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MCLENA-1: A Phase II Clinical Trial for the Assessment of Safety, Tolerability, and Efficacy of Lenalidomide in Patients with Mild Cognitive Impairment Due to Alzheimer's Disease

Boris Decourt[1], **Jeffrey Wilson**[2], **Aaron Ritter**[1], **Christopher Dardis**[3], **Frank P DiFilippo**[4], **Xiaowei Zhuang**[1], **Dietmar Cordes**[1], **Garam Lee**[1], **Nadia D Fulkerson**[1], **Tessa St Rose**[1], **Katurah Hartley**[1], **Marwan N Sabbagh**[1]

[1]Cleveland Clinic Lou Ruvo Center for Brain Health, Las Vegas, NV, USA;

[2]Department of Economics W. P. Carey School of Business, Arizona State University, Tempe, AZ, USA;

[3]Barrow Neurological Institute, Phoenix, AZ, USA;

[4]Cleveland Clinic Department of Nuclear Medicine, Cleveland, OH, USA

Abstract

With the general population reaching higher ages, a surge in Alzheimer's disease (AD) incidence will happen in the coming decades, putting a heavy burden on families and healthcare systems Worldwide. This emphasizes the pressing need for AD therapeutic interventions. Accumulating evidence indicates that inflammation is prominent both in the blood and central nervous system of AD sufferers. These data suggest that systemic inflammation plays a crucial role in the cause and effects of AD neuropathology. Capitalizing on our experience from a previous clinical trial with thalidomide, we hypothesize that modulating inflammation via the pleiotropic immunomodulator lenalidomide may alter AD if administered during a proper time window in the course of the disease. Thus, in this Phase II, proof-of mechanism study, 30 amnestic mild cognitive impairment (aMCI) subjects will be treated with lenalidomide at 10 mg/day for 12 months on a 1:1 ratio, followed by a 6 months washout period. The primary objective of this study is to investigate the effect of lenalidomide on cognition, which is assessed at regular intervals. The secondary objective is to assess the safety and tolerability of lenalidomide in aMCI patients evaluated through adverse events, vital signs, clinical biochemistry, and physical and neurological examinations. Tertiary objectives are to analyze the effects of lenalidomide on brain amyloid loads (Florbetapir PET imaging) and neurodegeneration (volumetric MRI) by comparing pre- and post-dosing data.

Correspondence: Marwan N Sabbagh Cleveland Clinic Lou Ruvo Center for Brain Health, 888 W.Bonneville Avenue, Las Vegas 89106, NV, USA, Tel +1-702-483-6029, Fax +1-702-722-6584, sabbagm@ccf.org.

Disclosure

Marwan Sabbagh receives consulting fees from Allergan, Biogen, Bracket, Cortexyme, Grifols, Sanofi, Neurotrope, vTv therapeutics and Roche-Genentech outside the submitted work. He has ownership interest in Brain Health Inc., Versanum Inc., Neurotrope Inc., Optimal Cognitive Health Company and uMethod Health. The authors report no other conflicts of interest in this work.

Finally, exploratory objectives will investigate whether blood inflammatory markers can serve as surrogate markers of therapeutic efficacy. Our study should determine whether lenalidomide is safe in AD subjects and whether it can alter the clinical progression of AD when administered before dementia onset. If effective, lenalidomide would become the first drug capable of delaying the trajectory of AD, which could lead the way to find additional, less toxic treatments in the near future.

Keywords

Alzheimer's disease; biomarkers; brain amyloid; brain imaging; clinical trial; cognition; cytokines; dementia; hematologic changes; immunomodulation; inflammation; lenalidomide

Introduction

Currently, there are no FDA-approved medications indicated for the treatment of AD. All monotherapy clinical trials with a focus on disease modification completed to date have failed to meet the clinical endpoint of significantly slowing cognitive decline in dementia due to Alzheimer's disease (AD). This includes trials examining BACE1 inhibitors,[1] γ-secretase inhibitors and modulators,[2] and active and passive immunization against monomeric, oligomeric, and protofibril amyloid beta (Aβ).[3] Surprisingly, the failure of BACE1 inhibitors in recent clinical trials was associated with an exacerbation of cognitive decline, in addition to displaying toxicities due to the inhibition of not only amyloid precursor protein processing, but also the blocking of processing of all BACE1 substrates.[4] These results emphasize the urgent need for novel therapeutic approaches that reduce several AD neuropathologies simultaneously, without worsening cognition.

Inflammation is prominent in many neurological disorders, yet no clinical trial has demonstrated the efficacy of anti-inflammatory agents for AD.[5,6] Interestingly, chronic peripheral low-grade inflammation is associated with aging and increases the risk for disease and mortality, including AD.[7] Accumulating evidence indicates that nuclear factor-κB (NF-κB), tumor necrosis factor alpha (TNF-α), interleukins (e.g., IL-1β, IL-2, and IL-6), and chemokines (e.g., IL-8) are elevated both in the blood and central nervous system (CNS) of AD patients.[7] These data strongly suggest that inflammation plays a critical role in the cause and effect of AD neuropathology.[8] Interestingly, the anti-cancer drug lenalidomide is one of the very few pleiotropic agents that not only lowers the expression of TNF-α, IL-6, and IL-8, but also increases the expression of anti-inflammatory cytokines (e.g., IL-10). Thus, lenalidomide modulates both innate and adaptive immune responses.[9]

