7)	☐	☐
Ticket (Word 8)	☐	☐
Grass (Word 9)	☐	☐
Engine (Word 10)	☐	☐

If any task not administered or not completed, check one:

☐ (1) Participant refused

☐ (2) Participant unable for physical reasons

☐ (3) Participant unable for cognitive reasons

☐ (4) Not done (for reasons other than physical/cognitive)

Used with permission from the NIA Alzheimer's Disease Cooperative Study (NIA Grant AG10483).

5. CONSTRUCTIONAL PRAXIS

Drawn Correctly?

	Yes (1)	No (2)
A. Circle.	☐	☐
B. Two overlapping rectangles.	☐	☐
C. Rhombus (Diamond).	☐	☐
D. Cube.	☐	☐

☐ (1) Check box if the subject did not attempt to draw any forms

If any task not administered or not completed, check one:
☐ (1) Participant refused
☐ (2) Participant unable for physical reasons
☐ (3) Participant unable for cognitive reasons
☐ (4) Not done for reasons other than physical/cognitive

6. IDEATIONAL PRAXIS

Action Correct?

	Yes (1)	No (2)
Fold letter	☐	☐
Put letter in envelope	☐	☐
Seal envelope	☐	☐
Address envelope	☐	☐
Indicate where stamp goes	☐	☐

If any task not administered or not completed, check one:
☐ (1) Participant refused
☐ (2) Participant unable for physical reasons
☐ (3) Participant unable for cognitive reasons
☐ (4) Not done for reasons other than physical/cognitive

7. ORIENTATION

Response Correct?

	Yes (1)	No (2)
Full name (First and Last Name)	☐	☐
Month (Current Month)	☐	☐
Date (Today's Date ± 1 Day)	☐	☐
Year (Current Year)	☐	☐
Day (Current Day of the week)	☐	☐
Season (Current Season Or Within 1 Week Of Upcoming Season Or Within 2 Weeks Of Previous Season)	☐	☐
Place (Partial Or Full Name Of Site)	☐	☐
Time (± 1 Hour)	☐	☐

If any task not administered or not completed, check one:

☐ (1) Participant refused

☐ (2) Participant unable for physical reasons

☐ (3) Participant unable for cognitive reasons

☐ (4) Not done for reasons other than physical/cognitive

8. WORD-RECOGNITION TASK   Word List: <7>

TRIAL 1			TRIAL 2			TRIAL 3		
	Subject's Response			Subject's Response			Subject's Response	
Word	"Yes" (1)	"No" (2)	Word	"Yes" (1)	"No" (2)	Word	"Yes" (1)	"No" (2)
Nurse (Word 1)	☐	☐	**Board** (Word 25)	☐	☐	**Coin** (Word 49)	☐	☐
Magazine (Word 2)	☐	☐	Turnip (Word 26)	☐	☐	Plank (Word 50)	☐	☐
Wizard (Word 3)	☐	☐	**Gem** (Word 27)	☐	☐	War (Word 51)	☐	☐
Van (Word 4)	☐	☐	**Institution** (Word 28)	☐	☐	Porch (Word 52)	☐	☐
Leopard (Word 5)	☐	☐	**Coin** (Word 29)	☐	☐	Toast (Word 53)	☐	☐
Sale (Word 6)	☐	☐	Master (Word 30)	☐	☐	Rope (Word 54)	☐	☐
Sea (Word 7)	☐	☐	**Magazine** (Word 31)	☐	☐	**Anchor** (Word 55)	☐	☐
Train (Word 8)	☐	☐	**Van** (Word 32)	☐	☐	**Board** (Word 56)	☐	☐
Coin (Word 9)	☐	☐	**Anchor** (Word 33)	☐	☐	**Leopard** (Word 57)	☐	☐
Ship (Word 10)	☐	☐	Lumber (Word 34)	☐	☐	Judge (Word 58)	☐	☐
Institution (Word 11)	☐	☐	Servant (Word 35)	☐	☐	**Magazine** (Word 59)	☐	☐
Map (Word 12)	☐	☐	Pond (Word 36)	☐	☐	Camp (Word 60)	☐	☐
Axe (Word 13)	☐	☐	Military (Word 37)	☐	☐	**Sea** (Word 61)	☐	☐
Board (Word 14)	☐	☐	Hospital (Word 38)	☐	☐	**Institution** (Word 62)	☐	☐
Carrot (Word 15)	☐	☐	**Sea** (Word 39)	☐	☐	Tack (Word 63)	☐	☐
Milk (Word 16)	☐	☐	Jungle (Word 40)	☐	☐	Emerald (Word 64)	☐	☐
Volume (Word 17)	☐	☐	Nail (Word 41)	☐	☐	**Van** (Word 65)	☐	☐
Forest (Word 18)	☐	☐	**Wizard** (Word 42)	☐	☐	Globe (Word 66)	☐	☐
Anchor (Word 19)	☐	☐	**Leopard** (Word 43)	☐	☐	**Train** (Word 67)	☐	☐
Gem (Word 20)	☐	☐	**Train** (Word 44)	☐	☐	**Fund** (Word 68)	☐	☐
Cat (Word 21)	☐	☐	Editorial (Word 45)	☐	☐	Coast (Word 69)	☐	☐
Fund (Word 22)	☐	☐	Bread (Word 46)	☐	☐	**Gem** (Word 70)	☐	☐
Edge (Word 23)	☐	☐	**Fund** (Word 47)	☐	☐	**Wizard** (Word 71)	☐	☐
Cake (Word 24)	☐	☐	Trade (Word 48)	☐	☐	Kitten (Word 72)	☐	☐

If any task not administered or not completed, check one:

☐ (1) Participant refused

☐ (2) Participant unable for physical reasons

☐ (3) Participant unable for cognitive reasons

☐ (4) Not done (for reasons other than physical/cognitive)

Used with permission from the NIA Alzheimer's Disease Cooperative Study (NIA Grant AG10483).

9. REMEMBERING TEST INSTRUCTIONS ADCRI

☐ 0 = subject never needs extra reminders of instructions

☐ 1 = very mild - forgets once

☐ 2 = mild - must be reminded 2 times

☐ 3 = moderate - must be reminded 3 or 4 times

☐ 4 = moderately severe - must be reminded 5 or 6 times

☐ 5 = severe - must be reminded 7 or more times

10. SPOKEN LANGUAGE ABILITY ADCSL

☐ 0 = no instances where it is difficult to understand the subject

☐ 1 = very mild - one instance of lack of understandability

☐ 2 = mild - patient has difficulty less than 25% of the time

☐ 3 = moderate - patient has difficulty 25-50% of the time

☐ 4 = moderately severe - patient has difficulty 50% of the time

☐ 5 = severe - one or two word utterance; fluent, but empty speech; mute

11. WORD-FINDING DIFFICULTY IN SPONTANEOUS SPEECH ADCDIF

☐ 0 = no evidence of word finding difficulty in spontaneous speech

☐ 1 = very mild - 1 or 2 instances, not clinically significant

☐ 2 = mild - noticeable circumlocution or synonym substitution

☐ 3 = moderate - loss of words without compensation on occasion

☐ 4 = moderately severe - frequent loss of words without compensation

☐ 5 = severe - nearly total loss of content of words; speech sounds empty; 1-2 word utterances

12. COMPREHENSION ADCCMP

- ☐ 0 = no evidence of poor comprehension
- ☐ 1 = very mild - 1 or 2 instances of misunderstanding
- ☐ 2 = mild - 3-5 instances of misunderstanding
- ☐ 3 = moderate - requires several repetitions and rephrasing
- ☐ 4 = moderately severe - patient only occasionally responds correctly; i.e., yes/no questions
- ☐ 5 = severe - patient rarely responds to questions appropriately, not due to poverty of speech

13. CONCENTRATION/DISTRACTIBILITY ADCCON

- ☐ 0 = no evidence of poor concentration or distractibility
- ☐ 1 = very mild - one instance of poor concentration
- ☐ 2 = mild - 2-3 instances of poor concentration/distractibility; signs of restlessness and inattentiveness
- ☐ 3 = moderate - 4-5 instances during interview
- ☐ 4 = moderately severe - poor concentration/distractibility throughout much of interview
- ☐ 5 = severe - extreme difficulty in concentration and extremely distractible, unable to complete tasks

14. NUMBER CANCELLATION			
	NUMBER CANCELLATION: CORRECT	⌴⌴	ADCNC01
	NUMBER CANCELLATION: ERRORS	⌴⌴	ADCNC02
	NUMBER CANCELLATION: REMIND	⌴⌴	ADCNC03
	NUMBER CANCELLATION SUMMARY	⌴⌴	
			ADCNC

15. EXECUTIVE FUNCTION MAZE			
	EXECUTIVE FUNCTION: NUMBER ERRORS	⌴⌴	ADCM0Z1
	EXECUTIVE FUNCTION: TIME	⌴⌴	ADCMZ02
	EXECUTIVE FUNCTION SUMMARY	⌴⌴	
			ADCMZ

ADCS - ACTIVITIES OF DAILY LIVING (ADL) INVENTORY

NOTES: (1) {P} refers to the participant and should be replaced by the participant's name or relationship to the study partner each time an ADL question is asked of the study partner.

