Metformin

in Alzheimer's dementia Prevention (MAP)

Protocol Number, Columbia University: AAAS6912

Protocol Number, Johns Hopkins University: JHUSIRB00000013

National Clinical Trial (NCT) Identified Number: NCT04098666

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Sponsor: Columbia University Irving Medical Center

Funded by: National Institute on Aging (R01 AG062624)

Version Number: v2.0

May 21, 2024

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and International Conference on Harmonization (ICH) Good Clinical Practice (GCP) Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Johns Hopkins Medicine (JHM) Institutional Review Board (IRB), which is acting as the single IRB (sIRB) of record for review and approval of this study. JHM sIRB approval must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the sIRB before the changes are implemented to the study. In addition, all changes to the consent form will be sIRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Title:	Metformin in Alzheimer's dementia Prevention (MAP)
Study Description:	Up to Protocol version 1.8, MAP was a 24-month phase II/III 1:1 randomized clinical trial of extended-release metformin (Glucophage XR [reduced mass]) 2000 mg (in 500 mg tablets) vs. matching placebo among 370 persons with amnestic mild cognitive impairment without diabetes in the prevention of cognitive decline among persons at risk for Alzheimer's dementia. Participants were assessed every six months for a total of 5 visits (baseline and months 6, 12, 18, and 24). Up to 186 participants were planned to undergo brain magnetic resonance imaging (MRI) at baseline and after the 24 months visit. Up to 186 participants were also expected to undergo amyloid (18F-florbetaben) positron emission tomography (PET) and tau (18F-MK-6240) PET at baseline and after the 24 months visit, independently of the MRI. For protocol version 1.9, the follow-up duration was decreased to 18 months (4 visits), and the sample size was decreased to 326, based on a reconsideration of the pilot data and assumptions supporting the original design. Follow-up and PET will be completed after the 18-month visit.
Objectives:	Primary Objective: To test the efficacy of metformin in the prevention of cognitive decline associated with Alzheimer's dementia, we will compare changes over 18 months in verbal memory performance, measured with the Total Recall Score of the Free and Cued Selective Reminding Test (FC-SRT) Secondary Objectives: 1) Examine changes in global cognitive performance measured with the Alzheimer's Disease Cooperative Study, Preclinical

Alzheimer Cognitive Composite (ADCS-PACC); 2) Compare changes in neurodegeneration (cortical thickness) ascertained on MRI between metformin and placebo; 3) Compare changes in cerebrovascular disease (white matter hyperintensities) ascertained on MRI between metformin and placebo; 4) Compare changes in whole brain amyloid β (Aβ) standardized uptake value ratio (SUVR) and in incident amyloid positivity from baseline to 18 months between the metformin and placebo arms ; 5) Compare changes in tau SUVR in a composite brain region comprising medial and inferolateral temporal cortex from baseline to 18 months between the metformin and placebo arms; 6) Compare changes in plasma Alzheimer's disease (AD) biomarkers over 18-month follow-up between metformin and placebo; 7) To examine APOE-ε4 genotype and COVID-19 history as a modifier of the efficacy of metformin.

Endpoints:

The primary endpoint is changes from baseline to 18 months in verbal memory performance, measured with the Total Recall Score of the FC-SRT, between the metformin and placebo arms, following an intent to treat (ITT) approach. **The secondary endpoints** are 1) changes in global cognitive performance, measured with the Alzheimer's Disease Cooperative Study Preclinical Alzheimer Cognitive Composite (ADCS-PACC); 2) changes in neurodegeneration, ascertained as cortical thickness in areas affected by AD on brain MRI; 3) changes in cerebrovascular disease, ascertained as white matter hyperintensities (WMH) volume on brain MRI; 4) Changes in whole brain amyloid ß (Aß) SUVR and in incident amyloid positivity; 5) Changes in tau SUVR in a composite brain region comprising medial and inferolateral temporal cortex; 6) Changes in plasma AD biomarkers. The FC-SRT and ADCS-PACC, and the plasma AD biomarkers will be measured 4 times in the study (baseline and months 6, 12, and 18). The brain imaging measures will be measured twice, at baseline and 18 months. We will also explore incident amyloid positivity at 18 months as determined by a cutoff of Aß from PET equal to 24 centiloids.

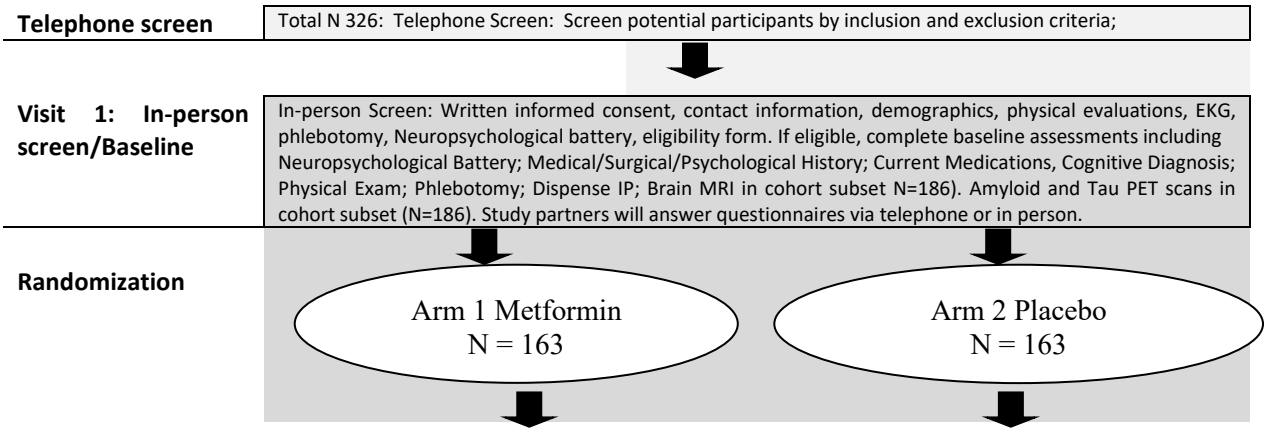
Study Population:

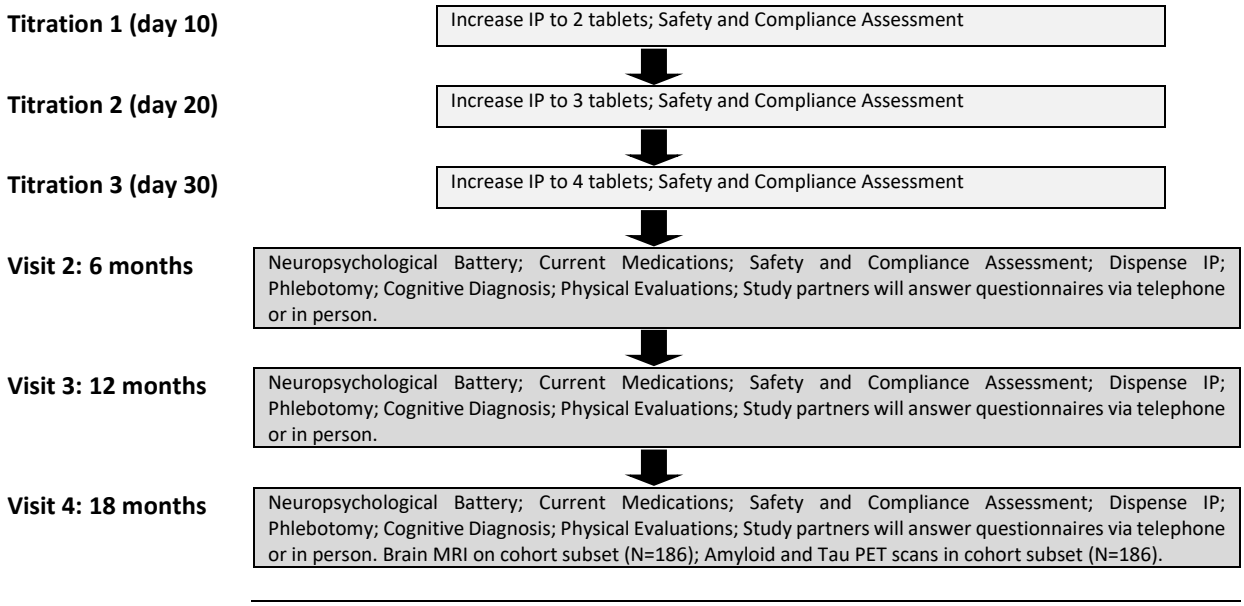
The target sample are 326 (163 per arm) men and women aged 55 years to 90 years, with early or late amnestic mild cognitive impairment (aMCI), without dementia, without diabetes, with a body mass index (BMI) of 20 k/m^2 or higher, not taking metformin, without contraindications to metformin use, and not taking any cognitive enhancers or medications that interfere with cognition. Biomarkers will not be used for the definition of aMCI. We will use the early aMCI and late aMCI criteria from the Alzheimer's Disease Neuroimaging Initiative (ADNI). The eligibility criteria, recruitment and retention strategies are described in detail in section 2.2 (Eligibility criteria). Persons without contraindications to MRI may undergo MRI, but inability or unwillingness to undergo MRI will not be a reason for exclusion. Participation will require the availability of a study partner (a person who knows the participant well), in order to answer questions about the participant in person or via telephone. Persons without contraindication to PET will also be invited to undergo amyloid and tau PET, independent of MRI.

Phase:	II /III
Description of Sites/Facilities Enrolling Participants:	There will be multiple sites in the United States chosen for their capacity to recruit, previous experience in Alzheimer's disease clinical trials, previous experience in National Institute on Aging's (NIA's) Alzheimer's Disease Cooperative Study (ADCS), and their affiliation with a local Clinical Translational Science Award (CTSA) funded by the National Center for Advancing Translational Sciences (NCATS).
Description of Study Intervention:	The trial will be preceded by a screening phase followed by randomization and a titration period in which extended-release metformin tablets or matching placebo (Glucophage XR, 500 mg/tablet [reduced mass]) will be titrated from 500 mg a day to 2000 mg a day in increments of 500 mg every 10 days. Enrolled study participants will undergo neuropsychological battery, clinical interviews, physical exam, and phlebotomy during four study visits at baseline, 6 months, 12-months, and 18-months. Brain MRI, amyloid PET, and tau PET will be conducted in up to 186 participants after the baseline visit and after the 18-month assessment. Adverse events and medication compliance will be checked every month. The placebo group will undergo the same study assessments and procedures, receiving placebo tablets rather than metformin.
Study Duration:	Up to 8 years
Participant Duration:	The length of follow-up is 18 months. This could be extended by four months to allow for completion of procedures between randomization and start of investigational product (IP) and after the last follow-up visit.

1.2 SCHEMA

Figure 1. The figure below represents a flow diagram of the study visits, from screening to the final visit. In addition to the visits described below, there will be monthly calls to assess safety and compliance. Visits 1 to 4 may be divided into a remote component (questionnaires only) and an in-person component (phlebotomy, physical evaluations [vital signs, anthropometric measures, brief neurologic exam], neuropsychological testing), or conducted entirely in person, following the preference of the study participant.





1.3 STUDY DURATION

Recruitment is planned to end such that data collection in the phase II study in 326 participants can be completed by approximately April 30, 2026. Analyses and decision to proceed to a phase III trial would occur within 6 months of end of the phase II study. The Figure above shows the proposed schedule of assessments per participant. After telephone screening and preliminary determination of eligibility, participants will have an in-person screening visit. If they meet criteria for amnestic mild cognitive impairment (aMCI), the baseline visit will be completed. If blood tests do not show exclusion criteria, participants will be randomized, followed by a drug titration period. Metformin/placebo, referred to as Investigational Product (IP) from here on, will be titrated every 10 days by one tablet (500 mg), up to 2000 mg a day. The neuropsychological battery, clinical interviews, physical exam, study partner interview, and phlebotomy, will be conducted at baseline and repeated every 6 months for 18 months, for a total of four visits. Brain imaging including brain MRI and amyloid and tau PET will be conducted in up to 186 participants (186). Baseline brain imaging will be conducted after the baseline visit and after the 18 months assessment. Medication compliance and adverse events will be checked every month via telephone call, text, or email, and in person during the scheduled in-person assessments every six months.

2 INTRODUCTION

2.1 STUDY RATIONALE

Our study focuses on late-onset (sporadic), not early onset (familial) Alzheimer's Dementia. We refer to Alzheimer's dementia as the clinical manifestation, and to Alzheimer's disease (AD) as the underlying pathologic process throughout the application. Alzheimer's dementia is the most common form of late onset dementia, accounting for 70% to 90% of cases in the U.S.[1] Nearly half of persons 85 years and older have Alzheimer's dementia,[2] and the prevalence worldwide will quadruple by mid-century.[3] The natural history leading to Alzheimer's dementia starts slowly in late middle age with mild memory deficits, the clinical hallmark of Alzheimer's dementia, progresses to amnestic Mild Cognitive Impairment (aMCI),[4] and

progresses to dementia with continued decline in memory.[5] Amnestic Mild Cognitive Impairment (aMCI) in considered a prevalent[6] high risk group for AD[4] and has become a target for secondary prevention for Alzheimer's dementia, as we propose in MAP. Memory impairment of slow onset and progression is the primary clinical hallmark of aMCI and Alzheimer's dementia.[7] Thus, the primary outcome of MAP will be changes in verbal memory performance. The amyloid hypothesis,[8] which posits that amyloid deposition in the brain is the key pathologic process underlying aMCI and Alzheimer's dementia, has dominated the experimentation of therapeutic approaches for prevention and treatment. However, interventions that increase the clearance of amyloid[9-11] or decrease its production[12,13] among persons with Alzheimer's dementia have thus far failed. These failures have led to clinical trials of anti-amyloid agents earlier in the natural history of Alzheimer's disease, in asymptomatic persons and persons at risk without dementia, but has also led to the questioning of amyloid as a therapeutic target.[14] An alternative approach for intervention is to target modifiable risk factors for Alzheimer's dementia.[15] Vascular risk factors (e.g. hypertension, dyslipidemia, type diabetes) have emerged as important predictors of Alzheimer's dementia risk, and among them, type 2 diabetes and hyperinsulinemia have emerged as some of the most consistent and strongest risk factors in epidemiologic studies.[16] We refer to type 2 diabetes as diabetes in the rest of this document.