Preliminary tests showed a significant decrease in brain TNF-α mRNA, BACE1 mRNA and protein levels, and Aβ plaque loads, as well as improved cognitive measures in APP23 mice administered lenalidomide (unpublished observations; work done under IACUC protocols at Banner Health #1102, at Arizona State University #16–1456R and 19–1669R, and at Cleveland Clinic #2019–2206). Therefore, capitalizing on our experience with a recent thalidomide clinical trial we conducted in house[10] and on our animal observations, in the current project, we aim to test the central hypothesis that lenalidomide reduces inflammatory and AD-associated pathological biomarkers and improves cognition. For this, we designed

an 18-month, Phase II, proof-of-mechanism, clinical study where patients with mild cognitive impairment (MCI) due to AD will be administered 10 mg/day lenalidomide for 12 months followed by a 6 month washout. Other concomitant medications will be allowed, as long as their dose has been stable prior to screening and remains stable throughout the study. This project is highly significant because it represents a novel method to lower several neuropathological features associated with AD with a single, pleiotropic therapeutic agent.

Methods

This study will adhere to the protocol and ethical principles stated in the most recent version of the Declaration of Helsinki, as well as all applicable federal, state, and local laws, and institutional rules, and regulations. This study was reviewed and endorsed by the Cleveland Clinic Institutional Review Board (IRB; protocol #19–658). The investigators will report regularly the progress of the study to a medical monitor, a data and safety monitoring board (DSMB), and the IRB for compliance and investigational drug safety purposes. The Food and Drug Administration authorized the use of lenalidomide for this project under Investigational New Drug Application (IND) #142121. This trial is registered on ClinicalTrials.gov ().

Participants: Inclusion and Exclusion Criteria

Inclusion—In order to be eligible for this study, subjects must satisfy the following inclusion criteria. The subjects must be outpatients; at least 50 years of age, but less than 90; unable to become pregnant (bilateral tubal ligation, oophorectomy, hysterectomy, or postmenopausal for 2 years); diagnosed with amnestic MCI based on the most recent NIA-AA criteria;[11] both the screening and baseline visits (visits 1 and 2) have a documented Mini Mental State Exam (MMSE) score between 22–28; vision and hearing must be satisfactory to complete study procedures; must be able to take oral medications; Hachinski ischemic score must be ≤ 4; Geriatric depression scale must be ≤ 10; must reside in the community; supervision must be available for the administration of all study medication; and, at the discretion of the Medical Monitor, patients with stable prostate cancer may be included. In addition, a CT or MRI scan of the brain obtained within 12 months prior to screening must be consistent with the diagnosis and show no evidence of significant focal lesions or of pathology that could contribute to dementia. If a CT or an MRI scan is unavailable from the past 12 months, a CT scan fulfilling the requirements must be obtained before randomization. Cholinesterase inhibitor and/or memantine administration is acceptable, as long as the dosage is stable for at least 90 days before screening and is expected to remain stable for the entire duration of the study or have demonstrated lack of efficacy of or intolerance to these medications. Each subject must have a collateral informant/study partner who has significant direct contact with the patient of at least 10 hrs per week and who is willing to accompany the patient to all clinic visits and to be present during all telephone visits/interviews. If the patient has a legally authorized representative (LAR), the LAR must review and sign the informed consent form. If the patient does not have an LAR, the patient must appear able to provide informed consent and must review and sign the informed consent form. In addition, the patient's informant/study partner (as defined above) must sign

the informed consent form. If the LAR and the patient's informant/study partner is the same individual, s/he should sign under both designations.

Exclusions—Subjects will be excluded if they have any of the condition listed below: current evidence or history within the last 3 years of a neurological or psychiatric disorder that could contribute to dementia, including (but not limited to) Parkinson's disease, focal brain lesion, seizure disorder, epilepsy, and head injury with loss of consciousness; diagnostic and Statistical Manual of Mental Disorders (DSM) IV criteria for any major psychiatric condition such as psychosis, bipolar disorder, and major depression; known history or self-reported alcohol or substance abuse; living alone; history of myocardial infarction or signs or symptoms of unstable coronary artery disease within the last year (including revascularization procedure/angioplasty); poorly controlled hypertension; severe pulmonary disease (including chronic obstructive pulmonary disease) requiring more than 2 hospitalizations within the past year; need for home oxygen use or sleep apnea; any thyroid disease (unless euthyroid and on treatment for at least 6 months prior to screening); active neoplastic disease (except for skin tumors other than melanoma) within five years; history of multiple myeloma; identification of serious hematologic or coagulation disorder including any unexplained anemia or a platelet count less than 100,000/μL at screening; absolute neutropenia of $<750/mm^3$, or a history of neutropenia; history of or current thromboembolism (including deep venous thrombosis); any clinically significant hepatic or renal disease (including presence of Hepatitis B or C antigen/antibody or an elevated transaminase levels of higher than two times the upper limit of normal (ULN) or creatinine greater than 1.5 × ULN); HIV positivity; use of any investigational drug within 30 days or within five half-lives of the tested agent, whichever is longer; use of any investigational medical device within two weeks before screening or after end of the present study; females who are at risk of pregnancy or are of child bearing age; unwilling or unable to undergo MRI and PET imaging; cardiac pacemaker or defibrillator or other implanted device; and any other disease or condition that, in the opinion of the investigator, makes the patient inadmissible to participate in this clinical trial.