(2) This ADL inventory must be given in the format of an interview of the study partner, either directly or by telephone. The form should NOT be given to a study partner to complete on his/her own.

READ THE FOLLOWING INSTRUCTIONS TO THE STUDY PARTNER:

I am going to ask you about a number of daily activities that {P} may have performed during the past 4 weeks. Please tell me about {P}'s actual performance, not about what he/she could have done if an opportunity had arisen. For each activity that {P} attempted, I'm going to ask you to choose one out of a number of descriptions that best fits his/her most usual performance.

For some activities, I'll ask about whether {P} performed independently, or with supervision or help. Let me explain how we are defining these words:

Independently means that {P} completed the activity without being helped. We still consider it independent if {P} was reminded or prompted to get started, or received a little prompting while performing the activity.

With supervision means that {P} required verbal reminders and instructions while doing the activity.

With help means that {P} was given some degree of <u>physical</u> <u>assistance</u> by another person to perform the activity.

INSTRUCTIONS FOR THE RATER:

If the study partner states that {P} had no opportunity to perform the task during the past four weeks (*e.g.*, {P} did not have access to a telephone, therefore could not possibly make phone calls), the response should be recorded as 'no.'

If either the study partner's answer or the questionnaire are unclear, please make notes on the case report form detailing the problem.

For questions regarding specific ADL items, please refer to the ADL Response Card.

ADCS-MCI Activities of Daily Living Inventory - Page 1 of 11
Month 6 Visit

Subject Number	Subject Initials	Examiner Initials	Examination Date
M C – ☐☐☐ – ☐☐☐	☐☐☐	☐☐☐	☐☐ ☐☐ ☐☐☐☐
			Month Day Year

INSTRUCTIONS: Complete questions 1-18 and 19-24. Then go back to page 8 of this ADL exam to calculate the total score for questions 1-18 and proceed to page 9 of this ADL exam to complete the "Don't Know" answers worksheet for questions 1-18.

Information obtained through: 1 ☐ Subject visit
2 ☐ Telephone call

Yes   No   Don't Know

☐   ☐   ☐   **1.** In the past 4 weeks, did {S} **usually** manage to <u>find his/her personal belongings</u> at home?

→ *If yes,* which best describes how he/she usually performed:

   3 ☐ without supervision or help

   2 ☐ with supervision

   1 ☐ with physical help

☐   ☐   ☐   **2.** In the past 4 weeks, did {S} <u>select </u>his/her first set of clothes for the day?

→ *If yes,* which best describes his/her **usual** performance:

   3 ☐ without supervision or help

   2 ☐ with supervision

   1 ☐ with physical help

☐   **3.** Regarding physically getting dressed, which best describes his/her **usual** performance in the past 4 weeks: (check one)

   4 ☐ dressed completely without supervision or physical help

   3 ☐ dressed completely with supervision, but without help

   2 ☐ needed physical help only for buttons, clasps, or shoelaces

   1 ☐ needed some help even if clothes needed no fastening or buttoning

   0 ☐ someone else dressed him/her

☐☐ **Subscore** for Page 1
(Range = 0-10)

ADCS-MCI Activities of Daily Living Inventory - Page 2 of 11
Month 6 Visit

Subject Number   Subject Initials

| M | C | – | | | | | – | | | | | | | | |

Yes   No   Don't Know

☐ ☐ ☐   **4.** In the past 4 weeks, did {S} clean a living-, sitting-, or family room?

→ *If yes,* which best describes how he/she **usually** performed:

2 ☐ without supervision or help

1 ☐ with supervision

0 ☐ with physical help

☐ ☐ ☐   **5.** In the past 4 weeks, did {S} <u>balance his/her checkbook</u> or a credit card statement?

→ *If yes,* which best describes how he/she **usually** performed:

2 ☐ without supervision or help

1 ☐ with supervision

0 ☐ with physical help

☐ ☐ ☐   **6.** In the past 4 weeks, did {S} ever <u>write</u> things down?

<u>Note:</u> *If {S} wrote things only after encouragement or with help, the response should still be 'yes.'*

→ *If yes,* which best describes the **most complicated** things that he/she wrote:

2 ☐ letters or long notes that other people understood

1 ☐ short notes or messages that other people understood

0 ☐ his/her signature or name

☐ ☐ ☐   **7.** In the past 4 weeks, did {S} clean a load of laundry ?

→ *If yes,* which best describes how he/she **usually** performed:

2 ☐ without supervision or help

1 ☐ with supervision

0 ☐ with physical help

☐   Subscore for Page 2
(Range = 0-8)

Subject Number   Subject Initials

| M | C | – | | | | | – | | | | | | | | |

Yes **No** **Don't Know**

☐ ☐ ☐   **8.** In the past 4 weeks, did {S} <u>keep appointments</u> or meetings with other people, such as relatives, a doctor, the hairdresser, etc.?

→ *If yes,* which best describes his/her awareness of the meeting ahead of time:

3 ☐ usually remembered without written or verbal reminders

2 ☐ usually referred to notes, a diary, or calendar

1 ☐ usually remembered the appointment after verbal reminders on the day

0 ☐ usually did not remember, in spite of verbal reminders on the day

☐ ☐ ☐   **9.** In the past 4 weeks, did {S} <u>use a telephone</u>?

→ *If yes,* which best describes his/her **highest** level of performance:

4 ☐ made any call necessary e.g., after looking up numbers in white or yellow pages, or by dialing directory assistance

3 ☐ made calls <u>only</u> to well-known numbers, <u>without</u> referring to a directory or list

2 ☐ made calls <u>only</u> to well-known numbers, <u>by using</u> a directory or list

1 ☐ answered the phone and spoke to callers; did not make calls

0 ☐ did not answer the phone, but spoke when put on the line

☐ ☐ ☐   **10.** In the past 4 weeks, did {S} make him/herself a meal or snack at home?

→ *If yes,* which best describes his/her **highest** level of food preparation:

3 ☐ cooked or microwaved food, with little or no help

2 ☐ cooked or microwaved food, with extensive help

1 ☐ mixed or combined food items for a meal or snack, without cooking or microwaving (e.g. made a sandwich)

0 ☐ obtained food on his/her own, without mixing or cooking it

Subscore for Page 3
(Range = 0-10)

ADCS-MCI Activities of Daily Living Inventory - Page 4 of 11
Month 6 Visit

Subject Number   Subject Initials

M | C | – | | | | | – | | | | | | | |

Yes   No   Don't Know

☐ ☐ ☐   **11.** In the past 4 weeks, did {S} <u>get around</u> (or travel) <u>outside of his/her home</u>?

→ *If yes,* which best describes his/her **optimal** performance:

3 ☐ traveled alone, went at least 1 mile away from home

2 ☐ traveled alone, but remained within 1 mile of home

1 ☐ traveled only with a chaperone for supervision regardless of the trip

0 ☐ traveled only with physical help, regardless of the trip

☐ ☐ ☐   **12.** In the past 4 weeks, did {S} talk about current events? (This means events or incidents that occurred during the past month.)

If yes, ask questions 12a, 12b, 12c and 12d :

→ **12a)** Did {S} talk about regional, national, or international events (including sports)?

1 ☐ Yes   0 ☐ No

→ **12b)** Did {S} talk about events <u>outside home</u> involving family, friends, or neighbors?

1 ☐ Yes   0 ☐ No

→ **12c)** Did {S} talk about events that occurred <u>at home</u> that he/she took part in or watched?

1 ☐ Yes   0 ☐ No

→ **12d)** Did {S} converse without repeating him/herself, or asking the same questions repeatedly?