2.2 BACKGROUND

2.2.1 PERIPHERAL HYPERINSULINEMIA – INCREASED RISK OF ALZHEIMER'S

Peripheral hyperinsulinemia is a plausible mechanism underlying the relation of diabetes with a higher risk of Alzheimer dementia. Diabetes is preceded and accompanied by insulin resistance causing hyperinsulinemia.[17] Insulin resistance is caused by increased adiposity (e.g., overweight and obesity) in most affected individuals, and accompanied by other important risk factors including high inflammation, hypertension, and dyslipidemia, a cluster referred to as the metabolic syndrome.[18] Hyperinsulinemia, diabetes, and the metabolic syndrome are known cerebrovascular risk factors.[19] Thus, it seems reasonable to postulate that hyperinsulinemia and diabetes could increase Alzheimer's dementia risk through cerebrovascular disease, a factor increasingly accepted to be important in the clinical manifestation of Alzheimer's disease.[20-23] However, peripheral hyperinsulinemia has also been demonstrated to result in lowering of insulin levels in the brain through decreased transport of insulin across the blood brain barrier,[24] which in turn may lower the expression of insulin degrading enzyme (IDE),[25] which is active in brain amyloid β (Aβ) clearance.[25-28] Low brain insulin signaling is increasingly accepted to be a feature of Alzheimer's disease pathology.[29,30] This plausible mechanistic pathway is supported by findings in observational,[31,32] brain imaging,[33,34] autopsy,[35] and experimental studies.[36] In addition, peripheral hyperinsulinemia is related to other factors important in Alzheimer's disease including increased inflammation,[37] oxidation,[38] and the accumulation of Advanced Glycation End Products (AGE).[39] In summary, hyperinsulinemia could increase the risk of Alzheimer's dementia through both cerebrovascular and Aβ related mechanisms,[40] and this hypothesis has prompted testing strategies related to hyperinsulinemia and diabetes in the prevention and treatment of Alzheimer's dementia.[15] These strategies usually entail improving insulin sensitivity to lower insulin and glucose levels,[41] an effective strategy for preventing diabetes that may be effective in decreasing the risk of Alzheimer's dementia.[15]

2.2.2 METFORMIN FOR ALZHEIMER'S PREVENTION

We propose to repurpose metformin, a medication with proven efficacy in decreasing hyperinsulinemia and preventing diabetes, for the prevention of Alzheimer's dementia. There are several proven diabetes

related pharmacological strategies that have been proposed for Alzheimer's dementia treatment or prevention, including intranasal insulin, thiazolidinediones (a.k.a. PPAR-γ agonists),[42] glucagon-like peptide agonists (GPA), and metformin.[43] Intranasal insulin, used with the purpose of increasing brain insulin (without effect on peripheral insulin), showed preliminary evidence of a cognitive benefit in a pilot study in persons with mild Alzheimer's dementia,[44] and is now being tested in a larger trial. The glucagon-like peptide agonists (GPA)[45] both increase peripheral insulin secretion and sensitivity, are used in diabetes treatment, and have been hypothesized to be of benefit in Alzheimer's dementia.[46] The thiazolidinediones are powerful insulin sensitizers effective in lowering insulin resistance[47] and preventing diabetes,[42] with efficacy similar to lifestyle strategies (diet and exercise that lead to weight loss) and greater than metformin.[48] However, thiazolidinediones have concerning side effects including edema, congestive heart failure (CHF), and in the case of rosiglitazone, myocardial infarction (MI) and stroke,[49] which led to a black box warning from the Food and Drug Administration (FDA).[50] Rosiglitazone was tested for secondary prevention of cognitive decline in mild Alzheimer's dementia among persons without diabetes and was found to be non-efficacious in a randomized trial in 511 subjects[51] after promising results in a pilot study.[52] However, there was evidence of benefit among non-carriers of APOE-ε4, similar to our pilot study (see 2.2.4). The thiazolidinedione pioglitazone seems to have a lower risk of MI and stroke compared with rosiglitazone, but shares class side effects such CHF.[53] A randomized placebo controlled trial of pioglitazone in 3,500 subjects at risk for aMCI (NCT01931566; Biomarker Qualification of Mild Cognitive Impairment Due to Alzheimer's Disease and Safety and Efficacy Evaluation of Pioglitazone in Delaying its Onset [TOMMORROW]) was recently stopped after an interim futility analysis.[54] It could be speculated that the adverse vascular effects of the thiazolidinediones [50] may have eclipsed the potential beneficial effects for Alzheimer's dementia related to the increase in peripheral insulin sensitivity and lowering of insulin and glucose levels.[55] Even if found efficacious, use of thiazolidinediones for Alzheimer's dementia prevention could be limited due to their adverse effects risk profile. Metformin is a medication belonging to the biguanide class.[56,57] It treats and prevents diabetes by suppression of hepatic glucose output, increasing insulin mediated glucose disposal, by increased intestinal glucose use, and by decreasing fatty acid oxidation; [58] these effects are accompanied by reduced pancreatic insulin secretion and lower insulin levels in blood in response to glucose loads. While the mechanisms for the action of metformin are not completely understood, it clearly reduces insulin levels,[41] inflammation and thrombosis,[59] and the risk of the metabolic syndrome[60] and diabetes[61] in persons at risk for diabetes. Metformin is usually the first step in pharmacological treatment of diabetes,[62] but it is increasingly used in persons without diabetes for diabetes prevention based on the findings of the Diabetes Prevention Program (DPP).[61] In the DPP, metformin was more effective than lifestyle (diet and exercise) intervention in preventing diabetes after 10 years.[63] The only common side effect of metformin in clinical trials has been gastrointestinal intolerance, occurring in 10% of subjects. In the DPP, the rate of serious adverse events for metformin was the same as for placebo. Metformin seems to be the most realistic and safe long-term strategy to reduce insulin levels and prevent diabetes when compared to lifestyle intervention[63] and thiazolidinediones,[49] because of its effectiveness and low risk of adverse events. Cerebrospinal fluid levels of metformin are approximately 10% of the plasma levels,[64] indicating some crossing of the blood brain barrier, but we postulate that it acts on the Alzheimer's disease process through reduction of peripheral insulin levels that affect brain clearance of Aβ. [25,65,66]In addition, Metformin also decreases AGE,[67,68] inflammation,[59] coagulation,[59] and prevents the metabolic syndrome (diabetes, hypertension, obesity, dyslipidemia),[60] factors that may also influence Alzheimer's dementia risk through cerebrovascular or neurodegenerative mechanisms.[55] The beneficial pleomorphic, metabolic, anti-inflammatory, and anti-thrombotic effects of metformin have led to the hypothesis that metformin is a wonder drug that may be effective in cancer prevention and treatment[69] as well as for the prevention of the adverse effects of aging.[70] Thus, we propose metformin as the ideal diabetes drug to repurpose for prevention of Alzheimer's dementia in persons at risk. This premise is further supported by our preliminary data. The most common

side effect of metformin in gastrointestinal intolerance (10%), which is significantly decreased with the extended-release form that we propose in MAP.[71] The most serious side effect, lactic acidosis, is very rare (< 0.01%),[72] and avoided by precluding its use in person with contraindications, as we will do in MAP. More recently, there is recognition that metformin might cause malabsorption of vitamin B12 (cobalamin),[73] which could impact cognition.[74] However, this side effect is very rare, and we will monitor B12 levels at all study visits in MAP.

2.2.3 OVERALL SUPPORTING EVIDENCE

There are conflicting data relating metformin with Alzheimer's dementia risk, but the best evidence favors the benefits of metformin on Alzheimer's dementia risk. Several laboratory and human studies have suggested that metformin increases the risk of Alzheimer's dementia, but this is countered by other studies indicating that it is beneficial. One study in a neuronal cell culture model reported that metformin increases the biogenesis of amyloid peptides via up-regulation of BACE1 Transcription.[75] Another study in a mouse model of Alzheimer's disease reported that metformin facilitates amyloid beta generation by beta and gamma secretases via autophagy activation.[76] These seemingly deleterious effects of metformin on Alzheimer's disease risk are countered by studies that have found that metformin activation of AMPK-dependent pathways is neuroprotective in human neural stem cells against amyloid beta induced mitochondrial dysfunction,[77] and that metformin attenuates cognitive impairments in hypoxia-ischemia neonatal rats by improving remyelination.[78] A recent study found that metformin prevented amyloid accumulation and memory impairment in Alzheimer's (APP/PS1) mice.[79] Studies in humans are conflictive as well. One case control study in an administrative dataset concluded that metformin was associated with an increased risk of dementia, but this association was not apparent in crude analyses, appeared after adjustment for demographics, and was seen for 10-29 metformin prescriptions and 60 and more metformin prescriptions (compared to no-prescriptions), but was null for 1-9 or 30 to 59 prescriptions.[80] In another cross-sectional study sampled by cognitive status (normal, mild cognitive impairment, dementia) metformin was reported to be associated with a higher risk of cognitive impairment.[81] The designs of these two studies were possibly subject to selection bias and confounding by indication, that is, that persons who had dementia and diabetes were more likely to be taking metformin; this is not surprising because elderly subjects with diabetes and dementia are switched from oral agents that can cause hypoglycemia (sulfonylureas) to those that do not cause hypoglycemia (e.g., metformin), in order to prevent this dangerous complication. Other studies with better designs have shown that metformin is not associated with a higher risk of cognitive impairment, and in fact, may be associated with a lower risk. In the Diabetes Prevention Program Outcomes Study (DPPOS), among persons with diabetes or pre-diabetes, longitudinal cumulative exposure to metformin was not associated with cognitive performance, and metformin showed a non-significant trend towards a benefit in memory performance in a second wave of cognitive testing.[82] The results of the third wave of cognitive testing in DPPOS are pending. The best epidemiologic study to date was carried out in a cohort of over 28,000 United States' veterans 65 years and older,[83] which reported that that the risk of dementia was significantly lower among those with diabetes taking metformin (which lowers insulin levels) compared with those taking sulfonylurea (which increases insulin levels). Compared to other studies this was a cohort study that used propensity scores to account for confounding by indication that is common in pharmacoepidemiologic studies.[84,85] A recent pilot placebo-controlled randomized cross-over study in 22 persons with MCI or mild Alzheimer's dementia[64] showed that metformin was associated with improved executive functioning, and trends suggesting improvement in learning, memory, and attention. Our preliminary data in humans (see B.1.5.1) and animals (see B.1.5.2) support the beneficial effects of metformin on Alzheimer's dementia risk.

2.2.4 BIOMARKERS OF ALZHEIMER'S DISEASE

Increased understanding of AD neuropathology and its natural history has enabled the development of brain imaging and cerebrospinal fluid AD biomarkers for the diagnosis and detection of dementia that can be used in clinical trials. Decades of advances in AD research, particularly in cerebrospinal fluid (CSF) and brain imaging biomarkers,[86,87] have led to the dominance of 3 neuropathological constructs: brain amyloid, brain tau, and neurodegeneration. Current understanding of the natural history leading to dementia due to AD can be summarized as follows:[86] the 2 main proteinopathies underlying AD, amyloid and tau, are separate processes, but amyloid deposition accelerates tau deposition; amyloid and tau deposition precede and cause neurodegeneration, which leads to the clinical syndromes of aMCI and dementia. The measurement of amyloid, tau, and neurodegeneration features prominently in the NIA/Alzheimer's Association (AA) 2018 research framework.[88] This framework proposes to conduct research in which individuals are classified by the presence or absence of evidence of amyloid, tau, and neurodegeneration (A/T/N), with or without clinical manifestations, for the purpose of better understanding the mechanisms and sequence of neuropathology. The 2018 research framework has been enabled by the widespread availability of accurate CSF and brain imaging markers of amyloid, tau, and neurodegeneration. As compared with cognitively normal individuals, persons with AD dementia show lower CSF Aβ42[89] and higher brain amyloid burden on amyloid PET,[90] higher CSF T-tau and P-tau[89] and higher brain tau burden on tau PET,[91] and lower cortical thickness and brain volumes on MRI.[92] More recently, neurofilament light (NFL) in CSF has also been reported to define neurodegeneration.[89] We do not propose to do lumbar puncture in MAP because, in our experience, it is perceived as invasive by study participants, and it risks recruitment and retention. We propose to conduct amyloid and tau PET in MAP participants undergoing MRI because we have good experience in terms of participant acceptability. PET also has the added advantage of providing information on brain regional distribution of amyloid and tau.

2.2.5 PLASMA BIOMARKERS OF ALZHEIMER'S DISEASE

Blood-based biomarkers of AD have lagged behind brain imaging and CSF biomarkers, but recent developments are enabling the use of blood-based biomarkers in AD research. The blood-brain barrier is altered in aging and AD.[93] The increased permeability between the brain and the periphery makes it possible for blood-based biomarkers to be representative of pre-clinical changes in AD.[94] Extant proteomic methods to measure blood-based biomarkers for AD include mass spectrometry, immunocapture, and aptamer-based techniques. However, issues around lower limit of detection, depletion of lower molecular weight proteins, and antibody availability have limited the use of these methods in particular.[95] More recently, ultrasensitive immunoassays coupled with mass spectrometry show greater promise.[96] The commercially available single-molecule array (Simoa™) is a novel method to measure Aβ40, Aβ42, tau and neurofilament light (NFL) in plasma,[97] which we propose to use. Below we provide a brief review of the literature for each plasma biomarker.

Plasma Aβ. Using Simoa™ technology in 248 participants with subjective cognitive decline from the SCIENCe project and Amsterdam Dementia Cohort, plasma Aβ42/Aβ40 ratio and plasma Aβ42, but not plasma total tau, identified abnormal CSF-amyloid status suggesting that plasma Aβ42/Aβ40 has the potential to be used as a screening measure to identify AD related neuropathological changes in cognitively normal individuals with subjective cognitive decline.[98] Our consultant to this project, Henrik Zetterberg, is an active collaborator in the Swedish BioFINDER study. In this cohort study, plasma Aβ42 and Aβ40 ascertained with Elecsys immunoassays (Roche Diagnostics) predicted Aβ status, as defined from cerebrospinal fluid Aβ42/Aβ40 ratio, with an area under the receiver operating curve (AUC)=0.80. This was found to be independent of age, ApoE, or cognitive status.[99] Plasma Aβ42/Aβ40, measured with Simoa™, was also predictive of cerebral amyloidosis in a sample of 276 cognitively intact individuals with

subjective memory complaints from the INSIGHT-preAD study, a French academic university-based cohort that is part of the Alzheimer Precision Medicine Initiative Cohort Program.[100]

Plasma tau. Plasma tau ascertained using the Simoa™ assay has been weakly or not correlated with CSF tau levels in both the BioFINDER study and Mayo Clinic Study of Aging.[101] However, in a sample of patients with AD, MCI and cognitively healthy controls from the ADNI, higher levels of Simoa™-based plasma tau were observed in AD dementia compared to both aMCI patients and cognitively healthy controls.[102] More recent data from the Framingham Heart Study examined the use of plasma total tau, measured with Simoa™, as a blood biomarker for dementia and related endophenotypes. In a sample of 1,453 participants, a 1 standard deviation (SD) increase in the log of plasma total tau level was associated with a 35% increase in AD dementia risk. Higher plasma total tau was also associated with poorer cognition, and smaller hippocampi and more neurofibrillary tangles and microinfarcts at autopsy.[103] An additional study also reported a significant correlation of plasma phosphorylated tau and total tau with brain tau deposition by PET imaging.[104]

Plasma NFL. Simoa™ based plasma NFL correlations with CSF levels are high[105] and additional reports from ADNI suggest that plasma NFL had high diagnostic accuracy for identifying patients with dementia vs. controls (AUC=0.87).[106] A recent study of 1,583 participants from ADNI reported the association between clinical diagnoses, CSF biomarkers, imaging measures and cognition with longitudinal Simoa™-based plasma NFL levels.[107] Levels of plasma NFL increased over 11 years in patients with MCI and AD dementia. The authors also reported that a longitudinal increase in plasma NFL correlated with CSF biomarkers (e.g., lower Aβ42, high total tau, high phosphorylated tau, and higher levels of neurodegeneration detected from MRI) and poorer cognitive performance. Our preliminary data in 34 participants shows that persons with dementia have appreciably higher levels of plasma NFL and tau compared with persons with normal cognition, with less appreciable differences for Aβ42/Aβ40 ratio. The fact that our pilot data agree with some of the recent literature for NFL and tau supports our proposal.

The field of AD plasma biomarkers is rapidly advancing,[108] and the biomarkers mentioned above may become obsolete or be replaced by better ones by the time that these biomarkers are measured, after the end of data collection. For example, p-tau 217 has emerged as the best plasma biomarker of amyloid burden. [109] Neuroinflammation can now be assessed in plasma with glial fibrillary acidic protein (GFAP).[110] Plasma measures of synaptic integrity and other neuropathologies are being developed [111] that could be validated by the time we measure plasma biomarkers. Given these ongoing advances in plasma AD biomarkers, we refer broadly to AD plasma biomarkers in this protocol.

2.2.6 COVID-19 AND COGNITION

Reports of the impact of COVID-19 on cognition and long-term cognitive sequelae are increasing, [112,113] but the cognitive impact of COVID-19 on cognition is not yet understood. Given that we are recruiting a sample that is at risk of both cognitive impairment and COVID, we added a questionnaire to explore COVID-19 history as a covariate and potential modifier of the effectiveness of metformin.

2.2.7 PRELIMINARY DATA

2.2.7.1. MAP is based on the results of the Phase II trial of metformin in aMCI (MetMCI). MetMCI (NCT00620191) was funded by the National Institute on Aging (NIA; AG026413; PI; Luchsinger; 05/2008-04/2013) and the Alzheimer's disease Drug Discovery Foundation (ADDF; # 270901; PI: Luchsinger; 12/2007-11/2011). MetMCI was a single-site, double-blind placebo-controlled 1:1 randomized pilot trial of short acting metformin 1000 mg (two 500 mg tablets) twice a day for 12 months in subjects with late

aMCI, defined by the Petersen criteria.[114] Randomization was stratified by the presence or absence of APOE-ε4 genotype, based on previous studies demonstrating a higher risk of dementia among persons with hyperinsulinemia who are heterozygous or homozygous for APOE-ε4,[31,32] and the results of a clinical trial of rosiglitazone showing cognitive benefits among persons without an APOE-ε4 allele.[51] Participants were seen once a week in the first 4 weeks of the study during metformin titration and were then evaluated at months 3, 6, 9, and 12. Half the sample (40 participants) were invited to participate in a brain imaging sub-study. Participants were 80 subjects aged 55 to 90 years with aMCI without treated diabetes, and with a body mass index (BMI) of 25 kg/m^2 or higher (overweight or obese by National Heart, Lung, and Blood Institute (NHLBI)) criteria[115]). Screening was conducted following a 2-step process. First, participants who were interested in participating were screened by telephone for demographic and medical inclusion and exclusion criteria. The Telephone Interview for Cognitive Status (TICS)[116] was administered to screen out persons who were unlikely to have any memory impairment. A TICS Score > 34 out of 41 was considered normal cognition. Persons with this score were not invited to participate. Persons who passed the telephone screen were invited for an in-person screening that included a physical exam, blood tests, and a neuropsychological battery. Participants were randomized to metformin or identical matching placebo, both provided by Merck-Lipha of France. The maximum dose of metformin was 1000 mg twice a day, as is commonly used in clinical practice. Metformin was as 500 mg tablets. Metformin was titrated weekly from 500 mg once a day to 1000 mg twice a day over 4 weeks. Subjects were maintained on the highest tolerated dose. Participants who did not tolerate the study drug were invited to continue in the study and were included in the ITT analyses. Metformin and placebo were supplied every 3 months, when participants were asked for side effects and contraindications to metformin in addition to undergoing safety laboratory tests. The primary outcomes of the study were changes from baseline to month 12 in total recall of the Buschke SRT[117] and the Alzheimer's Disease Assessment Scale-cognitive subscale (ADAS-cog).[118] The primary imaging outcome was changes from baseline to month 12 in relative glucose uptake (rCMRgl) in the posterior cingulate-precuneus measured by non-quantitative brain [^{18}] F-labeled 2-deoxy-2-fluoro-D-glucose (FDG) PET with magnetic resonance imaging (MRI) co-registration. We conducted a washout period of 2 weeks[119] at the end of the trial before performing FDG PET to ensure that changes in rCMRgl were not due to acute changes in glucose due to metformin. Analysis of Covariance (ANCOVA) was used to compare outcomes between the treatment groups following an ITT approach adjusting for variables that were different at baseline if necessary. Imputation with last-observation-carried-forward (LOCF) was used to account for missing follow-up data.