Study Procedures

Recruitment and Screening—The present clinical trial will enroll a total of 30 subjects at one single site in Las Vegas, NV. Our enrollment objective is to be close to 50% men and 50% women. Study subjects will be outpatients from the Cleveland Clinic Lou Ruvo Brain Health Center (CCLRCBH) diagnosed with amnestic mild cognitive impairment. We anticipate to screen 100 patients to find 30 meeting all eligibility criteria and displaying positive amyloid PET. Every year, CCLRCBH conducts several clinical trials for neurodegenerative disorders. CCLRCBH has 600 clinical trial participants in its current database. In addition, CCLRCBH follows approximately 2000 patients with memory disorders annually. These patients are continuously offered the possibility to be screened for clinical trials. When they are not eligible for a particular study, they are assessed to enter other studies.

Screen failures are described as patients who consent to participate in the clinical trial, but are not subsequently randomized to the study intervention or enrolled in the study. A

minimal set of screen failure information is needed to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to answer queries from regulatory authorities. Minimal information consists of demography, screen failure causes, eligibility criteria, and any serious adverse event (SAE).

Consent, Assessments, and Randomization—To participate in this study, all subjects must read and sign an IRB-approved informed consent. If the patient has a legally authorized representative, the LAR must review and sign the informed consent form. If the patient does not have an LAR, the patient must appear able to provide informed consent, and must review and sign the informed consent form. In addition, the patient's study partner/informant (as defined in inclusion criteria above) must sign the informed consent form. If the LAR and the patient's study partner/informant is the same individual, s/he should sign under both designations. Subjects can withdraw from the study at any time for any reason.

During the screening visit (Visit 1 at Weeks −6 to −2), subjects (or LAR, as appropriate) and study partner/informant (if not the legally-appointed representative) will sign the informed consent instrument prior to undergoing any study procedure. All screening tests and evaluations should be performed in 1 day. The tests and evaluations used to determine subject eligibility are indicated in Table 1. Patients who do not meet one or more of the entry criteria described above will not be randomized, will be considered screen failures, and will not be re-screened for this trial. If patients fulfil all of the above criteria and a CT scan or MRI is not available from the past 12 months, patients should be scheduled for a CT scan with results available by randomization. Similarly, if patients do not have an amyloid PET scan taken in the past 12 months, patients should be scheduled for amyloid PET imaging with results available by randomization.

When a subject has been determined to be eligible for enrollment, the site will contact the subject or their appropriate legal representative to confirm continued intent to participate in the study. If the subject or representative confirms intention to participate in the study, the investigator will randomize the subject and schedule Visit 2 at least 7–10 days before Baseline assessments. A randomization schedule is prepared by our biostatistician (Dr. Wilson), to which all investigators beside the clinical pharmacists are blind to until the completion of the study. Study subjects are randomized at the ration 1:1 (drug:placebo).

Baseline evaluations (Visit 2 at Week 0) will be performed on subjects randomized into the study. These evaluations will include an assessment of concomitant therapy and inclusion/exclusion criteria, vital signs and body weight, blood samples, Amyloid PET, vMRI, and cognitive assessments (Table 1). Upon leaving, patients will be given a sufficient supply of study medication (or placebo) to last until visit 3 along with instructions for their use. Patients will be instructed to take their first dose of study medication on the day of Visit 2. Patients should be instructed to take their medication every evening thereafter, at or around the same time. All assessments during subsequent visits are listed in Table 1.

Randomization will be carried out by our study biostatistician and the medication or placebo prepared accordingly by our clinical pharmacist. This procedure was developed to ensure

double blinding, in that neither the investigators nor the study subjects know the medication taken by study subjects until the completion of the study and the acquisition of all data.

Intervention Groups

Based on power calculations from our previous thalidomide trial and pre-clinical studies, and based on the limitation of recruitment at a single study site over the duration of the present project, we have decided to enroll a total of 30 subjects with single or multiple domain aMCI divided into a 1:1 ratio (drug:placebo). Fifteen subjects will receive a placebo control, and 15 subjects will receive lenalidomide 10 mg/day. These numbers are within the empirical range described previously for pilot pharmacological studies, with consideration of drop outs.[12]

This is a randomized, double-blind, placebo-controlled, parallel group study. The use of placebo is appropriate to minimize bias related to treatment expectations of the subject, study partner, and site investigator, as well as to changes in the relationship between the subject and study partners that might occur with the initiation of treatment and expectation of improvement in motor symptoms or cognition. Changes in subject/study partner interactions can impact subject mood and may introduce bias that cannot be quantified. The double-blind use of placebo will also prevent bias in the clinical and scientific assessments.

Drug and Dose Selection

Lenalidomide has lasting immunomodulatory effects, improving both cellular and humoral immune functions.[9] Its main modes of action are as follows: i) the destabilization of TNFα mRNA and the inhibition of IL-1, IL-6, and IL-12 production from human peripheral blood mononuclear cells; ii) the induction of T cell proliferation and the enhancement of IL-2 and interferon γ (IFNγ) production; and iii) the regulation of ubiquitination processes.[13] Additional mechanisms likely take place in non-cancer cells, but these mechanisms have not been fully identified.