1 ☐ Yes   0 ☐ No

☐   Subscore for Page 4
(Range = 0-7)

ADCS-MCI Activities of Daily Living Inventory - Page 5 of 11
Month 6 Visit

Subject Number

| M | C | | | | | – | | | | |

Subject Initials

| | | |

Yes **No** **Don't Know**

☐ ☐ ☐ **13.** In the past 4 weeks, did {S} <u>read a magazine, newspaper or book</u> for more than 5 minutes at a time?

If yes, ask questions 13a, 13b and 13c:

→ **13a)** Did {S} usually select or ask for something to read?

1 ☐ Yes   0 ☐ No

→ **13b)** Did {S} usually talk about what he/she read while or shortly after reading (less than 1 hour)?

1 ☐ Yes   0 ☐ No

→ **13c)** Did {S} usually talk about what he/she read 1–24 hours after reading?

1 ☐ Yes   0 ☐ No

☐ ☐ ☐ **14.** In the past 4 weeks, did {S} <u>watch television</u>?

If yes, ask questions 14a, 14b and 14c:

→ **14a)** Did {S} usually select or ask for different programs or his/her favorite show?

1 ☐ Yes   0 ☐ No

→ **14b)** Did {S} usually talk about the content of a program while watching it?

1 ☐ Yes   0 ☐ No

→ **14c)** Did {S} talk about the content of a program within a day (24 hours) after watching it?

1 ☐ Yes   0 ☐ No

☐ **Subscore** for Page 5
(Range = 0-6)

ADCS-MCI Activities of Daily Living Inventory - Page 6 of 11
Month 6 Visit

Subject Number   Subject Initials

| M | C | – | | | | | – | | | | | | | | | |

Yes   No   Don't Know

☐ ☐ ☐   **15.** In the past 4 weeks, did {S} ever <u>go shopping at a store</u>?

If yes, ask questions 15a and 15b:

→ **15a)** Did {S} usually select correct items without supervision or help?

1 ☐ Yes   0 ☐ No

→ **15b)** Did {S} usually pay for items on his/her own?

1 ☐ Yes   0 ☐ No

☐ ☐ ☐   **16.** In the past 4 weeks, was {S} ever <u>left on his/her own</u>?

If yes, ask questions 16a, 16b and 16c:

→ **16a)** Was {S} left **away** from home, for 15 minutes or longer, during the day?

1 ☐ Yes   0 ☐ No

→ **16b)** Was {S} **left** at home, for an hour or longer, during the day?

1 ☐ Yes   0 ☐ No

→ **16c)** Was {S} **left** at home, for less than 1 hour, during the day?

1 ☐ Yes   0 ☐ No

☐ Subscore for Page 6
(Range = 0-5)

ADCS-MCI Activities of Daily Living Inventory - Page 7 of 11
Month 6 Visit

Subject Number   Subject Initials

| M | C | – | | | | | – | | | | | | | | |

Yes **No** **Don't Know**

☐ ☐ ☐   **17.** In the past 4 weeks, did {S} <u>use a household appliance</u> to do chores?

(This does not include a TV.)

→ *If yes, ask about all of the following, and check those that apply:*

☐ washer   ☐ dryer   ☐ vacuum

☐ dishwasher   ☐ power tool(s)   ☐ toaster oven

☐ range   ☐ microwave   ☐ food processor

☐ other_____

If yes, ask question 17a:

→ **17a)** For <u>the 1 or 2 most commonly used</u> appliances, which best

describes how {S} **usually** used them:

4 ☐ without help, and operated all necessary controls

3 ☐ without help, but operated only on/off controls

2 ☐ with supervision (e.g., instructions), but no physical help

1 ☐ with physical help

<u>Note</u>: plugging in and switching on = 3;

switching on and adjusting volume/power = 4

☐ Subscore for Page 7
(Range = 0-4)

Subject Number   Subject Initials

| M | C | – | | | | | – | | | | | | | | |

Yes   No   Don't Know

☐   ☐   ☐   **18.** In the past 4 weeks, did {S} perform a <u>pastime, hobby or game</u>?

→ *If yes, ask about all of the following, check all that apply:*

☐ card or board games (including bridge, chess, checkers)

☐ bingo   ☐ crosswords   ☐ art

☐ musical instrument   ☐ knitting   ☐ sewing

☐ reading   ☐ gardening   ☐ golf

☐ tennis   ☐ workshop   ☐ fishing

☐ other_____

<u>Note:</u> Walking does <u>NOT</u> count as a hobby/pastime for this scale.

If yes, ask questions 18a and 18b:

→ **18a)** Did {S} require supervision, or help, to perform any of these hobbies?

3 ☐ no supervision required

2 ☐ supervision

1 ☐ help

→ **18b)** List any hobby(ies) that the subject has lost the ability to perform:

_____

_____

_____

☐ Subscore for Page 8
(Range = 0-3)

**Proceed to questions 19-24 on pages 10 and 11 of this ADL exam,
Then return to this page to total the scores for questions 1-18.**

ADL TOTAL SCORE

Sum the page subscores for
pages 1-8 of the ADL (items 1-18)

☐☐ **ADL Total Score.**
(Range = 0-53)

npiTEST

The Neuropsychiatric Inventory Questionnaire:

Background and Administration

By Jeffrey L. Cummings, MD

The Neuropsychiatric Inventory–Questionnaire: Background and Administration

The Neuropsychiatric Inventory–Questionnaire (NPI-Q) was developed and cross-validated with the standard NPI to provide a brief assessment of neuropsychiatric symptomatology in routine clinical practice settings (Kaufer et al, J Neuropsychiatry Clin Neurosci 2000, 12:233-239). The NPI-Q is adapted from the NPI (Cummings et al, Neurology 1994; 44:2308-2314), a validated informant-based interview that assesses neuropsychiatric symptoms over the previous month. The original NPI included 10 neuropsychiatric domains; two others, Nighttime Behavioral Disturbances and Appetite/Eating Changes, have subsequently been added. Another recent modification of the original NPI is the addition of a Caregiver Distress Scale for evaluating the psychological impact of neuropsychiatric symptoms reported to be present (Kaufer et al, JAGS, 1998;46:210-215). The NPI-Q includes both of these additions.

The NPI-Q is designed to be a self-administered questionnaire completed by informants about patients for whom they care. Each of the 12 NPI-Q domains contains a survey question that reflects cardinal symptoms of that domain. Initial responses to each domain question are "Yes" (present) or "No" (absent). If the response to the domain question is "No", the informant goes to the next question. If "Yes", the informant then rates both the Severity of the symptoms present within the last month on a 3-point scale and the associated impact of the symptom manifestations on them (i.e. Caregiver Distress) using a 5-point scale. The NPI-Q provides symptom Severity and Distress ratings for each symptom reported, and total Severity and Distress scores reflecting the sum of individual domain scores.

Most informants will be able to complete the NPI-Q in 5 minutes or less. It is recommended that responses to the NPI-Q be reviewed for completeness by a clinician and for clarifying uncertainties after each administration. The first time an informant completes the NPI-Q, it may be useful to verbally review the instructions. In some instances, it may be necessary to conduct the NPI-Q in part or entirely as an interview.

The NPI and NPI-Q are both copyright-protected by Jeffrey L. Cummings, MD. The NPI-Q was developed by Daniel Kaufer, MD with permission. **Use of the NPI or NPI-Q in investigational studies sponsored in whole or part by for-profit entities is prohibited without express written consent.**

For inquiries regarding the NPI-Q, contact:

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Los Angeles, CA 90095
jcummings@mednet.ucla.edu

The NPI-Q can be found at:
www.NPItest.net

Please answer the following questions based on <u>changes</u> that have occurred since the patient first began to experience memory problems.

Circle "Yes" <u>only</u> if the symptom(s) has been present <u>in the last month</u>. Otherwise, circle "No". For each item marked "Yes":

a) Rate the SEVERITY of the symptom (how it affects <u>the patient</u>):
> **1 = Mild** (noticeable, but not a significant change)
> **2 = Moderate** (significant, but not a dramatic change)
> **3 = Severe** (very marked or prominent, a dramatic change)

b) Rate the DISTRESS you experience due to that symptom (how it affects <u>you</u>):
> **0 = Not distressing at all**
> **1 = Minimal** (slightly distressing, not a problem to cope with)
> **2 = Mild** (not very distressing, generally easy to cope with)
> **3 = Moderate** (fairly distressing, not always easy to cope with)
> **4 = Severe** (very distressing, difficult to cope with)
> **5 = Extreme or Very Severe** (extremely distressing, unable to cope with)

Please answer each question carefully. Ask for assistance if you have any questions.