The most successful outreach strategy was newspaper ads, which was the source of 84% of all contacts. Sixty-five subjects (81%) completed 12 months, 6 subjects (7.5 %) completed 9 months, and 9 subjects (11.2%) had less than 9 months of follow-up. At 12 months, study completion was similar in the 2 arms (33 persons completed the placebo arm and 32 the metformin arm; p = 0.99). Based on this experience, we are proposing for MAP an in-person screen to recruitment ratio of 3, since MAP will include both early and late MCI. We also propose to use ads again as an effective strategy for recruitment.

Table 1. Comparison of baseline variables in the MetMCI study.	**Metformin (n=40)**	**Placebo (n=40)**	**p**
Age in years (SD)	65.3 (7.0)	64.1 (7.9)	0.49
Women (%)	18 (45)	24 (60)	0.21
Education in years (SD)	13.8 (3.4)	13.1 (4.5)	0.44
Ethnic group (%)			0.46
Hispanic	17 (42.5)	13 (32.5)	
Non-Hispanic Black	11 (27.5)	15 (37.5)	
Non-Hispanic White	12 (30.0)	12 (30.0)	
Apolipoprotein E ε4 (%)	10 (25.0)	11 (27.5)	0.79
Body Mass Index in kg/m^2 **(SD)**	30.9 (4.1)	31.3 (4.7)	0.65

Table 1. Comparison of baseline variables in the MetMCI study.	Metformin (n=40)	Placebo (n=40)	p
Systolic Blood Pressure in mmHg (SD)	130.8 (10.9)	132.1 (12.4)	0.62
Total Cholesterol in mg/dl (SD)	204.2 (43.6)	208.2 (46.7)	0.71
High Density lipoprotein in mg/dl (SD)	51.5 (14.1)	58.3 (17.7)	0.06
Hemoglobin A1C in % (SD)	6.1 (0.8)	6.1 (0.5)	0.92
Hemoglobin A1C > 6.5% (%)	7 (17.5)	6 (15.0)	0.76
High Sensitivity C-reactive protein in mg/dl (SD)	2.9 (3.6)	3.7 (2.9)	0.32
Insulin in IU/dl (SD)	16.3 (9.5)	13.4 (7.6)	0.20
ADAS-Cog Score (SD)	12.0 (4.0)	14.6 (6.1)	**0.02**
Selective Reminding Test Total Recall (SD)	34.2 (7.9)	36.1 (9.5)	0.32

Results: The only statistically significant difference between the groups at baseline (Table 1) was the ADAS-Cog score, which was lower (better) in the metformin group. Compliance with metformin was as follows: 7.5% stopped metformin but continued in the study following ITT, 15% remained on 500 mg a day (1 tablet), 35% remained on 1000 mg a day (2 tablets), 32.5% remained on 1500 mg a day (3 tablets), and 10% tolerated the maximum dose of 2,000 mg a day (4 tablets). There were no serious adverse events related to metformin and the 7.5% of persons who were not able to tolerate metformin reported gastrointestinal symptoms. Fasting insulin increased appreciably more in the placebo group compared with the metformin group as expected, and this difference was close to statistical significance (13.8 vs. 4.7 IU/mL; p = 0.09). High sensitivity C-reactive protein (hs-CRP), a measure of inflammation and vascular risk,[120] and a correlate of memory impairment in our center[121] decreased in the metformin group and increased in the placebo group (-0.3 vs. 1.0 mg/dL; p = 0.07). Weight decreased more in the metformin group (-2.7 ± 6.4 Kg) compared with the placebo group (-1.6 ± 4.5 Kg) but this difference was not statistically significant (p = 0.63). Table 2 below shows the comparison in the primary clinical outcomes of the study, changes from baseline to month 12 in the ADAS-Cog score, and the total recall of the Selective Reminding Test (SRT).

Table 2. Comparison of changes from baseline to 12 months in the ADAS-cog and total recall of the SRT between metformin and placebo. Crude (unadjusted) analyses are from T-tests. Adjusted analyses are from Analyses of Covariance adjusted for was the baseline score of the ADAS-cog.

	Metformin	Placebo	p
ADAS-Cog			
Baseline	12.0 ± 4.0	14.6 ± 6.1	**0.02**
Last visit	12.1 ± 3.8	12.8 ± 6.2	0.52
Crude difference	0.0 ± 3.3	-1.98 ± 5.5	0.06
Adjusted difference	-0.5 ± 4.1	-1.4 ± 4.1	0.34
Total recall SRT			
Baseline	34.2 ± 7.9	36.1 ± 9.5	0.32
Last visit	43.6 ± 9.1	41.5 ± 8.4	0.31
Crude difference	9.4 ± 8.5	5.7 ± 8.7	**0.05**
Adjusted difference	9.5 ± 6.1	5.4 ± 6.1	**0.05**

Both the SRT and ADAS-Cog scores improved in the placebo and metformin groups (increases in SRT total recall, decreases in ADAS-COG scores). Crude analyses showed a greater improvement in the SRT in the metformin group, but the difference for the ADAS-Cog favored the placebo group. However, after adjustment for baseline ADAS-Cog the metformin group showed significantly greater improvement in SRT total recall compared to placebo (difference in changes in total recall of the SRT of metformin vs. placebo = 4.4 ± 8.5 words) and the difference for the ADAS-Cog was attenuated and not significant. The results were similar for delayed recall of the SRT, in which the gain in words was higher in the metformin group (2.3 ± 2.5) compared to the placebo group (1.3 ± 2.3) and was close to statistical significance (p=0.06). There were no differences in delayed recall of the ADAS-cog (0.7 ± 1.8 for metformin vs. 0.0 ± 2.5 for placebo; p = 0.35).

There were no differences between metformin and placebo in changes in digit span backwards, the neuropsychiatric inventory, the mini-mental status exam (MMSE), paragraph recall, or ADCS Clinical Global Impression of Change for Mild Cognitive Impairment (CGIC-MCI).[122] One person in the placebo group and none in the metformin group converted to dementia. We conducted linear regression models examining the relation of the metformin dose with the primary outcomes, changes in total recall of the SRT and the total score of the ADAS-cog. The highest metformin dose (1000 mg twice a day) was associated with a statistically significant increase of 5.3 ± 10.0 more words in total recall of the SRT (p= 0.03) compared to those in the placebo group and those who could not tolerate metformin. There was no association between the highest metformin dose and changes in the ADAS-Cog (0.7 ± 4.8; p = 0.56). Based on these findings, we are proposing to change the metformin formulation to the better tolerated extended-release form (Glucophage XR® [reduced mass]), and to exclude participants who cannot tolerate at least 1000 mg of metformin a day, an approach adopted by the clinical trials of diabetes using metformin.[123]

Post-hoc subgroup analyses. We conducted post-hoc subgroups analyses: metformin showed better performance compared with placebo in younger persons, those without APOE-ε4, those with lower HbA1c, and those with higher insulin levels. There were no differences in sex strata. There were no differences for the ADAS-Cog in any of the strata. Based on these findings, and previous evidence of effect modification by APOE-ε4,[51] we will pay particular attention to APOE-ε4 in our decision to proceed to phase III. The results for younger persons and those with higher insulin levels are not surprising because metformin is known to be more effective in younger persons and in those with higher insulin resistance. The results for HbA1c may suggest that metformin is most effective while persons are insulin resistant with hyperinsulinemia, before pancreatic failure and increases in glucose. These subgroups findings should be interpreted with caution given the small strata, and we will explore these modifiers in exploratory analyses in MAP.

Table 3. Subgroup analyses

	Metformin		Placebo		
Total Recall Selective Reminding Test					
	N	Mean + SD	N	Mean + SD	P
Age group					
≤ 63.7 years	18	12.2 ± 7.5	22	6.3 ± 7.5	**0.02**
> 63.7 years	22	6.9 ± 9.8	18	5.4 ± 6.7	0.67
APOE-ε4					
Negative	30	10.7 ± 8.2	29	5.9 ± 8.6	**0.04**
Positive	10	5.7 ± 9.1	11	5.2 ± 9.2	0.89
Body Mass Index					
< 30 kg/m^2	21	9.0 ± 10.0	19	3.9 ± 10.0	0.13
≥ 30 kg/m^2	19	10.3 ± 6.9	21	6.9 ± 6.8	0.14
HbA1c					
≤ 6.0 %	23	12.2 ± 7.1	20	6.6 ± 7.1	**0.01**
> 6.0 %	17	6.3 ± 9.0	20	4.0 ± 9.8	0.49
Insulin					
≤ 9 IU/dl	21	6.7 ± 8.7	19	4.4 ± 8.7	0.42
> 9 IU/dl	19	12.4 ± 7.8	21	6.8 ± 8.0	**0.03**
ADAS-Cog					
Age group					
≤ 63.7 years	18	- 0.2 ± 3.5	22	-1.2 ± 3.5	0.38
> 63.7 years	22	-0.5 ± 5.2	18	- 1.8 ± 6.3	0.50
APOE-ε4					
Negative	30	0.1 ± 3.8	29	-1.2 ± 4.1	0.24
Positive	10	-2.3 ± 4.4	11	-2.2 ± 4.4	0.97
BMI					
< 30 kg/m^2	21	-0.8 ± 6.1	19	-2.2 ± 6.5	0.56
≥ 30 kg/m^2	19	-0.2 ± 3.7	21	-0.7 ± 3.6	0.72
HbA1c > 6.0%					
≤ 6.0 %	23	0.3 ± 3.9	20	-0.1 ± 3.9	0.66
> 6.0 %	17	-1.8 ± 4.4	20	-2.8 ± 4.3	0.51
Insulin					
≤ 9 IU/dl	21	-0.51 ± 4.5	19	-0.4 ± 4.4	0.93
> 9 IU/dl	19	-0.5 ± 3.9	21	-2.4 ± 3.9	0.15

Brain imaging outcomes: Follow-up MRI and PET were completed in 33 out of 40 participants (15 in the metformin group, 18 in the placebo group, 82.5% completion overall). Changes from baseline to 12-month in the posterior cingulate-precuneus rCMRgl, adjusted for cerebellar CMRgl uptake, showed a difference favoring metformin that was not statistically significant (2.0 ± 6.3% vs. 0.0 ± 6.0%; p = 0.36). Secondary regions of interest (ROI) including hippocampus (2.4 ± 4.7% vs. 1.0 ± 5.1%; p = 0.73), para-hippocampus (3.3 ± 5.5% vs. 2.0 ± 5.1%; p = 0.76), and entorhinal cortex (5.3± 9.0% vs. 1.3 ± 6.0; p = 0.16) favored metformin but were not statistically significant. Plasma Aβ-42 increased in the metformin group (0.69 ± 18.5 pg/mL) and decreased in the placebo group (-4.40 ± 23.51 pg/mL; p = 0.3). Although this difference was not statistically significant, it supported the beneficial findings for metformin (lowering of Plasma Aβ-42 is related to higher AD risk[124]). Post hoc MRI analyses. MRI parameters were not pre-specified outcomes in MetMCI. MRI was only used for FDG PET co-registration, and ROI were manually drawn. In collaboration with Adam Brickman, PhD, we processed the MRI data using FreeSurfer and obtained data brain volumes, with particular attention to hippocampal and entorhinal cortex volumes and thickness because they have been recommended for the longitudinal assessment of neurodegeneration and Alzheimer's disease severity.[125] Total (left + right) Hippocampal volume decreased less in the metformin group (-47.16 mm^3) as compared with the placebo group (-140.5 mm^3) but this difference was not statistically significant (p = 0.11). These appreciable differences remained after adjusting for age, sex, and

APOE-ε4. There were no differences for entorhinal cortical thickness (p = 0.51). Given these findings, we selected hippocampal volume as our outcome measure of neurodegeneration.

Post-hoc FDG PET analyses. We conducted post-hoc voxel-based analyses instead of the original ROI based analyses seeking additional evidence that supported metformin for AD prevention. rT1 MRIs were anatomically segmented with FreeSurfer and were linearly registered (MATLAB 2017a, SPM12) to the summed PET data in native PET space. The PET data was intensity normalized to the global mean and then each voxel was divided by the average in superior cerebellum (FreeSurfer defined mask) to create parametric standard uptake value ratio (SUVR) images.[126] The T1 MRI was spatially normalized (MAT2017a, SPM12) to the T1 template in MNI space, and the non-linear transformation matrix was applied to the SUVR image. SUVR images were spatially smoothed with an isotropic 8mm Gaussian kernel for voxel-wise analysis A voxel-wise 2x2 ANOVA was used to test for differences in FDG SUVR by treatment and by time point (MATLAB 2017a, SPM12). Statistical significance was defined at p<0.001, uncorrected for multiple comparisons, with no cluster size threshold. Descriptively, the metformin group had lower FDG SUVR compared to the placebo group at baseline (main effect of treatment), the baseline scan had higher FDG SUVR compared to the follow-up scan (main effect of time point), and the decrease in FDG SUVR from baseline to follow-up was greater in the placebo group compared to the metformin group (interaction between treatment and time point; Figure 2 below). Interestingly, the metformin group did not show decreases in posterior brain, but the placebo group did, suggesting that metformin could reduce FDG hypometabolism. In aggregate, these results for FDG-PET support the beneficial results of metformin found for the clinical outcomes.

2.2.7.2. Metformin improves cognition and memory deficits found in diabetic (db/db) mice. The PI has been conducting experiments examining cognition in diabetic mice and the effect of metformin on cognition, as part of grant RF1AG051556 (PI: Brickman, Luchsinger, Moreno), in collaboration with Herman Moreno at SUNY Downstate. We assessed cognitive and memory deficits using Active Place Avoidance (APA)[127] in db/db mice who were 10 months old, (n=18, 50% male) and 16 Heterozygous (db/wild type) mice (50% male) as controls. There was a significant difference between the groups in both APA (RM ANOVA F = 7, 21, p = 0.011) and APA conflict (RM ANOVA F = 5, 21 p = 0.021), indicating cognitive deficits in diabetic mice compared with controls. GLM repeated measures analysis demonstrated a significant sex effect in APA but not in APA conflict, with more abnormalities observed in female mice. The deficit in APA of old mice suggest that old female diabetic mice have deficits in hippocampal dependent spatial learning. The deficit in males and females observed in APA conflict suggests that db/db mice have deficits in set-shifting, a deficit that frequently occurs in Alzheimer's dementia.[128]

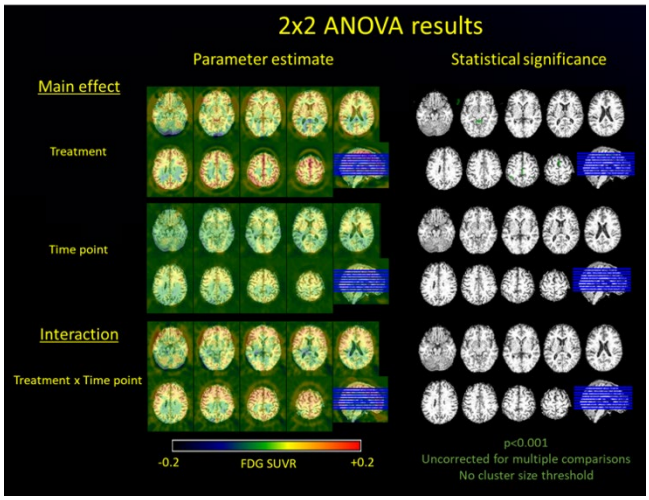


Figure 2. The parameter estimates and statistically significant clusters from the 2x2 ANOVA. Parameter estimates are all shown in positive direction (e.g., Placebo minus Metformin, Baseline minus Follow-up, Difference in Baseline and Follow-up for Placebo minus that for Metformin)

Comparison of 10 months old db/db mice treated with 0.1% metformin diet for 2 months with 10 months old heterozygous mice demonstrated that the differences seen before in APA or APA conflict were not observed, indicating that metformin reversed the cognitive deficits observed in db/db mice compared with the control mice (RM ANOVA F = 1,2; p = 0.21; F = 2, 1; p = 0.11 respectively). A significant difference was observed in db/db mice treated with metformin vs. control db/db without treatment in both APA and APA conflict (F = 5, 21; p = 0.021; F = 4.11; p = 0.02). These data strongly suggest that metformin provided chronically improves the cognitive deficits observed in old diabetic mice and lends further justification for the conduct of MAP.