Lenalidomide is prescribed in the clinic to treat relapsed and refractory multiple myeloma (25 mg/day) and transfusion-dependent anemia in patients with Low- or Intermediate-1-risk myelodysplastic syndromes with del (5q) (10 mg/day).[14] In May 2019, the FDA approved lenalidomide for previously treated follicular and marginal zone lymphoma (20 mg/day; https://www.biospace.com/article/celgene-s-revlimid-approved-for-follicular-lymphoma/). Its pharmacokinetic and pharmacodynamic (PD) properties have been studied in single-dose[15] and chronic[16] administration paradigms. For example, in myeloma patients, C_{max} occurs 0.5–4h post-dose both on days 1 and 28, and area under the concentration is 57% higher than in healthy male volunteers. Multiple dosing does not result in drug accumulation, elimination half-life is 3h, and most of the drug is excreted unchanged in the urine within 24 h.[16] In primates, lenalidomide crosses the blood brain barrier (BBB) and is detected in the cerebrospinal fluid (CSF) with PD properties paralleling those of plasma.[17] However, to our knowledge, no data have been reported in humans.

Drug dosing is crucial for this clinical trial. Our previous thalidomide trial failed because of too many adverse events (AEs) related to high drug doses.[10] When lenalidomide was licensed initially in 2005, doses of 40 mg/day were typical to treat relapsed multiple

myeloma (MM). In the past 5 years, lower doses (10–25 mg/day) have been shown to be similarly effective with a greatly reduced incidence of AEs in MM.[18] While AEs are far from trivial using 15 mg/day in elderly patients with MM, the context of the disease appears to be of fundamental importance. For example, MM toxicities are likely to be problematic at any dose sufficient to be effective in treatment.[19] However, when lenalidomide is used to treat non-hematological malignancies, the risks of cytopenias appear modest and the drug is well tolerated overall. For example, a systematic review of lenalidomide use in prostate cancer showed that grade 3/4 AEs occurred only with doses substantially higher than the range we propose to use in the present study.[20] In addition, and very importantly, the use of lenalidomide has not been studied in healthy subjects for more than one month. Thus, we want to use a dose range known to be effective (5–10 mg/day), but that induces the minimum toxicity possible in non-cancer subjects. Here, safety dictates a fixed dose rather than an escalating dose regimen.

Based on all these data, and after consulting with neuro-oncologists who have experience prescribing this drug, we propose to administer lenalidomide at 10 mg/day to 15 aMCI patients (and 15 placebo controls). This dose is within the best theoretical range described in clinical pharmacology, which is delimited by the area between the minimum effective dose and minimum toxic dose.

Outcome Measures

The endpoints selected for this study are appropriate measures of treatment effect. To date, the main measure for an effective treatment of AD is cognitive performance. Indeed, while they may be useful to help with the diagnosis of AD, there is no biomarker that has demonstrated theragnostic value yet. Therefore, any inflexion of cognitive decline, as measured by the several tests we have selected, will be the only clinical validation of disease improvement. Consequently, we will use a battery of tests recommended by the Alzheimer's Association for daily medical practice.[21] The tests will be used at Visits 1 (screening), 2 (baseline), 8 (Week 12 = 3 month assessment), 12 (Week 28 = 6 month assessment), 16 (Week 44 = 9 month assessment), 18 (Week 52 = End of Study assessment), 19 (Week 56 = End of Study+4 weeks washout assessment), and 20 (Week 78 = 6 month wash out assessment). The tests will be used in alternation to avoid that subjects memorize them simply by multiple administration, as specified in Table 1.

Cognitive Measures

Alzheimer's Disease Assessment Scale - Cognitive—Alzheimer's Disease Assessment Scale - Cognitive (ADAS-cog)[22] is a psychometric instrument that evaluates memory, attention, reasoning, language, orientation, and praxis. A higher score indicates more impairment; the range is 0 to 89. We will use the ADAS-cog14 because it is currently accepted by dementia clinicians to have a better ability to detect changes in mildly demented subjects. A score of 5 indicates cognitively normal individuals. A score around 12 suggests MCI. A score around 30 or above is typical of AD dementia.

Alzheimer's Disease Cooperative Study - Activities of Daily Living—In the Alzheimer's Disease Cooperative Study - Activities of Daily Living (ADCS-ADL) test,[23]

study partners are queried via a structured interview format as to whether subjects attempted each item in the inventory during the preceding four weeks, as well as their level of performance. The score range 0 to 56, with a lower score indicating more impairment. This will provide a reliable estimate of daily functioning.

Clinical Dementia Rating-Sum of Boxes—The Clinical Dementia Rating - Sum of Boxes (CDR-SOB) test[24] is a commonly used instrument in MCI studies. The CDR-SOB describes five degrees of impairment in performance on each of six categories of cognitive functioning including memory, orientation, judgment and problem solving, community affairs, home and hobbies, and personal care. The ratings of degree of impairment obtained on each of the 6 categories of function are synthesized into one global rating of dementia (ranging from 0 to 3), with more refined measure of change available by use of the Sum of Boxes. The CDR rater will be a trained clinician who will not have access to the ADAS-cog data, adverse events reports, or any other information that may unblind them to the treatment condition. Ideally the same CDR rater will be used throughout the course of the trial.