Delusions	Does the patient have false beliefs, such as thinking that others are stealing from him/her or planning to harm him/her in some way?

Yes   No   **SEVERITY: 1 2 3   DISTRESS: 0 1 2 3 4 5**

Hallucinations	Does the patient have hallucinations such as false visions or voices? Does he or she seem to hear or see things that are not present?

Yes   No   **SEVERITY: 1 2 3   DISTRESS: 0 1 2 3 4 5**

Agitation/Aggression	Is the patient resistive to help from others at times, or hard to handle?

Yes   No   **SEVERITY: 1 2 3   DISTRESS: 0 1 2 3 4 5**

Depression/Dysphoria Does the patient seem sad or say that he /she is depressed?

Yes   **No**   **SEVERITY: 1   2   3**   **DISTRESS: 0   1   2   3   4   5**

Anxiety   Does the patient become upset when separated from you?
Doeshe/she have any other signs of nervousness such as
shortness of breath, sighing, being unable to relax, or feeling
excessively tense?

Yes   **No**   **SEVERITY: 1   2   3**   **DISTRESS: 0   1   2   3   4   5**

Elation/Euphoria   Does the patient appear to feel too good or act excessively
happy?

Yes   **No**   **SEVERITY: 1   2   3**   **DISTRESS: 0   1   2   3   4   5**

Apathy/Indifference   Does the patient seem less interested in his/her usual
activities or in the activities and plans of others?

Yes   **No**   **SEVERITY: 1   2   3**   **DISTRESS: 0   1   2   3   4   5**

Disinhibition   Does the patient seem to act impulsively, for example,
talking to strangers as if he/she knows them, or saying
things that may hurt people's feelings?

Yes   **No**   **SEVERITY: 1   2   3**   **DISTRESS: 0   1   2   3   4   5**

Irritability/Lability   Is the patient impatient and cranky? Does he/she have
difficulty coping with delays or waiting for planned activities?

Yes   **No**   **SEVERITY: 1   2   3**   **DISTRESS: 0   1   2   3   4   5**

Motor Disturbance   Does the patient engage in repetitive activities such as
pacing around the house, handling buttons, wrapping string,
or doing other things repeatedly?

Yes   **No**   **SEVERITY: 1   2   3**   **DISTRESS: 0   1   2   3   4   5**

Nightime Behaviors　Does the patient awaken you during the night, rise too early

in the morning, or take excessive naps during the day?

Yes　No　　**SEVERITY: 1 2 3**　　**DISTRESS: 0 1 2 3 4 5**

Appetite/Eating　Has the patient lost or gained weight, or had a change in the type of food he/she likes?

Yes　No　　**SEVERITY: 1 2 3**　　**DISTRESS: 0 1 2 3 4 5**

NPI-Q SUMMARY

	No		Severity				Caregiver Distress					
Delusions	0		1	2	3		0	1	2	3	4	5
Hallucinations	0		1	2	3		0	1	2	3	4	5
Agitation/Aggression	0		1	2	3		0	1	2	3	4	5
Dysphoria/Depression	0		1	2	3		0	1	2	3	4	5
Anxiety	0		1	2	3		0	1	2	3	4	5
Euphoria/Elation	0		1	2	3		0	1	2	3	4	5
Apathy/Indifference	0		1	2	3		0	1	2	3	4	5
Disinhibition	0		1	2	3		0	1	2	3	4	5
Irritability/Lability	0		1	2	3		0	1	2	3	4	5
Aberrant Motor	0		1	2	3		0	1	2	3	4	5
Nighttime Behavior	0		1	2	3		0	1	2	3	4	5
Appetite/Eating	0		1	2	3		0	1	2	3	4	5
TOTAL												

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use ZOLINZA safely and effectively. See full prescribing information for ZOLINZA.

ZOLINZA® (vorinostat) Capsules
Initial U.S. Approval: 2006

---------------------------INDICATIONS AND USAGE ---------------------------
ZOLINZA is a histone deacetylase (HDAC) inhibitor indicated for the treatment of cutaneous manifestations in patients with cutaneous T-cell lymphoma (CTCL) who have progressive, persistent or recurrent disease on or following two systemic therapies. (1)

----------------------- DOSAGE AND ADMINISTRATION-----------------------
- 400 mg orally once daily with food. (2.1)
- If patient is intolerant to therapy, reduce the dose to 300 mg once daily with food. If necessary, reduce the dose further to 300 mg once daily with food for 5 consecutive days each week. (2.2, 5)
- Reduce dose in patients with mild or moderate hepatic impairment. (2.2)

--------------------- DOSAGE FORMS AND STRENGTHS ---------------------
Capsules: 100 mg (3)

------------------------------CONTRAINDICATIONS ------------------------------

None (4)

-----------------------WARNINGS AND PRECAUTIONS-----------------------
- Pulmonary embolism and deep vein thrombosis: Monitor for pertinent signs and symptoms. (5.1)
- Thrombocytopenia and anemia: May require dose modification or discontinuation. Monitor blood counts every 2 weeks during the first 2 months of therapy and monthly thereafter. (2.2, 5.2, 6)

- Gastrointestinal Toxicity: Nausea, vomiting and diarrhea; patients may require antiemetics, antidiarrheals, and fluid and electrolyte replacement to prevent dehydration. (5.3, 6, 17.1)
- Hyperglycemia: Monitor blood glucose every 2 weeks during the first 2 months of therapy and monthly thereafter. (5.4)
- Clinical chemistry abnormalities: Measure and correct abnormal electrolytes, creatinine, magnesium and calcium at baseline. Monitor every 2 weeks during the first 2 months of therapy and at least monthly during treatment. (5.5)
- Severe thrombocytopenia with gastrointestinal bleeding has been reported with concomitant use of ZOLINZA and other HDAC inhibitors (e.g., valproic acid). Monitor platelet counts more frequently. (5.6, 7.2)
- Fetal harm can occur when administered to a pregnant woman. Women should be apprised of the potential harm to the fetus. (5.7)

------------------------------ ADVERSE REACTIONS------------------------------
- The most common adverse reactions (incidence ≥20%) are diarrhea, fatigue, nausea, thrombocytopenia, anorexia and dysgeusia. (6)

To report SUSPECTED ADVERSE REACTIONS, contact Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., at 1-877-888-4231 or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

------------------------------DRUG INTERACTIONS------------------------------
- Coumarin-derivative anticoagulants: Prolongation of prothrombin time and International Normalized Ratio (INR) have been observed with concomitant use. Monitor INR frequently. (7.1)

See 17 for PATIENT COUNSELING INFORMATION and FDA-approved patient labeling.

Revised: 12/2015

FULL PRESCRIBING INFORMATION

1   INDICATIONS AND USAGE

ZOLINZA® is indicated for the treatment of cutaneous manifestations in patients with cutaneous T-cell lymphoma who have progressive, persistent or recurrent disease on or following two systemic therapies.

2   DOSAGE AND ADMINISTRATION

2.1   Dosing Information

The recommended dose is 400 mg orally once daily with food.

Treatment may be continued as long as there is no evidence of progressive disease or unacceptable toxicity.

ZOLINZA capsules should not be opened or crushed *[see How Supplied/Storage and Handling (16)]*.

2.2   Dose Modifications

If a patient is intolerant to therapy, the dose may be reduced to 300 mg orally once daily with food. The dose may be further reduced to 300 mg once daily with food for 5 consecutive days each week, as necessary.

Hepatic Impairment

Reduce the starting dose to 300 mg orally once daily with food in patients with mild to moderate hepatic impairment (bilirubin 1 to 3 x ULN or AST > ULN). There is insufficient evidence to recommend a starting dose for patients with severe hepatic impairment (bilirubin > 3 x ULN) *[see Use in Specific Populations (8.6) and Clinical Pharmacology (12.3)]*.

3   DOSAGE FORMS AND STRENGTHS

100 mg white, opaque, hard gelatin capsules with "568" over "100 mg" printed within radial bar in black ink on the capsule body.

4   CONTRAINDICATIONS

None.