2.2.7.3. MAP was prepared with a comprehensive consultation with the Trials Innovation Center (TIC) at Johns Hopkins University, one of the three hubs of the NCATS trial innovation network (TIN). In December of 2016 we applied for a consultation with the TIC at JHU-Tufts proposing to conduct a phase III trial of metformin in aMCI. The changes in design from the MetMCI study to the current proposal prompted the recommendation to conduct a phase II/III study instead of a phase III study.[129] The observation that a minority of participants achieved the maximum dose in the pilot study led us to propose the use of the better tolerated extended release metformin. The observation that there were significant practice effects for both outcomes led us to do repeated testing in the titration period in order to establish a cognitive baseline at the end of titration. The increasing acceptance of early MCI as part of the continuum of prodromal AD[130,131] led us to extend the study sample to include both early and late MCI. Our finding that the SRT was the outcome that showed a signal led us to choose a related outcome that is more sensitive to early change, the FC-SRT. Given these changes in the design we propose a larger phase II study with an a-priori rule to advance to phase III, instead of directly proposing a phase III study. The advantage of this design is that a phase II/III trial is an adaptive approach that decreases the time, number of participants, and cost needed to make a decision about the efficacy of a treatment.[129] Although there will participant enrollment will be suspended while the data of the phase II portion is analyzed to make a decision to move to phase III, the design and outcomes of the phase III study will be similar to phase II, allowing inclusion of the phase II participants as part of the analytic sample for the final phase III study.[129] As part of the TIC consultation, we shared our MetMCI dataset with the statistical team at JHU-Tufts, who replicated our findings and analyzed all data points (the primary MetMCI analysis only compared changes between baseline and 12 months between study arms, using LOCF for missing 12 month data). The differences in all time points for the SRT can be seen in Table 4 (below). The metformin arm had lower total recall at baseline compared with placebo but went on to have better performance at months 3, 6, and 12 compared with placebo, and similar performance at month 9. Both study arms showed improvements in recall, but the improvement was strongest in the metformin group. In order to determine the sample size of for the phase II/III study at the time of the original grant application, the TIC team used the MetMCI data to estimate the initial sample size for MAP (n=370, 185 per arm). The approach to the sample size calculation is detailed in section 4.4 (statistical design and power). The

consultation with the TIC also included a consultation for recruitment and retention strategies with the NCATS Recruitment Innovation Center (RIC) at Vanderbilt University. The RIC assisted the PI in choosing sites according to their capacity to recruit, their previous experience in Alzheimer's disease clinical trials, and their affiliation with a local Clinical Translational Science Award funded by NCATS.

	Baseline	Month 3	Month 6	Month 9	Month 12	P-value**
Total Recall of the Bushcke Selective Reminding Test (SRT) – Mean ± SE						
Metformin	34.6 ± 1.23	40.6 ± 1.57	42.6 ± 1.42	42.0 ± 1.53	44.0 ± 1.59	0.0002
Placebo	36.2 ± 1.50	39.1 ± 1.56	40.0 ± 1.76	42.2 ± 1.68	41.9 ± 1.33	0.0059
P-value*	0.613	0.531	0.213	0.864	0.248	

Table 4. Comparison of the SRT at all time points between metformin and placebo in MetMCI

2.2.7.3.1. Design changes in Protocol version 1.9 based on MetMCI data. The assumptions used for the original study design were reconsidered for protocol version 1.9 based on the MetMCI data, resulting in the following changes: (1) The sample size was reduced to 326 from 370; (2) the follow-up time was reduced to 18 months from 24 months. The rationale for the reduction in sample size is found in section 4.4. The rationale for reducing the study follow-up is that the great majority of recent and ongoing clinical trials of MCI and early AD have a follow-up period of 18 months. In addition, the effect in MetMCI was observed at 12 months, thus requiring only an additional 6 months to observe if the effect persists.

2.2.7.4. Middle aged persons diabetes treated with metformin have lower amyloid SUVR than persons with pre-diabetes. We compared amyloid levels (positive, intermediate, low) between persons taking and not taking metformin in a cohort of 266 persons aged 64 years of age from an ongoing study of AD biomarkers in Northern Manhattan. Brain Aβ was measured with 18F-florbetaben PET. We examined the association of metformin use with Aβ levels using ordinal logistic regression. Persons who reported metformin use had lower levels of Aβ (Odds ratio [OR] = 0.48; 95 confidence interval [CI] = 0.24, 0.97) after adjustment for age, sex, and APOE-ε4, and diabetes status. This association remained evident after excluding persons with normal glucose tolerance (NGT; OR = 0.35; 95 % CI = 0.16, 0.75), and excluding both persons with pre-diabetes and NGT (OR = 0.44; 95% CI = 0.16, 1.22). These results come from a cross-sectional analysis, which limits the inferences that can be made. However, they suggest that metformin use may be associated with lower brain Aβ.

2.2.7.5. We conducted a pilot study of Simoa™ plasma NFL, tau, and Aβ40, Aβ42 in a study of dementia detection among persons with cognitive concerns that shows that NFL and tau are promising plasma biomarkers. We tested plasma biomarkers in an ongoing study of dementia detection in primary care (AG057898; PI: Devanand, Luchsinger). Consultant Zetterberg advised us to use the Simoa™ platform with the company Quanterix (Lexington, MA, USA), with whom they have worked in the development of ultrasensitive AD fluid biomarkers.[96] Thus, we conducted a pilot study of these plasma biomarkers in a subsample (n=34) of participants, using frozen plasma. The rationale of the sample size of 34 was that this is the number of duplicate samples that can be tested in a single Simoa™ multiplex plate. A multiplex immunoassay kit was used for the quantification of total tau, Aβ40, and Aβ42 in plasma. Plasma NFL was measured with a single immunoassay. Table 5 below shows the mean (SD) of the plasma AD biomarker levels by cognitive diagnosis: normal (n=9), amnestic MCI single domain (n=4), amnestic MCI multiple domain (n=10), and dementia (n=11). A univariate linear regression was used to test for differences in the mean plasma biomarker levels across the cognitive diagnoses. Although the tests did not reach statistical significance ($p<0.05$), there are appreciable differences between persons with normal cognition and dementia for NFL and tau. Persons with dementia had 81% higher mean NFL and 37% higher tau as compared with persons with normal cognition. Data from ADNI also suggests higher plasma tau[102] and

plasma NFL[106] levels in participants with dementia compared to cognitively healthy controls. Thus, we propose to use these plasma biomarkers in MAP and hypothesize metformin will have stronger effects on tau and NFL than on Aβ42/Aβ40 ratio.

2.2.7.6. We have experience with imaging of amyloid, tau, neurodegeneration (ATN) brain biomarkers.

Table 5. Unadjusted means and standard deviations of plasma AD biomarker levels by cognitive diagnosis

	N	Normal (n=9)	aMCI-single domain (n=4)	aMCI-multi domain (n=10)	Dementia (n=11)	p-trend	*p*-value*
NFL, pg/mL	32	20.10 (8.18)	18.23 (17.09)	17.12 (12.45)	36.50 (32.46)	0.132	0.097
Tau, pg/mL	31	2.94 (1.26)	2.79 (0.72)	3.33 (1.47)	4.03 (2.18)	0.146	0.191
Ab40, pg/mL	31	311.16 (69.36)	289.55 (81.89)	279.18 (87.11)	351.47 (115.96)	0.421	0.392
Ab42, pg/mL	30	13.10 (4.69)	11.89 (2.50)	11.44 (4.11)	15.61 (5.14)	0.333	0.272
Ab42/Ab40 ratio	30	0.042 (0.008)	0.042 (0.006)	0.040 (0.005)	0.044 (0.005)	0.617	0.517
Data are mean (SD), *p-value for test of significant differences between dementia and normal groups							

Dr. Luchsinger has successfully implemented imaging of ATN brain biomarkers in currently funded grants (R01AG050440; RF1AG051556; R01AG055299). These studies are funding brain magnetic resonance imaging (MRI), amyloid (^{18}F-florbetaben, ^{11}C-PIB) positron emission tomography (PET) scans, and tau (^{18}F-MK-6240) PET scans in community dwelling participants with an average age of 64 years. As of 10/28/19 we had completed 477 amyloid PET scans and brain MRIs, and 304 tau PET scans. Dr. Luchsinger has investigational new drug (IND) protocols approved by the FDA for the use of ^{18}F-MK-6240 for brain tau imaging with PET (137,482) and ^{11}C-PIB for brain amyloid imaging with PET (142,117).

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 KNOWN POTENTIAL RISKS AND THEIR MITIGATION

Quality and risk management plan

To mitigate risks, an Integrated Quality & Risk Management Plan has been developed. The plan provides a summary of highest-scoring risks: delay in investigational products availability; failure to meet enrollment target; and high rate of missed assessments. In addition, the Risk Matrix provides details on risks to quality, along with mitigation strategies and risk scores.

Risks from metformin

Metformin is contraindicated in persons with an estimated glomerular filtration rate (eGFR) of less than 30 mL/min. For persons with an eGFR of 30 to 45 mL/min, metformin can be continued with caution but a reduction of the dose or discontinuation of the medication can be considered; in this range, it is also recommended that persons do not initiate metformin. Thus, participants with eGFR < 45 mL/min will not be eligible to participate. The risk of lactic acidosis is increased in persons with liver disease and class III or IV congestive heart failure. Thus, persons with liver disease other than non-alcoholic-fatty liver disease or class III or IV congestive heart failure will not be eligible to participate due to the risks of side effects. Non-alcoholic fatty liver disease is allowed given that it may benefit from metformin, unless it is at the cirrhosis stage.

Participants will be cautioned that IP be discontinued the day before any surgery or administration of contrast agents and may be resumed 48 hours after the procedure. We will monitor these contingencies on a monthly basis, and ad-lib as needed by the participant. We will also tell participants to avoid excessive intake or binge intake of alcoholic beverages. The Centers for Disease Control (CDC) define excessive alcohol intake as 15 drinks or more a week in men, and 8 drinks or more in women. The CDC defines binge

drinking as 5 or more drinks consumed on one occasion for men, and 4 or more drinks consumed in one occasion for women. The United States standard drink sizes are 12 ounces of 5% alcohol by volume (ABV) beer, 8 ounces of 7% ABV malt liquor, 5 ounces of 12% ABV wine, and 1.5 ounces of 40% ABV (80-proof) distilled spirits or liquor (e.g., gin, rum, vodka, whiskey).

Hypoglycemia should not occur because participants are not diabetic and should not be on other diabetes medications. If participants develop diabetes by HbA1c criteria (6.5% or higher) they will be referred to their physicians to decide about treatment for diabetes. This may lead to discontinuation of IP upon consultation with the study's safety officer at the DCC. However, participants will be invited to remain in the study and will be analyzed following intent to treat. We will call these participants every month to follow-up.

As mitigation, B12 levels, complete blood count, hepatic function, and kidney function will be tested every six months. Development of suspected side effects or contraindications to metformin will lead to stoppage of the medication upon consultation with the medical monitor.

We will call participants every month to ask about side effects such as the following:

- Very common (>1/10)
  - Gastrointestinal disorders such as nausea, vomiting, diarrhea, abdominal pain, and loss of appetite.
    These undesirable effects occur most frequently during initiation of therapy and resolve spontaneously in most cases. A slow increase of the dose may also improve gastrointestinal tolerability. Gastrointestinal disorders are the most common reason for metformin non-tolerance.
- Common side effects (>1/100):
  - Taste disturbance.
- Very rare side effects (<1/10,000):
  - The most severe but very rare side effect of metformin is lactic acidosis.
  - Metformin has also been reported to cause vitamin B12 (cobalamin) malabsorption and decrease in serum levels that can lead to megaloblastic anemia.
  - Liver function abnormalities
  - Skin reactions such as erythema or pruritus
  - Tachycardia

Adequacy of protection against risks

a. Informed Consent

Potential participants may contact the study coordinator/research assistant, or the latter may contact potential participants using available contact information. The usual contact will be via telephone, but could include email, or mail. At first contact by telephone the study will be briefly explained and the interest to participate will be explored. If the individual wants to participate, he/she will be screened for inclusion and exclusion criteria. If the individual is eligible, an appointment for a screening visit will be made. At that time, the consent form will be reviewed and if appropriate, signed by the participant and coordinator. A copy of the consent form will be provided to the participant for his or her records. The consent form will specifically request consent for the future use of data and stored serum and plasma for ancillary studies.

b. Protections Against Risk

Privacy/confidentiality. The EDC will automatically assign each participant with an ID number that will be used for all study documents.

Risks from neuropsychological testing

Participants will be clearly told as part of the written consent and verbally that they can opt out of the study at any time if they do not wish to carry out the testing. We will reschedule the testing as desired by participants if they wish to continue.

Risks from interviews

The interviews and cognitive testing may cause psychological distress in participants. Our staff in charge of data collection will be bilingual as needed, trained in cultural competence and have at least 3 years of experience in the administration of cognitive batteries. We have never had to respond to a contingency or complaint related to the administration of interviews or cognitive testing.

Risks from physical assessments

The measurement of vital signs and anthropometrics may cause psychological distress and physical discomfort.

Risks from phlebotomy

The total amount of blood drawn for clinical diagnostic evaluation will not exceed 60 mL. Venipuncture could result in transient discomfort, pain, bleeding, an ecchymosis (a.k.a. bruising), and in the worst-case scenario, a hematoma. Significant bleeding is highly unlikely in the absence of a bleeding or coagulation disorder. The study personnel will be instructed to maintain pressure with gauze on the venipuncture site for at least 10 minutes. In the case of persistent bleeding, the investigators will be called immediately. A hematoma is highly unlikely, but in a worst-case scenario could result in a compartment syndrome.

If participants report any discomfort or complication from phlebotomy or other physical assessment (e.g., hematoma), site PIs or study physicians will be called immediately to assess the problem and decide on further actions including referral to the emergency room.

Risks from brain imaging procedures

We will exclude from the study any participant who reports contraindications to MRI. If a participant becomes anxious and cannot complete any imaging procedures, or if a contraindication is found at the time of the imaging procedures, they will be excluded from undergoing brain MRI. Brain MRI may show abnormalities during a safety read conducted by a site radiologist within 24 hours of the scan. These findings are communicated to site investigators who take the appropriate action. Incidental MRI findings are classified in four levels following a standard protocol:

- Level 1: No medically significant findings. No referral necessary.
- Level 2: Minor findings without medical significance (e.g., white matter hyperintensities). No referral necessary.
- Level 3: Expedited, but non-urgent medical evaluation recommended within 2 weeks (e.g., apparent meningioma without signs of mass effect).
- Level 4: Acute abnormal findings requiring immediate medical attention (e.g., acute stroke or space occupying lesion with mass effect).

The estimated total radiation a participant will receive from the maximum of 2 PET scans repeated after approximately 18 months is 24.88 mSv (including the radiation of the 2 injections of florbetaben and MK-6240 and the radiation from the 2 CT scans, repeated after 18 months), similar to the radiation that the average person in the United States is exposed to in 48 months (4 years).

Potential risks from MRI. All participants willing to undergo MRI will be questioned for exclusion criteria, including claustrophobia and the presence of foreign bodies not acceptable for MRI. While there have been no reports of any harmful long-term effects caused by a 3.0 T magnet, or magnets of even higher

strength, the long-term effects of being placed in a magnet of this strength are not known. Some people may experience nervousness or discomfort due to the scanner's small space and the need to lie still during the scans. Other than for pacemakers, some types of metallic implants and medication patches (which are contraindications to MRI participation), we are not aware of any other potentially dangerous interactions with the MRI scan. Since the MRI scanner produces loud "knocking" noises; participants will be provided with earplugs for their comfort. If any discomfort is experienced and participants wish to stop the scan, they can inform the MRI technologist and the scan will be stopped immediately. In our experience, no one has had ongoing sensations from the MRI scan once the scanning has stopped.