Mini Mental State Examination—The Mini Mental State Examination (MMSE) is a screening instrument frequently used in Alzheimer's disease drug studies.[25] It evaluates orientation, memory, attention, concentration, naming, repetition, comprehension, and the ability to create a sentence and copy two intersecting pentagons. Scores range from 0 to 30, with 30 being the score assign to cognitively normal individuals. The range for aMCI is 22–28.

Safety and Tolerability

Routine Clinical Laboratory Tests—Routine hematology, blood chemistry and urinalyses will be performed as described in Table 1. At the screening visit, hematology, blood chemistry, HIV, hepatitis B and C, anti-HCV, and urinalyses will also be completed. Because lenalidomide is a very potent immunomodulator, patients with preexisting viral infections are more likely to be harmed by the study drug than benefiting from it.

Electrocardiograms—For each subject, 12-lead digital electrocardiograms (EKGs) will be collected according to Table 1. The EKG at screening will be a single EKG. EKGs should be collected at approximately the same time of day, if possible, to minimize diurnal variation. Patients must be supine for approximately 5 to 10 mins before EKG collection and remain supine, but awake during EKG collection. EKGs will be interpreted by a qualified physician (the investigator or qualified designee) at the site, as soon after the time of EKG collection as possible, and, ideally, while the subject is still present, to determine whether the subject meets entry criteria and for immediate subject management should any clinically relevant findings be identified.

After enrollment, if a clinically significant increase in the QT/corrected QT (QTc>500) interval from baseline, or other clinically significant quantitative or qualitative change from baseline is present, the investigator will assess the subject for symptoms (for example, palpitations, near syncope, syncope) and to determine if the subject can continue the study. The investigator (or qualified designee) will be responsible for determining if any change in

patient management is needed. The investigator (or qualified designee) will also document his/her review of the EKG printed at the time of evaluation, the final overread EKG, and any alert report.

Physical Examination—A complete physical examination, will be performed per Table 1. An abbreviated physical examination, to include an ophthalmic (routine fundoscopic) examination and routine examination of the heart, lungs, abdomen, skin, and oral cavity will also be performed. Sitting vital signs (blood pressure, heart rate, respiratory rate, and temperature) after each subject sits quietly for 5 mins and body weight will be assessed at the Screening Visit and at each subsequent visit.

Vital Signs—Body temperature (oral or tympanic) will be measured as specified in Table 1, and as clinically indicated. Blood pressure and pulse rate (PR) will be measured as specified in Table 1, and as clinically indicated. Blood pressure and PR should be measured after at least 5 mins sitting.

Body Weight and Height—Body weight and height will be recorded as specified in Table 1, and as clinically indicated.

Neurological Examination—A directed neurological examination will be performed by the investigator at the time points specified in Table 1. Any clinically significant change from baseline on follow-up physical/neurological examinations should be recorded as an AE. If additional amyloid-related imaging abnormalities - edema/effusions, or amyloid-related imaging abnormalities - hemosiderin deposition are detected, an additional neurological exam may be performed by the investigator.

Tolerability Assessment and Management - Adverse Events—The condition of each study subject will be monitored throughout the study. Signs and symptoms of possible AEs may be observed by the staff, elicited by asking the patient and/or study partner/informant an open or indirect question (e.g., “How have you felt since your last clinic visit?”), or volunteered by the subject and/or study partner. All adverse events, whether observed by the investigator or staff, elicited from the subject or study partner, or volunteered by the subject or study partner will be recorded. Data will include start and end dates, investigator-specified severity and relationship to study drug, and action taken. Whether the event resulted in death, required (or prolonged) hospitalization with persistent or significant disability/incapacity, required intervention to prevent any of the above outcomes, and/or whether it was reported as serious to the medical monitor, DSMB, IRB, and study sponsor will also be recorded.

Knowledge of a serious adverse event occurring or worsening in a study subject at any time during the trial must be reported by telephone within 24 hrs (if physically possible) to the investigator who will immediately inform the medical monitor, DSMB, and IRB. At any time following the study, the investigator should immediately notify the medical monitor, DSMB, and IRB if he learns of the occurrence of any malignancy or pregnancy involving the participant of this clinical trial, of any serious adverse event that could possibly be related to study drug, or of any congenital anomaly in an offspring of a participant.

Tolerability of lenalidomide in study subjects will be derived from the frequency of AEs and study withdrawal motivated by subjects' discomfort during the dosing period. During visits at the study site, we will collect blood samples and carry out blood counts and comprehensive metabolic panels to monitor hematologic and metabolic toxicities. The results will be shared with neuro-oncologists for expert opinions on whether or not the study subjects suffer lenalidomide-related toxicities. If toxicity is identified, we will follow clinical recommendations published previously,[26] i.e., if platelets fall below 50,000/μL and/or neutrophils fall below 1,000/μL, we will interrupt lenalidomide treatment (10 mg initial dose) for a minimum of 7 days. Then, we will confirm that platelets return to ≥50,000/μL and/or neutrophils return ≥1,000/μL before re-administering lenalidomide at 5 mg/day for the remaining of the trial. If study subjects continue developing neutropenia and thrombocytopenia under 5 mg/day dosing, the treatment will be stopped, and the patient retested 7 days later. After two successive failures to maintain normal counts of platelets and/or neutrophils under 5 mg/day, the subject will be terminated and recorded as early termination without replacement.