5   WARNINGS AND PRECAUTIONS

5.1   Thromboembolism

Pulmonary embolism occurred in 5% (4/86) of patients receiving ZOLINZA, and deep vein thrombosis has also been reported. Monitor for signs and symptoms of these events, particularly in patients with a prior history of thromboembolic events *[see Adverse Reactions (6)]*.

5.2   Myelosuppression

Treatment with ZOLINZA can cause dose-related thrombocytopenia and anemia. Monitor blood counts every 2 weeks during the first 2 months of therapy and monthly thereafter. Adjust dosage or discontinue treatment with ZOLINZA as clinically appropriate *[see Dosage and Administration (2.2), Warnings and Precautions (5.6) and Adverse Reactions (6)]*.

5.3   Gastrointestinal Toxicity

Gastrointestinal disturbances, including nausea, vomiting and diarrhea, have been reported *[see Adverse Reactions (6)]* and may require the use of antiemetic and antidiarrheal medications. Fluid and electrolytes should be replaced to prevent dehydration *[see Adverse Reactions (6.1)]*. Pre-existing nausea, vomiting, and diarrhea should be adequately controlled before beginning therapy with ZOLINZA.

5.4 Hyperglycemia

Hyperglycemia has been observed in patients receiving ZOLINZA and was severe in 5% (4/86) of patients [see Adverse Reactions (6.1)]. Monitor serum glucose every 2 weeks during the first 2 months of therapy and monthly thereafter.

5.5 Clinical Chemistry Abnormalities

Obtain chemistry tests, including serum electrolytes, creatinine, magnesium, and calcium, every 2 weeks during the first 2 months of therapy and monthly thereafter. Correct hypokalemia and hypomagnesemia prior to administration of ZOLINZA. Monitor potassium and magnesium more frequently in symptomatic patients (e.g., patients with nausea, vomiting, diarrhea, fluid imbalance or cardiac symptoms).

5.6 Severe Thrombocytopenia when Combined with Other Histone Deacetylase (HDAC) Inhibitors

Severe thrombocytopenia leading to gastrointestinal bleeding has been reported with concomitant use of ZOLINZA and other HDAC inhibitors (e.g., valproic acid). Monitor platelet counts more frequently [see Drug Interactions (7.2)].

5.7 Pregnancy

Pregnancy Category D

ZOLINZA can cause fetal harm when administered to a pregnant woman. There are no adequate and well-controlled studies of ZOLINZA in pregnant women. Results of animal studies indicate that vorinostat crosses the placenta and is found in fetal plasma at levels up to 50% of maternal concentrations. Doses up to 50 and 150 mg/kg/day were tested in rats and rabbits, respectively (~0.5 times the human exposure based on $AUC_{0-24\ hours}$). Treatment-related, developmental effects including decreased mean live fetal weights, incomplete ossifications of the skull, thoracic vertebra, sternebra, and skeletal variations (cervical ribs, supernumerary ribs, vertebral count and sacral arch variations) in rats at the highest dose of vorinostat tested. Reductions in mean live fetal weight and an elevated incidence of incomplete ossification of the metacarpals were seen in rabbits dosed at 150 mg/kg/day. The no observed effect levels (NOELs) for these findings were 15 and 50 mg/kg/day (<0.1 times the human exposure based on AUC) in rats and rabbits, respectively. A dose-related increase in the incidence of malformations of the gall bladder was noted in all drug treatment groups in rabbits versus the concurrent control. If this drug is used during pregnancy, or if the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus.

6 ADVERSE REACTIONS

The following serious adverse reactions have been associated with ZOLINZA in clinical trials and are discussed in greater detail in other sections of the label:

- Thromboembolism [see Warnings and Precautions (5.1)]
- Myelosuppression [see Warnings and Precautions (5.2)]
- Gastrointestinal Toxicity [see Warnings and Precautions (5.3)]
- Hyperglycemia [see Warnings and Precautions (5.4)]
- Clinical Chemistry Abnormalities [see Warnings and Precautions (5.5)]
- Severe thrombocytopenia when combined with other Histone Deacetylase (HDAC) Inhibitors [see Warnings and Precautions (5.6)]

The most common drug-related adverse reactions can be classified into 4 symptom complexes: gastrointestinal symptoms (diarrhea, nausea, anorexia, weight decrease, vomiting, constipation), constitutional symptoms (fatigue, chills), hematologic abnormalities (thrombocytopenia, anemia), and taste disorders (dysgeusia, dry mouth). The most common serious drug-related adverse reactions were pulmonary embolism and anemia.

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice.

The safety of ZOLINZA was evaluated in 107 CTCL patients in two single arm clinical studies in which 86 patients received 400 mg once daily.

The data described below reflect exposure to ZOLINZA 400 mg once daily in the 86 patients for a median number of 97.5 days on therapy (range 2 to 480+ days). Seventeen (19.8%) patients were exposed beyond 24 weeks and 8 (9.3%) patients were exposed beyond 1 year. The population of CTCL patients studied was 37 to 83 years of age, 47.7% female, 52.3% male, and 81.4% white, 16.3% black, and 1.2% Asian or multi-racial.

Common Adverse Reactions

Table 1 summarizes the frequency of CTCL patients with specific adverse reactions, using the National Cancer Institute-Common Terminology Criteria for Adverse Events (NCI-CTCAE, version 3.0).

Table 1: Clinical or Laboratory Adverse Reactions Occurring in CTCL Patients
(Incidence ≥10% of patients)

Adverse Reactions	ZOLINZA 400 mg once daily (N=86)			
	All Grades		Grades 3-5*	
	n	%	n	%
Fatigue	45	52.3	3	3.5
Diarrhea	45	52.3	0	0.0
Nausea	35	40.7	3	3.5
Dysgeusia	24	27.9	0	0.0
Thrombocytopenia	22	25.6	5	5.8
Anorexia	21	24.4	2	2.3
Weight Decreased	18	20.9	1	1.2
Muscle Spasms	17	19.8	2	2.3
Alopecia	16	18.6	0	0.0
Dry Mouth	14	16.3	0	0.0
Blood Creatinine Increased	14	16.3	0	0.0
Chills	14	16.3	1	1.2
Vomiting	13	15.1	1	1.2
Constipation	13	15.1	0	0.0
Dizziness	13	15.1	1	1.2
Anemia	12	14.0	2	2.3
Decreased Appetite	12	14.0	1	1.2
Peripheral Edema	11	12.8	0	0.0
Headache	10	11.6	0	0.0
Pruritus	10	11.6	1	1.2
Cough	9	10.5	0	0.0
Upper Respiratory Infection	9	10.5	0	0.0
Pyrexia	9	10.5	1	1.2

* No Grade 5 reactions were reported.

The frequencies of more severe thrombocytopenia, anemia *[see Warnings and Precautions (5.2)]* and fatigue were increased at doses higher than 400 mg once daily of ZOLINZA.

Serious Adverse Reactions

The most common serious adverse reactions in the 86 CTCL patients in two clinical trials were pulmonary embolism reported in 4.7% (4/86) of patients, squamous cell carcinoma reported in 3.5% (3/86) of patients and anemia reported in 2.3% (2/86) of patients. There were single events of cholecystitis, death (of unknown cause), deep vein thrombosis, enterococcal infection, exfoliative dermatitis, gastrointestinal hemorrhage, infection, lobar pneumonia, myocardial infarction, ischemic

stroke, pelviureteric obstruction, sepsis, spinal cord injury, streptococcal bacteremia, syncope, T-cell lymphoma, thrombocytopenia and ureteric obstruction.

Discontinuations

Of the CTCL patients who received the 400-mg once daily dose, 9.3% (8/86) of patients discontinued ZOLINZA due to adverse reactions. These adverse reactions, regardless of causality, included anemia, angioneurotic edema, asthenia, chest pain, exfoliative dermatitis, death, deep vein thrombosis, ischemic stroke, lethargy, pulmonary embolism, and spinal cord injury.

Dose Modifications

Of the CTCL patients who received the 400-mg once daily dose, 10.5% (9/86) of patients required a dose modification of ZOLINZA due to adverse reactions. These adverse reactions included increased serum creatinine, decreased appetite, hypokalemia, leukopenia, nausea, neutropenia, thrombocytopenia and vomiting. The median time to the first adverse reactions resulting in dose reduction was 42 days (range 17 to 263 days).

Laboratory Abnormalities

Laboratory abnormalities were reported in all of the 86 CTCL patients who received the 400-mg once-daily dose.