Potential risks from PET scans. The risks from PET scans include risks from phlebotomy, similar to those described above, and from exposure to radiation. The procedures involving radiation in this research study will expose each participant to a very small amount of radiation (24.88 mSv total for the maximum of two florbetaben scans and two MK-6240 scans over approximately two years), in addition to the amount that they might receive from normal medical care. There may be an increase in the chances of developing cancer many years after this study. The additional risk from this research study is less than 0.15% (1 in 705). At this very low level, scientists are uncertain as to the actual risk from research and there may be no risk at all.

Risks to confidentiality

Health Insurance Portability and Accountability Act (HIPAA), and data safety and security: only the PI and approved study personnel will have access to individually identifiable private information.

ID assignment. In compliance with HIPAA, individual participant confidentiality is assured through the use of ID codes. These ID numbers will be automatically assigned by the EDC (managed by the DCC) and will not contain protected health information (PHI) (e.g., social security number, medical records number, etc.). Case report forms will be identified only by ID numbers. Data processing and analyses will not permit identification of any individual. Linkage between individual and ID number will only be accessible by site personnel and the monitoring personnel.

Data transfer. It is assumed that all PHI will be collected after informed consent; as a result, certain PHI (e.g., date of birth) that are necessary for analyses may be entered as part of the data set.

Monitoring risks

Routine monitoring of study data will take place to ensure timely entry of study data, data quality and data integrity. The monitoring team will compile and track the following quality metrics:

- **Major protocol violations** – number of major protocol violations per participant at a site and the total count of violation divided by the number of participants in the study.
- **Major audit findings** – number of major audit findings per site and across the sites in the study.
- **Time to query resolution** – the average number of days between query issuance and resolution on a per-site and overall study basis.
- **Visit entry time** – the average time within a site for entry of visit data, calculated as elapsed days between the Date of Screening/Date of Visit and the first date that the status of that page was set to IN REVIEW.
- **Actual vs**. **planned spending** – the ratio of actual costs (both quarterly and annual) as a percentage of the planned budget for that year.
- **Enrollment rate** – the rate of enrollment by site and for the entire study, annualized and normalized by the duration of a site's participation to allow cross-site comparisons. Specifically, RND/Site-Year is calculated as #RND*365/#days elapsed since site activation.

- **Missingness rate** – the number of missed data points divided by the number of expected data points given the participants' status and progress. The denominator is difficult to calculate in an automated manner; a visual estimate on a sampling of participants is sufficient for the purposes of assessing site data quality.
- **Query rate** – the number of queries for a site divided by the number of case report form (CRF) pages accrued by the participants at that site as of a specific point-in-time.

Addressing medical and mental health contingencies. It is possible that during a study visit the staff or investigators encounter a medical contingency. The Table of Medical Contingencies (below) describes the protocol for such contingencies.

CONDITION	ACTION
Blood pressure problems as reported by participant	
High or low blood pressure	Refer to emergency room/call 911, or walk-in PMD's office immediately
Diabetes	
If dizzy and/or hypoglycemic by fingerstick	Administer juice, other sweet beverage, or food by mouth; if improved within 15 minutes, advise to consult with PMD the same day; if not better, refer to emergency room/call 911
Complaining of polydipsia polyuria, FS >200 mg/dl	Refer to emergency room/call 911
FS >200 mg/dl and asymptomatic	If treatment has not been taken, advise to take it If treatment has been taken, advise consultation with PMD the same day
Falls	
Fall during motor assessment or any other circumstance	Call PI or study physician; take to emergency room.
Respiratory	
Chest pain	If cardiac etiology cannot be ruled out clinically, refer to emergency room/call 911 If accompanied by diaphoresis or respiratory distress, refer to emergency room/call 911 If chest pain clearly not cardiac, consultation with PMD the same day
Asthma/COPD exacerbation	If improved with treatment available at home, consultation with PMD the same day. Otherwise, refer to emergency room/call 911
Other respiratory distress	Refer to the emergency room/call 911
Psychiatric/social	
Depression/anxiety	If no potential harm to self or others, consult with PMD or mental health provider the same day Take prescribed medications not already taken If potential harm to self or others, refer to the emergency room/call 911
Any condition with acute changes in mental status	Refer to the emergency room/call 911
Neglect	If potential harm to self-apparent within 24 hours, refer to emergency room/call 911 If medical condition suspected as cause, refer to emergency room/call 911

CONDITION	ACTION
	In other situations, notify social worker and next of kin
Other	
Leg edema	If acute, refer to emergency room/call 911 or walk-in clinic If sub-acute, consult with PMD the same day
Failure to thrive	Refer to emergency room/call 911
Apparent dehydration	Refer to emergency room/call 911
Fever	Consultation with PMD the same day
Vomiting/diarrhea	If unable to hydrate by mouth, refer to emergency room/call 911; otherwise, advice consultation with PMD the same day. Withhold study medication until vomiting and diarrhea have stopped, and dehydration has resolved.
Headache	If clearly migraine or tensional headache, advise to take prescribed medications; if no improvement, consult with PMD same day. If no changes in mental status or neurologic deficits, if blood pressure <160, consultation with PMD same day; otherwise, refer to emergency room/call 911
Weight loss/no medical follow-up	Consult with PMD the same day If no PMD, see contacts below
Acute renal insufficiency (azotemia), for any reason, including post-renal (e.g., obstruction), pre-renal (e.g., dehydration), or intrinsic (e.g., acute tubular necrosis)	Stop IP until the cause of acute renal insufficiency has resolved and renal function has improved to at least a GFR of 45 mL/min or higher
Any other condition treated	Advise consultation with MD. Provide contact numbers (see below)
IF IN DOUBT	Contact PI or study physician

Table 6. Medical Contingencies: Summary of Conditions and Actions

Vulnerable participants

No vulnerable populations will be included in this study.

2.3.2 POTENTIAL BENEFITS

Metformin may improve the risk of diabetes and improve features of the metabolic syndrome.

Potential benefits of the proposed research to participants and others

In principle, participants will not derive any benefits from participation in the study. However, participants will undergo actionable blood tests at screening and during follow-up that might uncover an underlying medical problem not related to the study that might otherwise gone unnoticed. We will share study results with participants, who in turn will be able to share the results with their physicians. If participants do not have a primary physician and need guidance, the site PIs will facilitate referral to a primary care physician at each of the study sites. The results of brain MRI will not be shared with participants. However, if there are level 3 or 4 findings, participants will be notified as soon as possible by the pertinent site PI or designated physician. Level 3 findings require evaluation that is not urgent. Level 4 findings require urgent evaluation and possibly an immediate emergency room referral. Participants on metformin may derive metabolic benefits and desired weight loss from participation in the study. The benefit to others comes from learning about whether metformin can prevent Alzheimer's dementia.

Importance of the knowledge to be gained

MAP is responsive to NIA's Funding Opportunity Announcement PAR-18-028 "Phase III Clinical Trials for the Spectrum of Alzheimer's Disease and Age-related Cognitive Decline". MAP addresses the following example of interventions listed in PAR-18-028 "repurposed drugs that have promise for AD treatment such as chemotherapeutic agents or drugs for insulin dysregulation/diabetes. MAP is responsive to goal 1 of the National Plan to Address Alzheimer's Disease to "Prevent and Effectively Treat Alzheimer's Disease and Related Dementia by 2025". There are no known preventive or curative strategies for Alzheimer's disease at the moment. Recent clinical trials of amyloid based therapies have failed. MAP provides an opportunity to test a medication that has metabolic benefits and a low-risk side effect profile for the prevention of the cognitive declines associated with conversion to Alzheimer's dementia among persons with prodromal Alzheimer's dementia.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
1. To compare changes from baseline to 18 months in verbal memory performance, measured with the Total Recall Score of the FC-SRT, between the metformin and placebo arms, following an ITT approach.	Changes in Total Recall Score of the Free and Cued Selective Reminding Test (FC-SRT)	The justification for using the FC-SRT as the primary outcome is that a similar test of verbal learning, the SRT, was the co-primary outcome in the MetMCI study that demonstrated a difference between the metformin and placebo arms. The FC-SRT is a more advanced version of the SRT that is sensitive to memory changes in prodromal Alzheimer's disease.[132]
Secondary		
1. We will examine global cognitive performance, measured with the ADCS-PACC, as a secondary outcome.	Changes from baseline to 18 months in global cognitive performance, measured with the ADCS-PACC score, a composite that includes 4 tests: The FC-SRT, 2. The Delayed Recall score on the Logical Memory IIa subtest from the Wechsler Memory Scale, The Digit Symbol Substitution Test score, from the Wechsler Adult Intelligence Scale–Revised, and the Mini Mental Status total score.	The ADCS-PACC is being used in clinical trials in persons with prodromal AD.
2. To compare changes in neurodegeneration, ascertained as cortical thickness ascertained on brain MRI from baseline to 18 months between metformin and placebo.	Changes from baseline to 18 months in cortical thickness ascertained on brain MRI	Cortical thickness is a measure of neurodegeneration.
3. To compare changes in cerebrovascular disease, ascertained as WMH volume on brain MRI, from baseline	Changes in from baseline to 18 months in cerebrovascular disease, ascertained as white matter	WMH is a measure of cerebrovascular disease.

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
to 18 months between metformin and placebo.	hyperintensities (WMH) volume (in mL) on brain MRI.	
4. To compare changes in brain amyloid from baseline to 18 months between the metformin and placebo arms.	Changes from baseline to 18 months in whole brain Ab standardized uptake value ratio (SUVR) and in incident amyloid positivity	whole brain Ab SUVR is a marker of in-vivo brain amyloid burden
5. To compare changes in brain tau burden from baseline to 18 months between the metformin and placebo arms.	Changes from baseline to 18 months in tau SUVR in a composite brain region comprising medial and inferolateral temporal cortex	tau SUVR in a composite brain region is a biomarker of in-vivo brain tau
6. To compare changes in AD plasma biomarkers, between metformin and placebo during follow-up	Changes from baseline to 18 months in plasma AD biomarkers. These biomarkers will be measured in plasma from baseline and months 6, 12 and 18	AD plasma biomarkers are markers of neuropathology.
Exploratory		
1.To examine APOE-ε4 genotype and COVID 19 history as a modifier of the interventions.	All outcomes listed above. Persons without an APOE-e4 allele will be compared with those homozygous or heterozygous for APOE-e4. COVID-19 history will be ascertained by self-report.	Primary and secondary endpoints. Previous studies have demonstrated a higher risk of dementia among persons with hyperinsulinemia who are heterozygous or homozygous for APOE-ε4. COVID-19 may cause long term cognitive sequelae that could affect the effectiveness of metformin.

4 STUDY DESIGN

4.1 OVERALL DESIGN

We propose to conduct a phase II/III, multisite 1:1 randomized double-blinded placebo-controlled trial of extended-release metformin (maximum dose of 2,000 mg, four Glucophage XR® 500 mg tabs [reduced mass]) in 326 men and women, aged 55 to 90 years of age, with amnestic mild cognitive impairment (including early and late amnestic mild cognitive impairment). The trial will last 18 months. After screening for eligibility and consent, participants will have a baseline visit, followed by randomization to metformin or matching placebo, and a titration phase. Metformin (or matching placebo) will be titrated from 500 mg a day to 2,000 mg a day at increments of 500 mg every 10 days, as recommended by the manufacturers. Participants will be seen every 6 months thereafter for study assessments. Study procedures will include phlebotomy and laboratory tests, general health questionnaires,

neuropsychological testing, physical exam comprising a brief neurologic exam, measurement of vital signs and anthropometric measures. Study partners will also undergo questionnaires. Up to 186 participants will undergo brain magnetic resonance imaging (MRI) before IP is initiated (a window of two months is allowed to complete brain imaging), and after the last assessment at 18 months. Similarly, up to 186 participants will undergo amyloid and tau Positron Emission Tomography (PET). MAP will be a multisite study.

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

Alzheimer's dementia is a common disease with no current cure. One in ten Americans over the age of 65 years will develop Alzheimer's dementia. Participation in this study will help advance knowledge of this disease. It is unknown if metformin will prevent cognitive decline associated with Alzheimer's dementia. A control group administered placebo is necessary to determine if changes in verbal memory performance, as measured with the FC-SRT and global cognitive performance, as measured with the ADCS-PACC, are related to the use of metformin or not. The placebo control group will not be subject to risks greater than the intervention arm.

4.3 JUSTIFICATION FOR DOSE

The justification for the dose is that it is the maximum approved dose for the treatment of diabetes, also used off label for diabetes prevention among persons at risk.

4.4 END OF STUDY DEFINITION

A participant is considered to have completed the study if he or she has completed all phases of the study including the last visit or the last scheduled procedure shown in the flow diagram in Section 1.2 Schema. The end of the study is defined as completion of the last visit or procedure by all participants.

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

- Diagnosis of aMCI: in general, the diagnosis of aMCI follows the definition in the 2011 National Institute on Aging (NIA)/ Alzheimer's association (AA) guidelines, without biomarkers.[133] Participants must have:
  - Subjective memory concerns reported by the participant, study partner, or clinician.
  - A mini-mental state exam ≥ 22 for participants with more than 8 years of education. For participants with less than 8 years of education, a MMSE ≥ 20 will be allowed.
  - Clinical Dementia Rating (CDR) = 0.5. The memory box score must be at least 0.5. Information from the formal University of Washington CDR instrument, report by the participant of subjective cognitive complaints, and findings from the screening neuropsychological battery, can be used for this determination by the investigative team. For example, the University of Washington CDR can be 0, but the CDR memory box score can be deemed to be 0.5 based on cognitive complaints at screening and meeting the MCI neuropsychological criteria.
  - General cognition and functional performance sufficiently preserved such that a diagnosis of dementia cannot be made by the site physician at the time of the screening visit.

- Abnormal memory function documented by scoring within the education adjusted ranges on the Logical Memory II subscale (Delayed Paragraph Recall, Paragraph A only) from the Wechsler Memory Scale-Revised.
  - For early MCI:
    9-11 for 16 or more years of education
    5-9 for 8-15 years of education
    3-6 for 0-7 years of education
  - For late MCI
    ≤ 8 for 16 or more years of education
    ≤ 4 for 8-15 years of education
    ≤ 2 for 0-7 years of education

- Age range: 55 years to 90 years.
- Sex distribution: all eligible men and women will be included, and no one will be excluded because of gender.
- Languages: fluent in English or Spanish. We have reliable, well-validated Spanish tests for all outcome measures.
- Participants without a known history of diabetes. If diabetes is diagnosed during screening (hemoglobin A1c [HbA1c] of 6.5% or greater) participants will be excluded. The main justification for this exclusion is the potential for these participants to be placed on other diabetes medications that may confound our study.
- General cognition and functional performance such that a diagnosis of dementia cannot be made at the time of screening based on DSM-V criteria.
- Vision and hearing must be sufficient for compliance with testing procedures.
- Must have a study partner to come to all appointments or be available by telephone at follow-up visits.

Study Partner Inclusion Criteria

- The study partner can provide an independent evaluation of functioning for a person enrolled in the MAP study as a participant.
- The study partner agrees to attend study visits with the MAP participant or be available by telephone.

5.2 EXCLUSION CRITERIA

- Use of metformin or any class of medication approved for the treatment of diabetes,[134] even if it is used for an indication other than diabetes (e.g. obesity), within 1 year of screening. These medications include GLP-1 agonists used for weight loss.
- Body mass index < 20 k/m^2.
- Metformin is contraindicated in persons with an estimated glomerular filtration rate (eGFR) of less than 30 mL/min. For persons with an eGFR of 30 to 45 mL/min, a reduction of the dose or discontinuation of metformin may be considered. It is also recommended that persons do not initiate metformin in this range. Thus, participants with eGFR < 45 mL/min will not be eligible to participate.
- The risk of lactic acidosis is increased in persons with liver disease and class III or IV congestive heart failure. Thus, persons with liver disease other than non-alcoholic-fatty liver disease or class III or IV congestive heart failure will not be eligible to participate due to the risks of side effects.