Brain Imaging Measures

Amyloid PET Imaging—Amyloid PET imaging will allow the quantification of brain amyloid loads before and after drug dosing. If post-dosing signals are lower than pre-dosing, it will suggest that not only lenalidomide is able to lower brain amyloid loads, but also that PET imaging can be used to measure a decrease in Aβ in the brain of living subjects, which is controversial currently.

In this study, we will follow a protocol similar to the Alzheimer's Disease Neuroimaging Initiative (ADNI). For brain amyloid loads assessment, subjects will undergo 18F-florbetapir imaging on a Siemens Biograph mCT PET/CT scanner. Subjects will receive a single intravenous bolus injection target dose of 370 MBq (10 mCi) of 18F-florbetapir, followed by a saline flush. At 50 mins following injection, a continuous 20 min brain scan (4 frames of 5 min duration) will begin. If at any point during the imaging session it is determined that the subject is not able to continue, or that it is not in the best interest of the subject to continue, imaging will be discontinued. The image data that has been collected up to that point will be analyzed. Safety assessments will be conducted prior to injection and upon completion of the imaging session. Standard attenuation correction using CT scan data will be applied. PET images will be corrected for motion by co-registering each frame to the participant's T1-weighted MRI images prior to summing the PET frames. The SUVr map with whole cerebellum as the reference region will be generated for each subject after the florbetapir scan is linearly and nonlinearly warped to the Montreal Neurological Institute (MNI) template. SUVr for six pre-defined regions of interest (frontal cortex, temporal cortex, parietal cortex, anterior cingulate gyrus, posterior cingulate gyrus, and the precuneus region) will be computed. The mean SUVr over all six regions will be combined to derive mean cortical uptake as the primary measure for florbetapir measurement of whole brain fibrillar amyloid burden.

Adverse events will be monitored continuously during the imaging session. A physician or a licensed/credentialed medical professional (e.g., a PET technologist, imaging center nurse,

or a regional equivalent) designated by the site principal investigator must assess or evaluate the subject for adverse events prior to injection and prior to discharge from the imaging center. Subjects who experience any adverse event during an imaging session will not be discharged until the event has resolved or stabilized.

Volumetric MRI—Participants will receive MRI scans on a GE 3T Excite scanner. Sequences will include a high resolution 3D T1-weighted magnetization prepared rapid acquisition gradient echo (MPRAGE) (TE=Min Full, Flip Angle=8, NEX=1, FOV=22, imaging matrix=192×192, slice thickness=1.2 mm). For clinical safety and secondary measures, a T2 weighted FLAIR image sensitive to strokes and edema, and long TE gradient echo acquisition for microhemorrhage will be performed. Hippocampal, ventricular, and whole brain volumes will be determined by automated segmentation using Freesurfer 6.0. Cortical thicknesses of each brain region are also determined from this automated segmentation. Voxel-wise measures of regional gray matter volumes (corrected for the total intracranial volume) are determined using voxel-based morphometry in SPM12, and the longitudinal whole brain atrophy are estimated using iterative principal component analysis.[27] Furthermore, voxel-wise whole brain morphological changes are determined using deformation-based morphometry with high resolution 3D T1 images as input.

Exploratory Blood and CSF Measures

Optional CSF Collection Sub-Study—A small sub-study will be offered to participants. They will have the choice to donate cerebrospinal fluid at both baseline (Week 0) and at the end of dosing (Week 52) visits. This donation is fully elective and patients will continue the study even if they are not willing to donate CSF. The CSF collected will be used to measure Aβ40, Aβ42, tau, phospho-tau, and the same inflammatory markers as in the blood (detailed below), in order to assess whether lenalidomide engages CNS molecular targets.

Lumbar Puncture (LP) is a standard neurodiagnostic procedure for the collection of CSF, but may be associated with pain during the performance of the procedure. This is usually temporary and confined to the lower back. A persistent low-pressure headache may develop after LP, probably due to leakage of CSF. Although the frequencies of post-LP headaches have been reported to be as high as 10% using standard 20 gauge spinal needles, rates of less than 2% have been reported in elderly subjects when atraumatic (Sprotte) needles are used.[28] If a post-LP headache persists, it may need additional treatment, e.g., with fluids and analgesics. Uncommonly, a blood patch (injection of some of the subject's blood to patch the CSF leak) may be needed. Potential, but rare risks of lumbar puncture include infection, damage to nerves in the back, bleeding into the CSF space, and death. The risk of these is less than 1%.

Because CSF collection is not possible at each site visit, we will explore the possibility of using blood inflammatory markers as surrogate markers of lenalidomide efficacy. For this, we will collect whole blood (cubital vein); maximum 20 mL in glass tubes containing ethylenediaminetetraacetic acid (EDTA). After differential centrifugations, we will measure

plasma C-reactive protein, TNF-α, IL-1β, IL-6, IL-8, and IL-10 by multiplex ELISA (Meso Scale Discovery platform).

Data and Sample Storage

Data and samples collected for this study will be saved at the Lou Ruvo Brain Health Center, Las Vegas, NV. Data and sample storage is consented by participants. All electronic data will be de-identified, stored on secured servers with encryption, and with access limited to a few users. Our procedures will seek IRB approval for compliance. Similarly, all biospecimens collected during the course of the study will be de-identified, and processed and analyzed blindly until the completion of the study. After the study is concluded, the de-identified, archived data and specimens will be made available for use by other researchers, including investigators not part of the study, upon request to the Principal Investigator.