Increased serum glucose was reported as a laboratory abnormality in 69% (59/86) of CTCL patients who received the 400-mg once daily dose; only 4 of these abnormalities were severe (Grade 3). Increased serum glucose was reported as an adverse reaction in 8.1% (7/86) of CTCL patients who received the 400-mg once daily dose *[see Warnings and Precautions (5.4)]*.

Transient increases in serum creatinine were detected in 46.5% (40/86) of CTCL patients who received the 400-mg once daily dose. Of these laboratory abnormalities, 34 were NCI CTCAE Grade 1, 5 were Grade 2, and 1 was Grade 3.

Proteinuria was detected as a laboratory abnormality (51.4%) in 38 of 74 patients tested. The clinical significance of this finding is unknown.

Dehydration

Based on reports of dehydration as a serious drug-related adverse reaction in clinical trials, patients were instructed to drink at least 2 L/day of fluids for adequate hydration *[see Warnings and Precautions (5.3, 5.5)]*.

Adverse Reactions in Non-CTCL Patients

The frequencies of individual adverse reactions were substantially higher in the non-CTCL population. Drug-related serious adverse reactions reported in the non-CTCL population which were not observed in the CTCL population included single events of blurred vision, asthenia, hyponatremia, tumor hemorrhage, Guillain-Barré syndrome, renal failure, urinary retention, cough, hemoptysis, hypertension, and vasculitis.

In patients recovering from bowel surgery and treated perioperatively with ZOLINZA, anastomotic healing complications including fistulas, perforations, and abscess formation have occurred.

7   DRUG INTERACTIONS

7.1   Coumarin-Derivative Anticoagulants

Prolongation of prothrombin time (PT) and International Normalized Ratio (INR) were observed in patients receiving ZOLINZA concomitantly with coumarin-derivative anticoagulants. Physicians should monitor PT and INR more frequently in patients concurrently administered ZOLINZA and coumarin derivatives.

7.2   Other HDAC Inhibitors

Severe thrombocytopenia and gastrointestinal bleeding have been reported with concomitant use of ZOLINZA and other HDAC inhibitors (e.g., valproic acid). Monitor platelet count every 2 weeks for the first 2 months *[see Warnings and Precautions (5.6)]*.

8   USE IN SPECIFIC POPULATIONS

8.1   Pregnancy
Pregnancy Category D [see Warnings and Precautions (5.7)]

8.3   Nursing Mothers
It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants from ZOLINZA, a decision should be made whether to discontinue nursing or discontinue the drug, taking into account the importance of the drug to the mother.

8.4   Pediatric Use
The safety and effectiveness of ZOLINZA in pediatric patients have not been established.

8.5   Geriatric Use
Of the total number of patients with CTCL in trials (N=107), 46 % were 65 years of age and over, while 15 % were 75 years of age and over. No overall differences in safety or effectiveness were observed between these subjects and younger subjects, and other reported clinical experience has not identified differences in responses between the elderly and younger patients, but greater sensitivity of some older individuals should be considered, reflecting the greater frequency of decreased hepatic, renal, or cardiac function, and of concomitant disease or other drug therapy.

8.6   Use in Patients with Hepatic Impairment
ZOLINZA was studied in 42 patients with non-CTCL cancer and varying degrees of hepatic impairment after single and multiple-dose administration. Compared to patients with normal liver function, AUC increases of 50 to 66% were observed in patients with hepatic impairment. The incidence of Grade 3 or 4 thrombocytopenia increased in patients with mild (bilirubin of 1 to 1.5 x ULN and AST < ULN, or bilirubin ≤ ULN and AST > ULN) and moderate (bilirubin 1.5 to ≤ 3 x ULN) hepatic impairment treated daily at doses of 300 and 200 mg respectively.

Patients with severe hepatic impairment (bilirubin > 3 x ULN) have not been treated at doses greater than 200 mg a day. Reduce the initial dose of ZOLINZA in patients with bilirubin 1 to 3 x ULN or AST > ULN *[see Dosage and Administration (2.2) and Clinical Pharmacology (12.3)]*.

8.7   Use in Patients with Renal Impairment
Vorinostat was not evaluated in patients with renal impairment. However, renal excretion does not play a role in the elimination of vorinostat. Patients with pre-existing renal impairment should be treated with caution *[see Clinical Pharmacology (12.3)]*.

10   OVERDOSAGE

No specific information is available on the treatment of overdosage of ZOLINZA.

In the event of overdose, it is reasonable to employ the usual supportive measures, e.g., remove unabsorbed material from the gastrointestinal tract, employ clinical monitoring, and institute supportive therapy, if required. It is not known if vorinostat is dialyzable.

11   DESCRIPTION

ZOLINZA contains vorinostat, which is described chemically as *N*-hydroxy-*N'*-phenyloctanediamide. The empirical formula is $C_{14}H_{20}N_2O_3$. The molecular weight is 264.32 and the structural formula is:
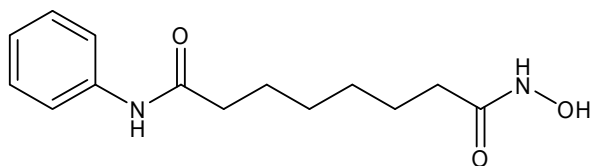
Vorinostat is a white to light orange powder. It is very slightly soluble in water, slightly soluble in ethanol, isopropanol and acetone, freely soluble in dimethyl sulfoxide and insoluble in methylene chloride. It has no chiral centers and is non-hygroscopic. The differential scanning calorimetry ranged from 161.7 (endotherm) to 163.9°C. The pH of saturated water solutions of vorinostat drug substance was 6.6. The pKa of vorinostat was determined to be 9.2.

Each 100 mg ZOLINZA capsule for oral administration contains 100 mg vorinostat and the following inactive ingredients: microcrystalline cellulose, sodium croscarmellose and magnesium stearate. The capsule shell excipients are titanium dioxide, gelatin and sodium lauryl sulfate.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Vorinostat inhibits the enzymatic activity of histone deacetylases HDAC1, HDAC2 and HDAC3 (Class I) and HDAC6 (Class II) at nanomolar concentrations (IC_{50}<86 nM). These enzymes catalyze the removal of acetyl groups from the lysine residues of proteins, including histones and transcription factors. In some cancer cells, there is an overexpression of HDACs, or an aberrant recruitment of HDACs to oncogenic transcription factors causing hypoacetylation of core nucleosomal histones. Hypoacetylation of histones is associated with a condensed chromatin structure and repression of gene transcription. Inhibition of HDAC activity allows for the accumulation of acetyl groups on the histone lysine residues resulting in an open chromatin structure and transcriptional activation. *In vitro*, vorinostat causes the accumulation of acetylated histones and induces cell cycle arrest and/or apoptosis of some transformed cells. The mechanism of the antineoplastic effect of vorinostat has not been fully characterized.

12.2 Pharmacodynamics

Cardiac Electrophysiology

A randomized, partially-blind, placebo-controlled, 2-period crossover study was performed to assess the effects of a single 800-mg dose of vorinostat on the QTc interval in 24 patients with advanced cancer. This study was conducted to assess the impact of vorinostat on ventricular repolarization. The upper bound of the 90% confidence interval of the placebo-adjusted mean QTc interval change-from-baseline was less than 10 msec at every time point through 24 hours. Based on these study results, administration of a single supratherapeutic 800-mg dose of vorinostat does not appear to prolong the QTc interval in patients with advanced cancer; however the study did not include a positive control to demonstrate assay sensitivity. In the fasted state, oral administration of a single 800-mg dose of vorinostat resulted in a mean AUC and C_{max} and median T_{max} of 8.6±5.7 µM•hr and 1.7±0.67 µM and 2.1 (0.5-6) hours, respectively.

In clinical studies in patients with CTCL, three of 86 CTCL patients exposed to 400 mg once daily had Grade 1 (>450-470 msec) or 2 (>470-500 msec or increase of >60 msec above baseline) clinical adverse reactions of QTc prolongation. In a retrospective analysis of three Phase 1 and two Phase 2 studies, 116 patients had a baseline and at least one follow-up ECG. Four patients had Grade 2 (>470-500 msec or increase of >60 msec above baseline) and 1 patient had Grade 3 (>500 msec) QTc prolongation. In 49 non-CTCL patients from 3 clinical trials who had complete evaluation of QT interval, 2 had QTc measurements of >500 msec and 1 had a QTc prolongation of >60 msec.