- A history of intolerance to metformin.
- History of cerebrovascular accident with residual neurological deficits.
- Moderate to severe depression, indicated by a score in the Geriatric Depression Scale of 9/15 or higher.
- Dementia diagnosis
- Lack of capacity to consent
- Participants with neurologic diseases associated with neurologic deficits on clinical examination.
- Participants with other current Axis I psychiatric diagnoses such as bipolar disorder or schizophrenia.
- Alcohol or substance abuse or dependence in the past 6 months.
- Use of medications rated as being the likely cause of cognitive impairment. These include benzodiazepines in dose equivalents greater than 2 mg daily of lorazepam, and regular use of prescription narcotics.
- Normal individuals without cognitive complaints.
- Participants with uncontrolled hypertension (systolic blood pressure ≥ 160 mmHg or diastolic blood pressure ≥ 95 mmHg).
- Participants with active cancer or a history of cancer within the last two years, with the exception of squamous or basal cell carcinoma of the skin.
- Participants who for any reason may not complete the study as judged by the study physician.
- Participants planning to move to another city or state during the duration of the study.
- Participants with a known history of diabetes. The rationale for this exclusion is persons with diabetes may already be on metformin or on other medications that increase insulin levels and could confound the trial.
- Participants with diabetes discovered on screening based on American Diabetes Association criteria using HbA1c (HbA1c of 6.5% or greater). Although metformin could be a first treatment of diabetes for these participants, addition of treatments for diabetes by physicians could confound the study.
- Use of any amyloid modifying treatment for AD such as lecanemab, either experimental or approved by the Food and Drug Administration, is exclusionary. Previous use of amyloid targeting therapy that was shown to be non-efficacious (e.g. solunazemab) is not exclusionary.
- Not able to undergo phlebotomy as reported by the participant or determined by the study coordinator or physician.
- Participants with known, suspected, or plan for becoming pregnant.
- The presence of a medical condition, and/or use of a medication and/or any substance, individually or in aggregate, that in the judgement of the study team, is the primary cause of cognitive impairment. For example, if hypothyroidism, cobalamin deficiency, or tertiary syphilis are reported or found during screening, they could be deemed as being likely contributors to cognitive impairment, and thus be exclusionary. Combinations of multiple medications with anti-cholinergic effects with or without other central nervous system depressants could also be considered as being causative of cognitive impairment and exclusionary.

<u>Exclusion Criteria for MRI</u>

Contraindications for MRI include inability to lie flat, claustrophobia, or presence of indwelling metal objects or implants that are not MRI compatible.

<u>Exclusion Criteria for PET</u>

History of adverse reactions to radiocontrast agents.

5.3 LIFESTYLE CONSIDERATIONS

Participants will be informed that the risk of complications from metformin, particularly lactic acidosis, can increase with excessive alcohol intake. In addition, study medication should be stopped in other acute conditions that could lead to lactic acidosis, including diarrhea and vomiting, acute renal failure, and hospitalizations for acute infection that could lead to sepsis or involve sepsis. IP may be resumed when these situations have resolved.

5.4 SCREEN FAILURES

Screen failures will be excluded from the study.

5.5 STRATEGIES FOR RECRUITMENT AND RETENTION

To ensure the trial accrues and retains the number and diversity of participants required to assess the primary and secondary endpoints, a recruitment and retention risk and needs assessment to identify areas of concern and opportunities for engagement will be conducted. Our approach will recognize and support the diverse needs of study sites (clinicians, research staff, and informatics staff), meaningfully involve participants, families, community groups and clinicians in all stages of the study. Site-specific recruitment planning will be done with guidance from the master recruitment and retention plan allowing adaptation to the sites' local IRB requirements and institutional marketing practices. We will use an ongoing evaluation process, which will include iterative feedback from the recruitment reports, sites, and study participants and will guide implementation activities and adapt as needed. A portfolio of recruitment materials such as flyers, posters, phone and email scripts, and social media ads will be available to be personalized for local sites. All recruitment materials and scripts will be approved by the IRB prior to use. These assets will be available electronically. Each participating site will be able to choose recruitment and retention strategies that fit their local setting such as:

- Broad targeted advertisements (e.g., brochures/flyers, web advertisements and notices, social network advertisements including but not limited to Facebook, Twitter and Instagram, and media advertisements on television, radio and newspapers).
- Direct-to-participant (e.g., mail, health system portals, calls, waiting rooms, or clinical team)
- Social and community networks (e.g., social media, support group newsletters or community events)

Potential strategies to be employed regarding screening and recruitment include:

Pre-Screen/Screening

- Phone calls will be made to potential participants identified through EHR, registries, and those who respond to ads.
- Unsolicited "cold calls" to potential participants will only be made at sites where this practice is approved by the local IRB. If approved, the study coordinator will use the T*elephone Screening Script* to discuss the study with the potential participant.
- If unsolicited "cold calls" are not permitted by the local IRB, such calls will not be conducted.
- An alternative to the unsolicited "cold call" is the use of MyChart (an Epic EHR product) or similar EHR-based communication tool. Use of EHR based communication will only be conducted based on local site practices and with local IRB approval. The communication text is outlined in the document, *EHR-based Recruitment Communication*.

- Potential participants will be encouraged to share study information and study contact phone number or website with friends/family who may interested in participating in the study
- Potential participants will be asked for recommendations on possible recruitment sites.
- Potential participants will be invited to register on Alzheimer's registries, general research recruitment registries including but not limited to ResearchMatch
- Potential participants will be asked, "How did you hear about the study?"
- Additional centralized pre-screening may occur through recruitment websites using a dedicated call-in center at Columbia University.

Consenting

- Potential participants will be given opportunities to ask questions about study
- Recruitment and retention team members will begin building rapport with participants
- Medical care providers will be informed of participant enrollment (when possible, or as required) and provided with study brochure or one-page flyer.

Potential strategies to promote engagement and retention of participant could include:

- Return of laboratory results
- Phone calls
- Reminder cards
- Holiday Birthday cards
- All participants and study partners will be offered a perk for participation, such as an Amazon Prime membership for the duration of the study or a gift card of similar value.

The DCC and CCMC will compile reports to generate a master log of participant attrition and missed visits. This log will be monitored to guide and inform continuous process improvement.

6 STUDY INTERVENTION

6.1 STUDY INTERVENTION(S) ADMINISTRATION

6.1.1 STUDY INTERVENTION DESCRIPTION

There will be 2 study arms, extended-release metformin (Glucophage XR® 500 mg tablets [reduced mass]; 2,000 mg a day maximum dose), and matching placebo with identical appearance. IP will be supplied by EMD Serono Research & Development Institute and delivered in bulk to the research pharmacy at the University of Iowa, which will prepare bottles with IP. These will be sent to the University of Rochester, which will prepare kits of IP for dispensation. The University of Rochester will send these kits to the local pharmacies of participating clinical sites for dispensation.

6.1.2 DOSING AND ADMINISTRATION

IP will be titrated every 10 days by one tablet (500 mg), up to 2000 mg a day. Participants will remain in the study on the maximum tolerated dose and will be considered in the analyses on an intent to treat basis.

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 ACQUISITION AND ACCOUNTABILITY

The central research pharmacy for the study will be located at the Clinical Materials Services Unit (CMSU) at the University of Rochester. The University of Iowa Pharmaceuticals (UIP) will receive Glucophage XR® 500 mg tablets [reduced mass] and matching placebo in bulk (drums of 24,828 tablets for metformin, drums of 13,800 for placebo) from the EMD Serono Research & Development Institute, who will supply the IP. The UIP will prepare bottles of IP with a 140 count (35-day supply at maximum dose). The CMSU will receive approximately 4,900 x 140-count bottles each of metformin 500 mg tablets and matching placebo tablets from the UIP divided over three shipments. These quantities of study drug will support 326 participants, randomized 1:1 (active:placebo) and includes an overage factor of 40% for baseline kits and 10% for resupply kits. Upon receipt of the investigational products, the CMSU will perform a visual inspection and quality assurance (QA) release of all incoming materials to confirm that the appropriate quantities were received in good condition. Using the randomization code provided by the DCC), the CMSU will generate the necessary bottle and kit box labels that will support the double-blind, 1:1 (active:placebo) design of the study.

6.2.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

The CMSU will label the study drug and configure the 140-count bottles into six-month kits delivered to the site local research pharmacy containing two three-month kits with 3 bottles each. The study site local research pharmacy will dispense the study drug to participants by mail or will dispense the study drug to the CRC who will provide it to the participant at the in-person study visit or by mail. Study drug may be dispensed as two 3-month kits every six months or one kit every 3 months, whichever is preferred by the participant. The CMSU will generate the necessary bottle and kit box labels that will support the double-blind, 1:1 (active:placebo) design of the study.

Sites will maintain accurate records of study kits in the EDC inventory form. When inventory falls below par (periodic automatic replenishment) level, study kits will be replenished by CMSU.

6.2.3 PRODUCT STORAGE AND STABILITY

IP tablets are to be kept in the container in which they are supplied and are to be kept tightly closed. To protect young children from poisoning, we advise that safety caps be kept locked, and the medication be immediately placed in a safe location out of sight and reach of children. Tablets should be stored in the medication bottle at room temperature and away from light, excess heat, and moisture (not in the bathroom). Any unused tablets are to be returned to study personnel.

6.2.4 PREPARATION

For this study, the IP tablets will be prepared by the study pharmacy. No preparation is required by study staff or study participants. The IP will be administered as an extended-release tablet taken by mouth, taken once daily with the evening meal. Participants will be instructed to follow the directions on the prescription label carefully and ask the doctor or study pharmacist to explain any part not understood. Participants will be instructed to take the IP exactly as directed and to not take more or less of it as prescribed by the healthcare provider. The IP tablets are to be swallowed whole. They are not to be split, chewed, or crushed.

6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

Randomization: eligible participants will be randomized in 1:1 ratio to receive either metformin or placebo, using randomly permuted block randomization stratified by each site to achieve balance of treatment assignment overall and by site.

Blinding: participants, providers, outcome assessors and biostatisticians will be blinded to the treatment assignment. One group of biostatisticians will be unblinded to the treatment assignment to provide reports to the MAP data and safety monitoring board (DSMB).

6.4 STUDY INTERVENTION COMPLIANCE

Compliance with the study intervention will be monitored by direct discussion at monthly telephone calls and face-to-face study visits (every six months).

6.5 CONCOMITANT THERAPY

For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Medications to be reported in the CRF are concomitant prescription medications (up to two months prior to baseline), over-the-counter medications and supplements.

All concomitant prescription medications will be recorded in the CRF at screening and at each study visit as outlined in Table 7. Study Assessments and Procedures.

6.5.1 RESCUE MEDICINE

Not applicable

7 DISCONTINUATION OF STUDY INTERVENTION AND WITHDRAWAL OF PARTICIPANT

7.1 DISCONTINUATION OF STUDY INTERVENTION

Discontinuation of IP does not mean discontinuation from the study, and remaining study procedures should be completed as indicated by the study protocol. If a clinically significant finding is identified (including, but not limited to changes from baseline) after enrollment, the investigator or qualified designee will determine if any change in participant management is needed. Any new clinically relevant finding will be reported.

If participants develop diabetes by HbA1c criteria (6.5% or higher), they will be referred to their physicians to decide about treatment for diabetes. This may lead to discontinuation of IP upon consultation with the study's safety officer at the DCC. However, participants will be invited to remain in the study and will be analyzed following intent to treat.

If the medication is not discontinued due to an SAE and the investigator believes that the participant could be re-challenged with the discontinued medication per standard clinical practice, then this may be attempted after discussion with the DSMB and approval by the Medical Monitor.

If the medication was discontinued due to an SAE, then there should not be an attempt to restart the medication.

Any discontinuation of study drug will be recorded on the Dose Change Log eCRF.

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Participants are free to withdraw from participation in the study at any time upon request.

An investigator may discontinue or withdraw a participant from the study for the following reasons:

- Pregnancy
- Significant study intervention non-compliance
- If any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant
- Disease progression which requires discontinuation of the study intervention
- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation

The reason for participant discontinuation or withdrawal from the study will be recorded on the CRF. Participants who sign the informed consent form (ICF) and are randomized but do not receive study intervention may be replaced. Participants who sign the ICF, are randomized, receive study intervention, and are withdrawn or discontinued from the study will not be replaced, and will be included in the final statistical analyses.

7.3 LOST TO FOLLOW-UP

A participant will be considered lost to follow-up if he or she fails to return for two consecutive scheduled 6-month-visits and is unable to be contacted by the study site staff.
The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit within 30 days and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, three telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

The site will record the last date of contact (due to study completion, participant withdrawal from the study, or death) in the eCRF as the termination date.

8 STUDY ASSESSMENTS AND PROCEDURES

The table below summarizes the study procedures during the 18 months of the study. There are procedures (questionnaires) that can be done remotely or in person, listed under the "Remote Module", and there are procedures that can only be conducted in person, listed under the "in-person" module. The window to complete screening procedures is 28 days. The window for 6-month follow-up visits is $\pm$ 30 days. The window for monthly contacts is $\pm$ 7 days. If a visit or contact is missed due to force majeure (e.g., hospitalization), a participant may continue in the study with the visit after the one missed. All brain imaging procedures should be conducted prior to starting study drug. Participants who completed the 18 months visit before the implementation of protocol 1.9 will be informed of the change in study duration and that they have completed the study. Participants in this situation who are awaiting follow-up brain

imaging (originally after the 24-month visit) will be invited to undergo the brain imaging procedures as soon as protocol 1.9 is approved and implemented.

		Screening/ Baseline	Titration 1,2, 3	Month 1,2	Month 3	Month 4,5	Month 6	Month 7,8	Month 9	Month 10,11	Month 12	Month 13,14	Month 15	Month 16,17	Month 18
REMOTE MODULE															
1	Telephone Screen	X													
2	UBACC[a]	X													
3	Remote Consent	X													
4	Demographics[f]	X													
5	Medical history[f]	X													
6	Medications[b]	X					X				X				X
7	MAC-Q	X					X				X				X
8	COVID-19 questionnaire	X					X				X				X
9	GDS	X					X				X				X
10	CDR (Participant)[c]	X					X				X				X
11	ADCS-ADL-PI (Participant)	X					X				X				X
IN-PERSON MODULE															
12	In-Person Consent (if not done remotely)	X													
13	Vital Signs[d]	X					X				X				X
14	Phlebotomy[d]	X					X				X				X
15	Laboratory tests[d]	X					X				X				X
16	ECG[d]	X													
17	MMSE	X					X				X				X
18	Paragraph Recall	X					X				X				X
19	Brief neurological exam	X					X				X				X
20	DSST	X					X				X				X
21	TMT A and B	X					X				X				X
22	Eligibility and Randomization	X													
23	FC-SRT	X					X				X				X
24	MRI	X													X
25	Amyloid PET	X													X
26	Tau PET	X													X
27	Titration		X												
28	Monthly Telephone Call		X	X	X	X	X	X	X	X	X	X	X	X	X
29	Drug Dispense	X			X		X		X		X		X		
30	Dose Log	X	X	X	X	X	X	X	X	X	X	X	X	X	X
31	Adverse Events		X	X	X	X	X	X	X	X	X	X	X	X	X
32	Protocol Deviation		X	X	X	X	X	X	X	X	X	X	X	X	X
STUDY PARTNER															
33	Study Partner Consent[e]	X													
34	CDR (Partner)[c]	X					X				X				X
35	ADCS-ADL-PI (Partner)	X					X				X				X
36	MBI	X					X				X				X
37	Final Status														X

Table 7. Study Assessments and Procedures

a: The UBACC test should only be performed if clinically indicated or if study personnel feel the participant does not have capacity to consent at any of the study visits, Screening included. Please refer to Section 8.1 for more information.
b: The study team should take particular care to note if any diabetes medication has been taken during the study duration.
c: A CDR will be completed with each study participant and their respective study partner at all visits (Screening/Baseline, Month 6, Month 12, and Month 18). A total composite CDR score will be calculated using the sum of the CDR scores of both the participant and the partner. An ADAS-PAC certified team member should perform the CDR.
d: Screening labs, vitals, and ECGs must be collected before randomization. Baseline labs, vitals, and ECGs do not need to be collected if performed within 14 days of the Baseline visit. All neuropsychological batteries, questionnaires, and the brief neurologic exam do not need to be performed again if collected during Screening. The screening tests will serve as the baseline collection.
e: Study partners must provide verbal consent. The investigator or designee (consent designee) will read a consent script to the study partner and document that the study partner verbally consented to the MAP study.
f: Study teams may stop screening assessments if the participant clearly does not meet eligibility.

8.1 EFFICACY ASSESSMENTS

The study will have the following visits/calls: (1) telephone screening; (2) in person-screening and baseline study visit; (3) telephone calls for titration; (4) Follow up visits at months 6, 12, 18 months; (5) monthly calls between 6-month visits; (6) Brain Magnetic Resonance Imaging (MRI) at baseline and month 18. Primary and secondary clinical (non-MRI) efficacy data will be collected at baseline and the 6-month visits. Secondary MRI efficacy data will be collected at baseline and 18 months. The following is a brief description of these visits and the assessments included in these visits.