Planned Analyses

Data Analyses—All subjects receiving at least 1 dose of study drug will be included in the safety analyses.

For efficacy analyses, subjects will be included only if they reach Visit 7 (Week 8) of the dosing. Patients withdrawing or withdrawn from the study before Visit 7 are unlikely to demonstrate a change in the cognitive markers investigated here given the effects of lenalidomide in the CNS take several weeks to be measurable (based on our previous thalidomide clinical trial[10] and animal observations). All intra and inter-subject outcome measures collected during the course of our study will be sent to our very experienced biostatistician for analyses.

Statistical analyses will be performed using PROC GLIMMIX in SAS. The data will be summarized using professional standards. The set of patients completing each study visit and their reasons for early terminations will be noted and compared across treatment groups. The duration of double-blind treatment within each treatment group and compliance rates will be summarized by descriptive statistics (mean, standard error, standard deviation, median, minimum, maximum, and sample size) for days of treatment and for total days of exposure, and by the distributions of patients exposed to various durations of treatment categories. Demographic and baseline characteristics will be summarized by descriptive statistics for all patients in each treatment group. Paired data tests will be used to assess eventual biomarker changes between pre- and post-treatment datasets. Measure of associations through bivariate correlations and generalized linear models will be employed to determine the impact of dose-response for lenalidomide on the biomarkers investigated. Missing data will not be imputed and all descriptive summary statistics will be reported based upon the observed data. For limited analyses where formal inferential statistics are needed, likelihood and Bayesian estimates will be employed for subjects missing follow-up assessments. A $p<0.05$ will be considered statistically significant.

Safety Analyses

Concomitant Medications.: Concomitant medications will be coded by the World Health Organization (WHO) Drug Dictionary and tabulated by treatment group.

Adverse Events.: The incidence of all treatment-emergent AEs (TEAEs) and treatment-related AEs will be tabulated by treatment received. These AEs will be classified by system organ class and preferred term using the Medical Dictionary for Regulatory Activities (MedDRA). For incidence reporting, if a subject reported more than one AE that was coded to the same system organ class or preferred term, the subject will be counted only once for that specific system organ class or preferred term. A TEAE is defined as an event that first occurs or worsens in intensity after the administration of study drug. Events recorded between the time the informed consent is signed and the first study drug administration will be listed. An overview of AEs, which includes subject incidence of TEAEs, treatment-related AEs, SAEs, deaths, and AEs leading to discontinuation, will be constructed. For AEs presented by severity, the worst severity during the study will be presented for each subject. A summary of values will be examined for significant differences using the appropriate generalized linear model.

Serious Adverse Events.: All SAEs will be listed and summarized in a similar manner to AEs.

Clinical Laboratory Results.: Clinical laboratory values will be measured by a central laboratory. Descriptive statistics for actual values and for changes from baseline will be tabulated for laboratory results by scheduled visit. Subjects with clinical laboratory values outside of the normal reference range at any post-baseline assessment will be noted and appropriate binary measures employed. Changes in baseline laboratory values (to worse severity per subject) will be noted so the appropriate test statistics can be used.

Electrocardiogram Results.: The EKG parameters (heart rate, PR interval, QRS interval, QT interval, and QTc interval) at each time recorded (scheduled for screening and study end), as well as the change from screening will be summarized with descriptive statistics. The overall EKG assessment will be reported as “Normal” or “Abnormal” with respect to relevant abnormalities by the investigator. A table providing a comparison of the EKG assessment over the treatment period (every abnormal) to screening will be presented. The values in the table will be analyzed to identify significant shifts.

Vital Signs.: The observed data at baseline and change from baseline for each measurement day will be summarized with descriptive statistics and the appropriate generalized linear mixed model used.

Aim 1

Because the only clinical validation for AD treatment efficacy is an inflexion in the trajectory of cognitive decline, our first aim is to test whether cognition is improved (or cognitive decline slowed down) in aMCI subjects treated with lenalidomide. For this, all subjects will be assessed by MMSE, CDR, and ADAS-cog 14 at baseline and after dosing to assess whether the drug has a positive effect on cognitive performance. Additional testing will be carried out after 6 months of drug wash out to assess the mid-term effect of the drug on cognition. These factors will be included in a generalized linear mixed model to address their impact.

Aim 2

Given therapeutic intervention may be given for a long period of time, it is important to assess the safety and tolerability of lenalidomide in aMCI patients. Interestingly, lenalidomide is used for more than 15 years for blood cancer treatment, thus, its adverse events are well documented and consist mainly of neutropenia (20–45%) and thrombocytopenia (30–55%), although its adverse events in non-cancer patients and at lower doses are much less known. All study subjects will be monitored regularly by blood counts during the course of the study. Per clinical recommendation for this drug, subjects showing abnormal blood counts will stop taking lenalidomide until blood counts come back to normal and will resume treatment with 5 mg/day until the following visit. These repeated measures along with the key factors are included in a repeated measures factor analysis with random effects to account for the inherent correlation.