12.3 Pharmacokinetics

Absorption

The pharmacokinetics of vorinostat were evaluated in 23 patients with relapsed or refractory advanced cancer. After oral administration of a single 400-mg dose of vorinostat with a high-fat meal, the mean ± standard deviation area under the curve (AUC) and peak serum concentration (C_{max}) and the median (range) time to maximum concentration (T_{max}) were 5.5±1.8 µM•hr, 1.2±0.62 µM and 4 (2-10) hours, respectively.

In the fasted state, oral administration of a single 400-mg dose of vorinostat resulted in a mean AUC and C_{max} and median T_{max} of 4.2±1.9 µM•hr and 1.2±0.35 µM and 1.5 (0.5-10) hours, respectively. Therefore, oral administration of vorinostat with a high-fat meal resulted in an increase (33%) in the extent of absorption and a modest decrease in the rate of absorption (T_{max} delayed 2.5 hours) compared to the fasted state. However, these small effects are not expected to be clinically meaningful. In clinical trials of patients with CTCL, vorinostat was taken with food.

At steady state in the fed-state, oral administration of multiple 400-mg doses of vorinostat resulted in a mean AUC and C_{max} and a median T_{max} of 6.0±2.0 µM•hr, 1.2±0.53 µM and 4 (0.5-14) hours, respectively.

Distribution
Vorinostat is approximately 71% bound to human plasma proteins over the range of concentrations of 0.5 to 50 µg/mL.

Metabolism
The major pathways of vorinostat metabolism involve glucuronidation and hydrolysis followed by β-oxidation. Human serum levels of two metabolites, O-glucuronide of vorinostat and 4-anilino-4-oxobutanoic acid were measured. Both metabolites are pharmacologically inactive. Compared to vorinostat, the mean steady state serum exposures in humans of the O-glucuronide of vorinostat and 4-anilino-4-oxobutanoic acid were 4-fold and 13-fold higher, respectively.

In vitro studies using human liver microsomes indicate negligible biotransformation by cytochromes P450 (CYP).

Excretion
Vorinostat is eliminated predominantly through metabolism with less than 1% of the dose recovered as unchanged drug in urine, indicating that renal excretion does not play a role in the elimination of vorinostat. The mean urinary recovery of two pharmacologically inactive metabolites at steady state was 16±5.8% of vorinostat dose as the O-glucuronide of vorinostat, and 36±8.6% of vorinostat dose as 4-anilino-4-oxobutanoic acid. Total urinary recovery of vorinostat and these two metabolites averaged 52±13.3% of vorinostat dose. The mean terminal half-life ($t_{1/2}$) was ~2.0 hours for both vorinostat and the O-glucuronide metabolite, while that of the 4-anilino-4-oxobutanoic acid metabolite was 11 hours.

Specific Populations
Gender, Race & Age
Based upon an exploratory analysis of limited data, gender, race and age do not appear to have meaningful effects on the pharmacokinetics of vorinostat.
Pediatric
Vorinostat was not evaluated in patients <18 years of age.
Hepatic Impairment
The single dose pharmacokinetics of a 400 mg ZOLINZA dose was evaluated in patients with non-CTCL cancers with varying degrees of hepatic impairment. The mean AUC of vorinostat in patients with mild (bilirubin > 1 to 1.5 x ULN or AST > ULN but bilirubin ≤ ULN) and moderate (bilirubin 1.5 to ≤ 3 x ULN) hepatic impairment increased by 50% compared to the AUC of vorinostat in patients with normal hepatic function. The mean vorinostat AUC in patients with severe hepatic impairment (bilirubin > 3 x ULN) increased by 66% compared to the AUC of patients with normal hepatic function.

The safety of multiple daily doses of ZOLINZA was also evaluated in patients with non-CTCL cancers with varying degrees of hepatic impairment. The highest dose studied in mild, moderate and severe hepatic impairment was 400, 300 and 200 mg daily respectively. The incidence of Grade 3 or 4 adverse reactions was similar among the hepatic function groups. The most common Grade 3 or 4 adverse reaction was thrombocytopenia.

Reduce the dose in patients with mild to moderate hepatic impairment. There is not enough data in patients with severe hepatic impairment to recommend a dose modification *[see Dosage and Administration (2.2) and Use in Specific Populations (8.6)]*.
Renal Insufficiency
Vorinostat was not evaluated in patients with renal impairment. However, renal excretion does not play a role in the elimination of vorinostat *[see Use in Specific Populations (8.7)]*.

Pharmacokinetic effects of vorinostat with other agents
Vorinostat is not an inhibitor of CYP drug metabolizing enzymes in human liver microsomes at steady state C_{max} of the 400 mg dose (C_{max} of 1.2 µM vs IC_{50} of >75 µM). Gene expression studies in human hepatocytes detected some potential for suppression of CYP2C9 and CYP3A4 activities by vorinostat at concentrations higher (≥10 µM) than pharmacologically relevant. Thus, vorinostat is not expected to affect

the pharmacokinetics of other agents. As vorinostat is not eliminated via the CYP pathways, it is anticipated that vorinostat will not be subject to drug-drug interactions when co-administered with drugs that are known CYP inhibitors or inducers. However, no formal clinical studies have been conducted to evaluate drug interactions with vorinostat.

In vitro studies indicate that vorinostat is not a substrate of human P-glycoprotein (P-gp). In addition, vorinostat has no inhibitory effect on human P-gp-mediated transport of vinblastine (a marker P-gp substrate) at concentrations of up to 100 μM. Thus, vorinostat is not likely to inhibit P-gp at the pharmacologically relevant serum concentration of 2 μM (C_{max}) in humans.

13   NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenicity studies have not been performed with vorinostat.

Vorinostat was mutagenic *in vitro* in the bacterial reverse mutation assays (Ames test), caused chromosomal aberrations *in vitro* in Chinese hamster ovary (CHO) cells and increased the incidence of micro-nucleated erythrocytes when administered to mice (Mouse Micronucleus Assay).

Effects on the female reproductive system were identified in the oral fertility study when females were dosed for 14 days prior to mating through gestational day 7. Doses of 15, 50 and 150 mg/kg/day to rats resulted in approximate exposures of 0.15, 0.36 and 0.70 times the expected clinical exposure based on AUC. Dose dependent increases in corpora lutea were noted at ≥15 mg/kg/day, which resulted in increased peri-implantation losses were noted at ≥50 mg/kg/day. At 150 mg/kg/day, there were increases in the incidences of dead fetuses and in resorptions.

No effects on reproductive performance were observed in male rats dosed (20, 50, 150 mg/kg/day; approximate exposures of 0.15, 0.36 and 0.70 times the expected clinical exposure based on AUC), for 70 days prior to mating with untreated females *[see Warnings and Precautions (5.7)].*

14   CLINICAL STUDIES

Cutaneous T-cell Lymphoma

In two open-label clinical studies, patients with refractory CTCL have been evaluated to determine their response rate to oral ZOLINZA. One study was a single-arm clinical study and the other assessed several dosing regimens. In both studies, patients were treated until disease progression or intolerable toxicity.

Study 1

In an open-label, single-arm, multicenter non-randomized study, 74 patients with advanced CTCL were treated with ZOLINZA at a dose of 400 mg once daily. The primary endpoint was response rate to oral ZOLINZA in the treatment of skin disease in patients with advanced CTCL (Stage IIB and higher) who had progressive, persistent, or recurrent disease on or following two systemic therapies. Enrolled patients should have received, been intolerant to or not a candidate for bexarotene. Extent of skin disease was quantitatively assessed by investigators using a modified Severity Weighted Assessment Tool (SWAT). The investigator measured the percentage total body surface area (%TBSA) involvement separately for patches, plaques, and tumors within 12 body regions using the patient's palm as a "ruler". The total %TBSA for each lesion type was multiplied by a severity weighting factor (1=patch, 2=plaque and 4=tumor) and summed to derive the SWAT score. Efficacy was measured as either a Complete Clinical Response (CCR) defined as no evidence of disease, or Partial Response (PR) defined as a ≥50% decrease in SWAT skin assessment score compared to baseline. Both CCR and PR had to be maintained for at least 4 weeks.

Secondary efficacy endpoints included response duration, time to progression, and time to objective response.

The population had been exposed to a median of three prior therapies (range 1 to 12).