1. Telephone screening. We propose a 2-tier approach for screening, including telephone pre-screening followed by in-person screening. Telephone screening includes questions about inclusion and exclusion criteria including demographics), social history, medical history, contraindications to metformin, and medications. Study sites have the option of conducting a telephone cognitive screen such as the TICS to exclude persons with normal cognition.

Only persons who meet inclusion criteria and do not meet exclusion criteria are invited for in-person screening/baseline visit.

2. In-person screening and baseline study visit. In-person screening includes written informed consent, demographics, anthropometric measurements (height, weight, waist and hip circumference), vital signs (blood pressure and heart rate), EKG, laboratory tests (thyroid stimulating hormone [TSH], rapid plasma reagin [RPR], vitamin B12, complete blood count [CBC], basic metabolic panel [BMP], hepatic panel, lipid panel, and HbA1c), the neuropsychological battery, and a COVID-19 questionnaire. At the time of screening the tester will complete other assessments if the participant meets the neuropsychological criteria for MCI. All evaluations will be recorded in the eCRF, as applicable. If the participant meets all the inclusion criteria and has no exclusion criteria, the participant will be randomized and mailed a randomization kit with IP.

3. Telephone calls for titration. There will be 4 calls, one at the beginning of titration (one tablet a day), and 3 more calls every 10 days when the dose is increased by one tablet a day. During these calls, adverse events and tolerance will be documented.

4. Follow-up visits at months 6, 12, and 18 will include data collection on anthropometric measurements (height, weight, waist and hip circumference), vital signs (blood pressure and heart rate), brief neurologic exam laboratory tests (vitamin B12, CBC, BMP, hepatic panel, lipid panel, and HbA1c), the neuropsychological battery, and the COVID-19 questionnaire. Adverse events and compliance will also be recorded. At the time of screening the tester will complete other assessments if the participant meets the neuropsychological criteria for MCI. All evaluations will be recorded in the eCRF, as applicable.

5. Monthly calls between 6-month visits: adverse events and compliance will be recorded

6. Brain MRI will be conducted at baseline and 18 months.

7. Amyloid PET will be conducted at baseline and 18 months.

8. Tau PET will be conducted at baseline and 18 months.

9. Plasma AD biomarkers will be measured in stored plasma from the 4 main visits (baseline and months 6, 12, and 18).

The following is a description of all assessments:

Capacity to consent

We will use the University of California, San Diego Brief Assessment of Capacity to Consent (UBACC)[135] to assess capacity in the study. The UBACC is a 10-item scale that assesses understanding and appreciation of the information related to a research protocol. The UBACC will be available in English and Spanish. It is important to point out that the study excludes participants with dementia at baseline (determined by a clinical dementia rating summary score of 1 or higher). Thus, all participants have in principle a cognitive status that allows for capacity to consent at baseline. There are two situations in which we will assess capacity to consent with the UBACC.

- Throughout the study if the study staff appreciate that the participant does not comprehend the study information during the consent process regardless of cognitive status. At baseline, lack of capacity to understand the study will constitute a reason for exclusion. During follow-up assessments, determination of lack of capacity to consent will lead to the need for a legal authorized representative to consent after the participant provides assent to participate.
- During follow-up, if the participant develops dementia, indicated by a clinical dementia rating summary score of 1 as determined in the previous follow-up assessment. If the participant demonstrates capacity, he/she may consent; if the participant lacks capacity but wants to continue participation, a legally authorized representative will need to provide consent after the participant's assent. It is important to point out that this approach respects the participant's decision at baseline to participate in the study, when he/she had the capacity to consent.

Participants will be given three trials to respond to the UBACC. If after three trials they do not have a perfect score, they will be determined to lack capacity to consent. The determination of lacking capacity to consent will lead to exclusion at baseline, and to the need for a legally authorized representative to provide consent during follow-up. We expect that the contingencies described above will occur in less than 5% of the participants in the study, who will have mild cognitive impairment at baseline.

Medical history, medications

Detailed medical, surgical, and psychological history, and all medications utilized, including a judgment as to whether they affect cognition (positively or negatively) will be collected at baseline. Psychiatric history, current and past history of depression, current anxiety, alcohol and other substance use, head injury, hypertension, cardiac disease, thyroid disease, other major medical conditions, and surgery are evaluated at the baseline visit. A full medical history will be obtained only at the baseline visit. Any report of events, or side effects will prompt a full history and physical exam at follow-up visit. A COVID-19 questionnaire will be administered at every visit.

Physical Assessments (vital signs, anthropometric measures, brief neurologic exam)

A brief neurologic exam will be conducted at the in-person screening visit. Participants must also be assessed for signs of congestive heart failure, pulmonary, liver or renal disease as contraindications to

metformin. Vital signs including blood pressure and heart rate will be measured. Standing height will be measured using a stadiometer calibrated in cm. Body weight is measured using a balance beam scale calibrated in kg. With the participant standing, measurements are taken to the nearest 0.1 kg of weight with a balance scale and height without shoes to nearest 0.5 cm, to calculate BMI (weight in k/height in m2). Waist circumference (WC) is measured at the level of the umbilicus. Hip circumference (HP) is measured at the level of maximal protrusion of the gluteal muscles. Resting blood pressure (BP) will be measured using an automated oscillometric device; three measurements will be obtained at 1-minute intervals in a seated position after 5 minutes of rest. The average of the 2nd and 3rd measurements will be recorded. Vital signs and anthropometric measures will be repeated at all visits. A full physical exam will be repeated at follow-up visits only in the case of a change in medical history.

Neuropsychological battery

The following battery will be administered at baseline and 6-month visits. For neuropsychological testing, verbatim instructions for administration and specific scoring guidelines will be available. All instruments in the neuropsychological battery are commonly used, validated, published instruments. Non-verified or unpublished instruments are not used in this research study. All tests are validated in English and Spanish. We will use different versions of the tests when available (e.g., different word lists) to account for learning effects. The following is an expanded description of the neuropsychological battery:

- Total Recall Score of the Free and Cued Selective Reminding Test (FCSRT). The FCSRT is a 16-item word list with visual and auditory presentation that uses semantic cuing to facilitate encoding and retrieval. The test has a score range of 0 to 48.
- Paragraph Recall on the Logical Memory IIa (episodic memory): Free recall of one short story that consists of 25 bits of information will be elicited immediately after it is read aloud to the participant and again after approximately 30-minute delay. The total bits of information from the story that are recalled immediately (maximum score = 25) and after the delay interval (maximum score = 25) are recorded. The delay score (0-25 story units) will be used in the composite and also for the inclusion criteria.
- Digit-Symbol Substitution Test: The Digit Symbol Substitution test is a subset from the WAIS-R. The test consists of 110 small blank squares presented in seven rows with one of nine numbers (1-9) randomly printed directly above each blank square. A "key" is printed above the rows of blank squares. The "key" pairs numbers 1 through 9 with an unfamiliar symbol. The participant must work as fast as possible for 90 seconds. The measure of interest is number of squares filled in correctly within the time limit (maximum raw score = 110).
- Mini Mental Status Exam (MMSE). The MMSE scale evaluates orientation, memory, attention, concentration, naming, repetition, comprehension, and ability to create a sentence and to copy two overlapping pentagons. The MMSE is scored as the number of correctly completed items with a lower score indicative of poorer performance and greater cognitive impairment. The total score ranges from zero (worse) to 30 (perfect).
- Trail-Making Test, Part A: This test of visuo-perceptual ability, attention and speed consists of 25 circles distributed over a white sheet of 8 1/2" X 11" paper that are numbered. The participant is instructed to connect the circles with a pencil line as quickly as possible all numbers in an ascending order (e.g., 1 to 2; 2 to 3; etc.). The participant's performance is judged in terms of the time (in seconds) required to complete the task and by the number of errors of commission and omission. The time to complete the trial will be the measures of interest.
- Trail-Making Test, Part B: This test of visuo-perceptual ability, attention and set-shifting ability consists of 25 circles distributed over a white sheet of 8 1/2" X 11" paper that either are numbered

(1 through 13) or contain letters (A through L). The participant is instructed to connect the circles with a pencil line as quickly as possible while alternating between numbers and letters in an ascending order (e.g., A to 1; 1 to B; B to 2; 2 to C). The participant's performance is judged in terms of the time (in seconds) required to complete the task and by the number of errors of commission and omission. The time to complete the trial will be the measures of interest. Trail-Making Test, Part B is available in multiple forms of equal difficulty for purposes of repeated evaluations.

- Functional abilities:
  - ADCS-ADL-PI: The ADCS-ADL-PI was developed in the ADCS Prevention Instruments Trial. The participant and study partner separately rate the participant's performance of 18 IADL tasks over the past 2 months. Questions about use of technology (e.g., computers and cell phones) are included. Responses for each IADL include improved IADL performance (fewer errors, faster completion, less need to refer to notes or instructions), no change ('as well as usual'), various levels of impaired performance, and non-performance.
  - Clinical Dementia Rating (CDR). The CDR is a clinical scale that rates the severity of dementia as absent, questionable, mild, moderate, or severe (CDR score of 0, 0.5, 1, 2, or 3, respectively). The score is based on interviews with the participant and study partner, using a structured interview that assesses six domains: 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 six categories of function are synthesized into one global rating of dementia (ranging from zero to 3), with more refined measure of change available by use of the Sum of Boxes (CDR-SB). Reliability and validity have been established, as has high inter-rater reliability.
- Memory Complaint Questionnaire (MAC-Q): The MAC-Q consists of six items. The first five items relate to specific situations that are frequently reported as troublesome for those with declining memory, and the last item broadly measures overall self-perceived memory decline.
- Mild Behavioral Impairment Checklist (MBI-C): the MBI-C is a rating scale for neuropsychiatric symptoms in a pre-dementia population. It has items in 5 domains: decreased motivation, emotional dysregulation, impulse dyscontrol, social inappropriateness, and abnormal perception or thought content. The MBI inquires about the presence of symptoms in these domains (yes, no) and their severity (mild=1, moderate=2, severe=3). It has 34 items in the 5 domains (maximum score =102). The MBI-C is administered to the study partner.
- Geriatric Depression Scale (GDS): We will administer the 15 item GDS at all visits to assess depressive symptoms. The GDS has 15 yes/no questions assessing depressive symptoms.

Brain imaging

- Magnetic resonance imaging (MRI). We will acquire MP-RAGE and FLAIR imaging according to ADNI2 specifications appropriate to site hardware and operating system. In addition, a Fast T2 Spin-Echo image will be acquired according to site hardware and operating system specifications for evaluation of MRI infarction and to exclude hemorrhage. Standard 2D sequence gradient echo imaging will be acquired to assess micro-hemorrhages as will multi-directional DTI to assess white matter integrity. These latter two image sequences will be standardized according to available hardware and operating systems and may be used for secondary analyses at a future date. The maximum imaging time will be 60 minutes. Following scan acquisition, the data are transferred to a PACS for clinical review of potentially significant incidental findings.

- Amyloid PET: Amyloid PET imaging will be obtained using ^{18}F-florbetaben. ^{18}F-florbetaben will be injected as 8.1 mCi, with image acquisition 90-110 min post injection (following CT or transmission scan) as 4 x 5 min frames.
- Tau PET: Tau PET acquisition will entail injection of 5 mCi of MK-6240 with imaging from 90-110 min after CT or transmission scan.[136]

All brain imaging must be completed before study intervention is initiated. To this end, a window of 2 months is allowed between enrollment and commencing titration of study drug to complete brain imaging procedures. For Brain MRI site qualification, a brain MRI with all sequences will be conducted on a volunteer, reviewed from an existing study, or evaluated by the MRI protocol based on the instructions provided during the study setup.

Plasma biomarkers

The plasma AD biomarkers will be measured using the commercially available ultrasensitive single-molecule array (Simoa™) assays.[137] We will ship frozen plasma aliquots for these procedures at the end of data collection. Compared to the more commonly used ELISA methods, the Simoa™ assays provide high sensitivity and precision, while eliminating the matrix interferences.[138] Tau, Aβ40, Aβ42 will be assayed together using a multiplex assay (Human Neurology 3-Plex Total). The Simoa™ Human Neurology 3-Plex Total Tau assay uses a combination of monoclonal antibodies that react with both normal and phosphorylated epitopes in the midregion of the molecule yielding an assay that is specific to all tau isoforms. With a mean range of 2.28-109 pg/mL, the lower limit of detection for tau is 0.02 pg/mL, the reproducibility coefficient of variation (CV) is 8.5% and repeatability CV is 7.7%.[102,137] The Simoa Aβ40 and Aβ42 assays target the N-terminus of beta amyloid and different C-terminus detection antibodies specific to Aβ40 and Aβ42. Aβ42 (mean range: 0-400 pg/mL) has a lower limit of detection of 0.019-0.034 pg/mL, a reproducibility CV=7.5% and repeatability CV=6.7%.[137,139-141] Aβ40 (mean range: 0-800 pg/mL) has a lower limit of detection of 0.16 pg/mL, a reproducibility CV=5.1% and repeatability CV=3.5%.[98,141,142] A separate NFL test will be run using the ultrasensitive Simoa™ assay (mean range: 34.7-51.0 pg/mL and lower limit of detection of 0.97 pg/mL, CV=4.3%).[106,143] Isolation and detection of single enzyme molecules using femtolitre-sized reaction chambers, known as Simoa™. This method detects the target at low concentrations by ensuring that the fluorophores are confined to small volumes, and hence, the concentration of fluorescently labeled target is high. The antibodies are conjugated to magnetic particles utilizing a standard EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride) coupling procedure. In the first step of the assay, antibody coated paramagnetic capture beads, biotinylated detection antibodies, and samples are combined, during which target molecules present in the sample are captured by the capture beads and labeled with the biotinylated detection antibodies. After washing, a conjugate of streptavidin-β-galactosidase (SβG) is mixed with the capture beads where SβG bound to the biotin, resulting in enzyme labeling of captured target molecules. Following a second wash, the capture beads are resuspended in a resorufin β-D-galactopyranoside (RGP) substrate solution and transferred to the Simoa™ array disc for detection. All samples will be diluted 4-fold for Aβ42 and 8-fold for Aβ40 using a proper sample diluent (PBS containing carrier protein and detergent) for measurement. Given the rapid advance in the field of plasma AD biomarkers,[111] other biomarkers developed using the Simoa™ platform or other methods may replace or be added to those mentioned above.

Cognitive diagnoses

Cognitive diagnosis will be examined for inclusion and exclusion purposes and will also be examined as an exploratory outcome. For cognitive diagnosis transitions (i.e., conversion from MCI to dementia), an adjudication panel at the CCMC will conduct a meeting to arrive at a consensus regarding diagnosis at the

end of the study. This panel will be comprised of investigators Luchsinger, Goldberg, and Devanand. During the meeting, information of all the cognitive, functional and other clinical information of the participants will be presented, blind to study allocation. Evidence of cognitive deficits (based on the neuropsychological scores), evidence of impairment in social or occupational function (as assessed by the functional measures), and evidence of cognitive and social-occupational function decline will be the criteria used for the diagnosis of dementia.

8.2 SAFETY AND OTHER ASSESSMENTS

General questionnaires. We will collect demographic data including date of birth, sex, education, ethnic and racial group, and country of origin.[144] We will measure depressive symptoms with the Geriatric Depression Scale[145]. Medical history (e.g., diabetes) and medications will be collected with questionnaires we currently use for other studies.

COVID-19 questionnaire. The questionnaire includes questions about history of COVID-19, positive testing for coronavirus, symptoms and sequelae including memory problems, reinfection, and vaccination. A history of COVID-19 will be examined as a covariate and modifier.

Laboratory tests. All laboratory tests will be conducted under fasting conditions. Screening (BMP, HbA1c, lipids, hepatic profile, CBC, RPR, B12 levels, TSH) will be conducted at the central laboratory at the Center for Advanced Laboratory Medicine (CALM) at Columbia. BMP, HbA1c, hepatic profile, lipids, B12 levels, and CBC will be repeated at each visit for safety purposes. TSH and RPR will not be repeated at the follow-up visits. APOE will be genotyped (ε2, ε3, and ε4 alleles) by LGC genomics (Beverly, MA; http://www.lgcgroup.com) using single nucleotide polymorphisms (SNPs) rs429358 and rs7412. APOE-ε4 will be examined as a covariate and potential modifier. At the end of the study, we will conduct the following laboratory tests in stored samples to examine the impact of the intervention on intermediate outcomes: Insulin levels will be measured using a solid-phase chemiluminescent enzyme immunoassay (Immulite, Diagnostic Products Co, Los Angeles, CA). Inflammatory markers will include hsCRP, serum Amyloid A, IL-6, IL-8, IL-1B, TNFa, WBC, MCP-1 will be measured using ELISA (Diagnostic Systems Laboratories, Inc., Webster, Texas). We will use the American Diabetes Association criteria for HbA1c to diagnose diabetes (HbA1c 6.5% or greater).[146] We will calculate the homeostatic model assessment (HOMA)[147] using glucose (from the BMP) and insulin values as a measure of insulin resistance.