Aim 3

As an exploratory effort to identify surrogate biological markers of treatment efficacy, we will test whether inflammatory and AD-specific markers are altered in aMCI subjects treated with lenalidomide. First, we will compare brain amyloid loads pre- and post-dosing via PET imaging to assess whether the regimen used in this study lowers amyloid burden. Second, we will measure the AD-related CSF markers Aβ40, Aβ42, tau, and phospho-tau via multiplex ELISA. Third, we will also measure CSF inflammatory markers, including TNF-α, IL-1β, IL-6, and IL-8, by multiplex ELISA. Finally, we will measure plasma inflammatory markers by multiplex ELISA on all samples collected during the study in order to confirm that lenalidomide modulates inflammation in the periphery. The pre- and post-measures, along with the AD and CSF markers, form the systematic component of a generalized linear mixed model used to identify significant drivers.

Discussion

Alzheimer’s disease is a multifactorial and complex neurodegenerative disorder with multiple symptoms and pathophysiological processes developing over time.[29] Consequently, the use of monotherapies with very precise targets is unlikely to slow down or cure the disease. Instead, the use of combination or pleiotropic therapies will likely be more successful to treat the disease.[30] Furthermore, because scientists are currently unable to regenerate brain tissue with of the ability to encode personal memories, the first successful AD therapies will likely be administered at early stages of the disease, i.e., before severe neurodegeneration develops. In the present clinical trial, we will use the pleiotropic immunomodulator lenalidomide on amyloid positive aMCI subjects to assess its potential at slowing down the clinical progression of AD over a year of treatment. Because nothing is known about the study drug in the context of neurological disorders, our first study is a proof of concept trial conducted on a small number of subjects to determine whether lenalidomide could be used as an AD prevention therapy in the future. To reach this goal, we have carefully selected clinically relevant endpoints, including cognitive measures, amyloid brain imaging, and volumetric MRI. Further, we will explore the theragnostic potential of minimally invasive and affordable blood biomarkers to assess treatment efficacy, and we will compare those peripheral markers to clinical markers (i.e. cognitive measures, amyloid

imaging, and volumetric MRI). Given the urgent need for therapeutic interventions to prevent the AD pandemic predicted to happen in the coming decades,[31] we strongly believe that repurposing clinically relevant drugs in well-defined pilot studies will dramatically accelerate the discovery of the first effective AD treatments, that will lead to the development of novel interventions, while minimizing toxicity. The present study is designed to obtain accurate data about the repurposing of lenalidomide for AD treatment in the shortest time possible.

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Table I

Schedule of Visit

	WK −6/−2	WK 0	WK 1	WK 2	WK 4	WK 6	WK 8	WK 12	WK 16	WK 20	WK 24	WK 28	WK 32	WK 36	WK 40	WK 44	WK 48	WK 52	WK 56	WK 78
	Screening	Baseline	Telephone			Telephone			Telephone		Telephone		Telephone		Telephone		Telephone	End of Treatment		Follow Up
	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10	Visit 11	Visit 12	Visit 13	Visit 14	Visit 15	Visit 16	Visit 17	Visit 18	Visit 19	Visit 20
Inclusion/Exclusion	x																			
Informed Consent	x																			
Medical History/Verify Diagnosis	x																			
Height, HIV test, Hepatitis B and C Tests	x																			
Pregnancy Test (women only)	x																			
Interim History		x		x	x		x	x		x		x		x		x		x		x
EKG	x				x			x				x						x		x
Vital Signs and Weight	x	x		x	x		x	x		x		x		x		x		x		x
Neuro and Physical Exam	x	x		x	x		x	x		x		x		x		x		x		x
Amyloid imaging (if none done in past 12 months)	x																	x		
Volumetric MRI (if none done in past 12 months)		x																x		
CT Scan (if none done in past 12 months)	x																			
LP (Optional)		x																x		
Labs (CBC, CMP, UA)	x	x		x	x		x	x		x		x		x		x		x		x
Blood Biomarkers		x		x	x		x	x		x		x		x		x		x	x	x
Cognitive Assessments	x[a]	x[b]						x[c]				x[d]				x[c]		x[e]	x[f]	x[g]
Concomitant Meds	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
AE Assessment		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x

	WK −6/−2	WK 0	WK 1	WK 2	WK 4	WK 6	WK 8	WK 12	WK 16	WK 20	WK 24	WK 28	WK 32	WK 36	WK 40	WK 44	WK 48	WK 52	WK 56	WK 78
	Screening	Baseline	Telephone			Telephone			Telephone		Telephone		Telephone		Telephone		Telephone	End of Treatment		Follow Up
	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10	Visit 11	Visit 12	Visit 13	Visit 14	Visit 15	Visit 16	Visit 17	Visit 18	Visit 19	Visit 20
Dispense Study drug		x		x	x		x	x		x		x		x		x				
Drug Accountability				x	x		x	x		x		x		x		x		x		

Notes:

[a]Screening: MMSE + Hachinski + Geriatric Depression Scale.

[b]Baseline: MSME + ADAS-cog + CDR-SOB + ADCS-ADL.

[c]3 and 9 months: ADAS-cog.

[d]6 months (half-way through drug treatment): ADAS-cog + CDR-SOB.

[e]12 months (end of drug treatment): ADAS-cog + CDR-SOB + ADCS-ADL.

[f]12 month + 4 weeks washout: MMSE + ADAS-cog + CDR-SOB + ADCS-ADL.

[g]18 months (6 months washout): MMSE + ADAS-cog + CDR-SOB + ADCS-ADL.