Table 2 summarizes the demographic and disease characteristics of the Study 1 population.

Table 2: Baseline Patient Characteristics
(All Patients As Treated)

Characteristics	Vorinostat (N=74)
Age (year)	
Mean (SD)	61.2 (11.3)
Median (Range)	60.0 (39.0, 83.0)
Gender, n (%)	
Male	38 (51.4%)
Female	36 (48.6%)
CTCL stage, n (%)	
IB	11 (14.9%)
IIA	2 (2.7%)
IIB	19 (25.7%)
III	22 (29.7%)
IVA	16 (21.6%)
IVB	4 (5.4%)
Racial Origin, n (%)	
Asian	1 (1.4%)
Black	11 (14.9%)
Other	1 (1.4%)
White	61 (82.4%)
Time from Initial CTCL Diagnosis (year)	
Median (Range)	2.6 (0.0, 27.3)
Clinical Characteristics	
Number of prior systemic treatments, median (range)	3.0 (1.0, 12.0)

The overall objective response rate was 29.7% (22/74, 95% CI [19.7 to 41.5%]) in all patients treated with ZOLINZA. In patients with Stage IIB and higher CTCL, the overall objective response rate was 29.5% (18/61). One patient with Stage IIB CTCL achieved a CCR. Median times to response were 55 and 56 days (range 28 to 171 days), respectively in the overall population and in patients with Stage IIB and higher CTCL. However, in rare cases it took up to 6 months for patients to achieve an objective response to ZOLINZA.

The median response duration was not reached since the majority of responses continued at the time of analysis, but was estimated to exceed 6 months for both the overall population and in patients with Stage IIB and higher CTCL. When end of response was defined as a 50% increase in SWAT score from the nadir, the estimated median response duration was 168 days and the median time to tumor progression was 202 days.

Using a 25% increase in SWAT score from the nadir as criterion for tumor progression, the estimated median time-to-progression was 148 days for the overall population and 169 days in the 61 patients with Stage IIB and higher CTCL.

Response to any previous systemic therapy does not appear to be predictive of response to ZOLINZA.

Study 2

In an open-label, non-randomized study, ZOLINZA was evaluated to determine the response rate for patients with CTCL who were refractory or intolerant to at least one treatment. In this study, 33 patients were assigned to one of 3 cohorts: Cohort 1, 400 mg once daily; Cohort 2, 300 mg twice daily 3 days/week; or Cohort 3, 300 mg twice daily for 14 days followed by a 7-day rest (induction). In Cohort 3, if at least a partial response was not observed then patients were dosed with a maintenance regimen of 200 mg twice daily. The primary efficacy endpoint, objective response, was measured by the 7-point Physician's Global Assessment (PGA) scale. The investigator assessed improvement or worsening in overall disease compared to baseline based on overall clinical impression. Index and non-index cutaneous lesions as well as cutaneous tumors, lymph nodes and all other disease manifestations were also assessed and included in the overall clinical impression. CCR required 100% clearing of all findings, and PR required at least 50% improvement in disease findings.

The median age was 67.0 years (range 26.0 to 82.0). Fifty-five percent of patients were male, and 45% of patients were female. Fifteen percent of patients had Stage IA, IB, or IIA CTCL and 85% of

patients had Stage IIB, III, IVA, or IVB CTCL. The median number of prior systemic therapies was 4 (range 0.0 to 11.0).

In all patients treated, the objective response was 24.2% (8/33) in the overall population, 25% (7/28) in patients with Stage IIB or higher disease and 36.4% (4/11) in patients with Sezary syndrome. The overall response rates were 30.8%, 9.1% and 33.3% in Cohort 1, Cohort 2 and Cohort 3, respectively. The 300 mg twice daily regimen had higher toxicity with no additional clinical benefit over the 400 mg once daily regimen. No CCR was observed.

Among the 8 patients who responded to study treatment, the median time to response was 83.5 days (range 25 to 153 days). The median response duration was 106 days (range 66 to 136 days). Median time to progression was 211.5 days (range 94 to 255 days).

15   REFERENCES

1. "OSHA Hazardous Drugs." *OSHA. [http://www.osha.gov/SLTC/hazardousdrugs/index.html]*

16   HOW SUPPLIED/STORAGE AND HANDLING

ZOLINZA capsules, 100 mg, are white, opaque hard gelatin capsules with "568" over "100 mg" printed within the radial bar in black ink on the capsule body. They are supplied as follows:
   NDC 0006-0568-40.
   Each bottle contains 120 capsules.

Storage and Handling

Store at 20-25°C (68-77°F), excursions permitted between 15-30°C (59-86°F) *[see USP Controlled Room Temperature]*.

Procedures for proper handling and disposal of anticancer drugs should be considered. Several guidelines on this subject have been published.[1] There is no general agreement that all of the procedures recommended in the guidelines are necessary or appropriate.

ZOLINZA (vorinostat) capsules should not be opened or crushed. Direct contact of the powder in ZOLINZA capsules with the skin or mucous membranes should be avoided. If such contact occurs, wash thoroughly as outlined in the references. Personnel should avoid exposure to crushed and/or broken capsules *[see Nonclinical Toxicology (13.1)]*.

17   PATIENT COUNSELING INFORMATION

See FDA-Approved Patient Labeling (Patient Information)

17.1 Instructions

Patients should be instructed to drink at least 2 L/day of fluid to prevent dehydration and should promptly report excessive vomiting or diarrhea to their physician. Patients should be instructed about the signs of deep vein thrombosis and should consult their physician should any evidence of deep vein thrombosis develop. Patients receiving ZOLINZA should seek immediate medical attention if unusual bleeding occurs. ZOLINZA capsules should not be opened or crushed.

Patients should be instructed to read the patient insert carefully.

Manuf. for: Merck Sharp & Dohme Corp., a subsidiary of
MERCK & CO., INC., Whitehouse Station, NJ 08889, USA

Manufactured by:
Patheon, Inc.
Mississauga, Ontario, Canada L5N 7K9

U.S. Patent Nos. RE 38,506 E; 6,087,367

Table 4. Study schedule

| | Enroll (48 weeks) | Intervention | | | | | | | | | | | | | | | Safety |
		Baseline (t0)	Week 2	Week 4	Week 6	Week 8	Week 12	Week 16	Week 20	Week 24 (t1)	Week 28	Week 32	Week 36	Week 40	Week 44	Week 48 (t2)	Week 72
Informed consent	X																
Patient number assignation	X																
Demographics (age, gender, origin, educational level, place of residence)	X																
Medical history	X																
Inclusion/exclusion criteria	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
aMCI and mild AD diagnostic criteria	X																
Physical/neurological examination	X	X		X		X	X	X	X	X	X	X	X	X	X	X	X
Previous and concomitant medications	X																
Allergies and/or previous adverse events	X																
Blood sample[iii]	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
MRI	X	X								X						X	
ECG: heart rate, PR, QRS, QT, QTc, and ST	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Patient medical history summary																	X
MoCA		X								X						X	
Modified ADAS-Cog		X								X						X	
ADCS-ADL		X								X						X	
NPI		X								X						X	
Lumbar puncture: CSF Aβ[iii]	X									X						X	
PET		X								X						X	
Apo E genotyping		X															
Other safety measures: weight, vital signs and temperature		X		X		X	X	X	X	X	X	X	X	X	X	X	X
Common side effects (20%): fatigue, diarrhea, nausea, dysgeusia, thrombocytopenia, anorexia[iv]			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Less common side effects: signs or symptoms of pulmonary embolism and deep vein thrombosis, gastrointestinal toxicity (nausea, vomiting and diarrhea), anemia and hyperglycemia.			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

[i] Blood sample will include general blood laboratory measures: chemistry and electrolytes (sodium, potassium, chloride, total bilirubin, direct bilirubin, alkaline phosphatase, GPT, GOT, GGT, BUN, creatinine, uric acid, phosphorus, calcium, glucose, total protein, albumin, cholesterol, CK), hematology (hemoglobin, hematocrit, erythrocyte count, MCV, MCHC, meukocytes, neutrophils, lymphocytes, monocytes, eosinophils, basophils, platelets) and PCR.

[ii] Samples collected will be destroyed when confirmed results will be available.

[iv] Specific side effects will be summarized throughout the clinical trial by instructing the participants (they will have access to a contact number exclusively intended for the prevention and treatment of adverse effects) and will be evaluated at each visit.