Adverse events. Gastrointestinal symptoms and development of contraindications to metformin will be queried. Reported symptoms will be further evaluated for severity and duration until they resolve. SAEs will be collected and reported on SAE CRF pages.

Measures used for screening. The Clinical Dementia Rating sum of boxes (CDR/CDRsob)[148] will be used primarily for aMCI inclusion criteria but will be assessed at every visit. The Logical Memory II subscale from the Wechsler Memory Scale-Revised will be used for establishing early and late MCI criteria.[149] The MMSE will also be used to establish entry criteria.

8.3 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.3.1 DEFINITION OF ADVERSE EVENTS (AE)

Adverse events

Adverse event (AE) means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention related. An AE can therefore be any undesirable sign,

symptom or medical condition occurring after starting IP, even if the event is not considered to be related to the pharmaceutical product. An AE can arise with any use of the drug (e.g., off-label use, use in combination with another drug) and with any route of administration, formulation, or dose, including an overdose (21 CFR 312.32 (a)). Headache, dizziness, lightheadedness, and gastrointestinal upset are AEs that will be specifically questioned about during phone calls and study visits.

8.3.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

An AE is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death
- A life-threatening adverse event that places the participant at immediate risk of death at the time of the event as it occurred.
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. This determination is based on the opinion of either the investigator or sponsor.

The following hospitalizations are not considered SAEs:

- Visits to the emergency room or other hospital department lasting less than 24 hours that do not result in admission (unless considered an "important medical event" or a life-threatening event);
- Elective surgery planned before or after signing consent;
- Medical/surgical admissions other than remedying ill health state that were planned before study entry. Appropriate documentation is required in these cases;
- Admissions encountered for another life circumstance that have no bearing on health status and require no medical/surgical intervention (e.g., lack of housing, economic inadequacy, caregiver respite, family circumstances, administrative reason).

8.3.3 CLASSIFICATION OF AN ADVERSE EVENT

8.3.3.4 Severity of Event

For AEs not included in the protocol defined grading system, the following guidelines will be used to describe severity.

Mild – Events require minimal or no treatment and do not interfere with the participant's daily activities.

Moderate – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.

Severe – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious".

8.3.3.5 Relationship to Study Intervention

All AEs must have their relationship to study intervention assessed by the clinician who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

Definitely Related – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study intervention (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.

Probably Related – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study intervention, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal.

Potentially Related – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related", as appropriate.

Unlikely to be related – A clinical event, including an abnormal laboratory test result, whose temporal relationship to study intervention administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the study intervention) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).

Not Related – The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.]

8.3.3.6 Expectedness

The study investigator will be responsible for determining whether an adverse event (AE) is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention, such as that described in the medication package insert.

8.3.4 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

Participants will have assessments including phlebotomy for safety laboratory every 6 months including baseline, 6-months, 12-months, and 18-months visits. In addition, participants will have monthly calls for monitoring of adverse events between visits. The safety laboratory tests will include complete blood count, basic metabolic panel, hepatic panel, and vitamin B12 (cobalamin). Cobalamin testing will allow to assess whether participants develop cobalamin deficiency and anemia, which could be due to metformin, or contraindications to metformin use such as hepatic insufficiency and severe renal insufficiency.

At each study visit, the investigator will inquire about the occurrence of AE and SAEs since the last visit. All AEs and SAEs will be captured on the appropriate eCRF. All AEs and SAEs occurring while on study drug

will be documented appropriately regardless of relationship. All AEs and SAEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded on the appropriate eCRF (e.g., AE or SAE).

Changes in the severity of an AE or SAE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs and SAEs characterized as intermittent require documentation of onset and duration of each episode.

8.3.5 ADVERSE EVENT REPORTING

The site investigator is responsible for monitoring the safety of participants enrolled into the study. If an adverse event necessitates modification of study drug dosing, this will be captured on the Dose Change Log eCRF.

All AEs and SAEs occurring from the first day of study enrollment (the day of randomization) to 30 days post last day of study drug dosing will be captured in the AE and SAE eCRF. Unless exempted as described in section 8.3.2, all SAEs, whether or not deemed drug-related or expected, must be reported to the CCC by the investigator or qualified designee **within 24 hours** of first becoming aware of the event. The investigator or qualified designee will enter the required information regarding the SAE into the appropriate module of the eCRF, which will automatically result in distribution of the information to the site-monitoring group, the Safety Event Adjudication Committee, and EMD Serono.

Pregnancy and cancer are not always serious by regulatory definition; however, these events must be submitted to the sponsor within the same time period as an SAE via the EDC.

If the eCRF system is temporarily unavailable, the event, including the investigator-determined causality to study drug should be reported via the back-up paper SAE form to Safety Surveillance. Upon return of the availability of the electronic data capture (EDC) system, the SAE information must be entered onto the eCRF.

8.3.6 SERIOUS ADVERSE EVENT REPORTING

The study clinician will immediately report to the sponsor any SAE, whether or not considered study intervention related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the study intervention caused the event. Study endpoints that are serious adverse events (e.g., all-cause mortality) must be reported in accordance with the protocol unless there is evidence suggesting a causal relationship between the study intervention and the event (e.g., death from anaphylaxis). In that case, the investigator must immediately report the event to the sponsor.

All SAEs will be followed until satisfactory resolution or until the site investigator deems the event to be chronic or the participant is stable. Other supporting documentation of the event may be requested by the DCC/study sponsor and should be provided as soon as possible.

The study sponsor will be responsible for notifying the FDA, IRB and DSMB of any suspected, (possibly, probably, definitely related) unexpected serious adverse reaction (SUSAR) as soon as possible, but in no case later than **7 calendar days** after the sponsor's initial receipt of the information. In addition, the sponsor must notify FDA and all participating investigators in an investigational new drug (IND) safety

report of potential serious risks, from clinical trials or any other source, as soon as possible, but in no case later than **15 calendar days** after the sponsor determines that the information qualifies for reporting.

8.3.7 REPORTING EVENTS TO PARTICIPANTS

We anticipate the following types of reports of events to participants:

- Reporting of results of safety laboratory tests: All participants will receive written full reports of the complete blood count, basic metabolic panel, hepatic panel, and cobalamin level. These reports will indicate the normal levels for each value and will encourage participants to call the site PIs with any questions and/or consult with their primary care physicians. If abnormalities in these laboratory tests are deemed to be due to the study intervention, or constitute a new contraindication to the study intervention, participants will be informed by the site PI by telephone, and in writing.
- Reporting of incidental findings in brain MRI. As indicated in section 2.3.3, there are 4 levels of incidental findings on MRI; level 3 requires non-urgent follow-up, and level 4 requires emergency follow-up. Site PIs will receive neuroradiology safety reads of the MRI within 72 hours. If level 3 findings are reported, the site PI will contact the participants via telephone, and with the permission of the participant, their medical provider, in order to inform of the incidental finding. In addition, a written report of the incidental finding will be mailed to the participant and medical provider. For level 4 incidental findings, the participant will be contacted on an urgent basis with the instruction to present to an emergency room or urgent care provider immediately.
- Reporting of new adverse events in our study, other studies, or the medical literature. In consultation with the DSMB and sIRB, we will compose a letter explaining the appearance of new information on the study drug reported in medical journals, other studies, and our study. This information will also be added to the consent form and participants will be re-consented at the next available visit.

8.3.8 EVENTS REQUIRING A CHANGE IN IP DOSING

At each study visit, the investigator will inquire about the occurrence of events that require a change in IP dosing, which may include, but are not limited to, hospitalizations, surgeries, and procedures that require holding metformin. Clinical events such as the development of anemia and/or B12 deficiency, and the development of contraindications to metformin, such as New York Heart Association Class III or IV congestive heart failure, and renal or hepatic insufficiency deemed to be of clinical significance are other examples of events that may require a change in IP dosing. Changes in IP dosing, and the associated reason, will be captured on relevant eCRF pages. Events above pre-specified thresholds as outlined in the Data Safety Monitoring Plan will trigger a review by the DSMB to investigate the presumed cause and impact of these events. Such events are initially reviewed by a Data Coordinating Center (DCC) staff member to determine if an event threshold has been reached, at which time the study investigators and the DSMB will be notified.

8.3.9 REPORTING OF PREGNANCY

Although our cohort will be between the ages of 55 and 90 years, there is the possibility of pregnancy. If, following initiation of the IP, it is subsequently discovered that a study participant is pregnant or may have been pregnant at the time of IP exposure, the IP will be permanently discontinued.

The Investigator must immediately notify the PI and the Safety Event Adjudication Committee of this event within 24 hours and in accordance with SAE reporting procedures.

Follow-up information regarding the course of the pregnancy, including perinatal and neonatal outcome must be reported. Any associated AEs or SAEs that occur to the mother or fetus/child will be recorded in the SAE CRFs.

Protocol-required procedures for study discontinuation and follow-up must be performed on the participant unless contraindicated by pregnancy.

8.4 UNANTICIPATED PROBLEMS

8.4.1 DEFINITION OF UNANTICIPATED PROBLEMS (UP)

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the single IRB (sIRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

8.4.2 UNANTICIPATED PROBLEM REPORTING

The investigator will report unanticipated problems (UPs) to the reviewing sIRB and to the DCC/lead principal investigator (PI). The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents a UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- Unanticipated problems that are SAEs will be reported to the DCC/study sponsor within 24 hours of the investigator becoming aware of the event, and a determination will be made in regard to their relation to the study drug. Those SAEs considered to be related to the study drug will be reported to the sIRB within 7 days of occurrence. Those SAEs not related to the study drug will be reported to the sIRB in annual reports. SAEs considered related to the study drug will be reported to the IRB and to the within 7 days.
- Any other UP will be reported to the DCC/study sponsor within 48 hours of the investigator becoming aware of the problem and will be reported to the sIRB annually.
- All UPs should be reported to appropriate institutional officials (as required by an institution's written reporting procedures), the supporting agency head (or designee), and the Office for Human Research

Protections (OHRP) within 7 days of the sIRB's receipt of the report of the problem from the investigator.

- For more information on sIRB reporting requirements, The JHM IRB Organizational Policy on Prompt Reporting of Reportable Events can be referenced here:

  https://www.hopkinsmedicine.org/institutional_review_board/guidelines_policies/organization_policies/prompt_reporting_policy.html

8.4.3 REPORTING UNANTICIPATED PROBLEMS TO PARTICIPANTS

If UPs possibly, probably, or definitely related to the study drug appear in the literature, other studies, or this study, which could change the risk of the study participation, the investigators will draft a letter to all participants describing this new information, the likelihood that it is related to the study drug, how this information alters the risk to participate in the study, and new measures taken to ensure the safety of participants. This process will be done with review and approval of the sIRB and DSMB. In addition, this new information will be added to the consent form and study participants already enrolled will be re-consented.

9 STATISTICAL CONSIDERATIONS

9.1 STATISTICAL HYPOTHESES

Primary efficacy endpoint(s)

The primary aim is to compare changes from baseline to 18 months in verbal memory performance, measured with the Total Recall Score of the Free and Cued Selective Reminding Test (FC-SRT), between the metformin and placebo arms, following an ITT approach. We hypothesize that the metformin arm will demonstrate less decline in the FC-SRT as compared with placebo.

Secondary efficacy endpoint(s):

- Examine global cognitive performance, measured with the Alzheimer's Disease Cooperative Study, Preclinical Alzheimer Cognitive Composite (ADCS-PACC). We hypothesize that the metformin arm will demonstrate less decline or improvement in the ADCS PACC as compared with placebo.
- Examine APOE-ε4 genotype as a modifier of the efficacy of metformin. We hypothesize that the benefit of metformin will be highest among APOE-ε4 carriers.
- Compare changes in neurodegeneration, ascertained as cortical thickness in areas affected by Alzheimer's disease acquired on brain MRI from baseline to 18 months between metformin and placebo. We hypothesize that the metformin arm will show less decrease in cortical thickness at 18 months compared with placebo.
- Compare changes in cerebrovascular disease, ascertained as white matter hyperintensities (WMH) volume on brain MRI, from baseline to 18 months between metformin and placebo. We hypothesize that the metformin arm will show less increase in WMH as compared with the placebo arm.
- Compare changes in whole brain Aβ standardized uptake value ratio (SUVR) and in incident amyloid positivity from baseline to 18 months between the metformin and placebo arms.
- Compare changes in tau SUVR in a composite brain region comprising medial and inferolateral temporal cortex from baseline to 18 months between the metformin and placebo arms.
- Compare changes in plasma AD biomarkers between metformin and placebo.

9.2 SAMPLE SIZE DETERMINATION

9.2.1. Original Sample size calculation. Sample size has been calculated for the difference in average change in SRT scores at 24 months from baseline between metformin and placebo arms. In the previous study, MetMCI, average change in SRT score at 12 months among metformin subjects was 9.8 (SD = 8.6) versus 5.9 (SD = 8.5) in the placebo arm, which corresponds to a standardized difference in means measured as Cohen's D of 0.46 (90%CI: 0.06 to 0.86). In the placebo arm, average change in SRT was similar at 12 months and 9 months. However, in the metformin arm, average SRT continued to improve from 9 to 12 months. These data are based on 33 (82.5%) vs. 37 (92.5%) subjects with non-missing outcome data at 12 months in metformin and placebo arms, respectively. Assuming that a similar standardized difference in means will be observed at 24 months, our calculation indicates that we will need 87 participants per arm to detect this effect size with 90% power, rejecting the null hypothesis at 0.08 level of statistical significance. This observed standardized difference corresponds to a "medium" effect size (Cohen, 1988). If, however, the observed difference at 24 months corresponds to low-to-medium effect sizes (i.e., Cohen's D between 0.35 and 0.4), the required maximum sample size is 302. The selected total sample size of 370 participants will be adequate to detect effect sizes 0.35 and larger, assuming overall drop-out/missingness of about 20%. Since the only difference between treatment arms was observed in APOEe4 negative participants, we performed sample size calculations for this subgroup. The observed standardized difference in average change in SRT score between metformin and placebo at 12 months was 0.6, (90%CI: 0.13 to 1.07). With a sample size of 302 (or adjusted for missing at 370) and assuming 60% participants will be APOEe4 negative, we will have 90% statistical power to detect this effect size or larger by rejecting the null hypothesis at 0.01 level of statistical significance.

9.2.2. New sample size calculation for protocol version 1.9. The sample size is calculated based on the data observed in phase II trial of metformin in aMCI (MetMCI) that followed 80 patients (40 per arm) with late aMCI up to 12 months. In order to determine the sample size of for the phase II/III study, the TIC team calculated the mean changes from baseline to all follow-up time points in total recall of the Selected Reminding Test (SRT) from the MetMCI pilot study and estimated the sample sizes necessary to find statistical significance for the differences between study arms at each time point, assuming □= 0.2 (80% power) and □ = 0.1. The observed effect sizes (Cohen's D)[1] ranged between a minimum of 0.21 for the month 9 difference to 0.52 for the month 6 difference. Clinical trials of interventions to prevent cognitive decline have yielded small effect sizes (e.g. 0.1 Cohen D) that are of dubious clinical significance. A Cohen D of 0.5 (moderate effect size) is usually considered clinically significant, but unlikely to be achieved in a clinical trial of interventions to prevent cognitive decline. Thus, we settled on a small to moderate target effect size (0.3 Cohen D). For an effect size of 0.3 Cohen D the calculated sample size is 278 for the phase II/III study. Considering a potential annual loss of follow-up of about 10%, the final sample size to 326. Is important to point out that given the proposed analytic approach, the study is powered to detect smaller differences. We assessed the statistical power for the proposed study based on differences in the slopes or trends between treatment arms in the SRT over 12 months (i.e. additional analysis for the primary outcome). A regression model with robust standard errors to account for within person clustering performed on these preliminary data, revealed an SRT slope (95% CI) of 0.84 (0.56, 1.13) points / month in the metformin group, an SRT slope (95% CI) of 0.52 (0.25, 0.79) points / month among the placebo patients, and a difference in slopes (e.g. interaction) of -0.33 points / month (p=0.10). Simulations were performed on replicates of these data, using the treatment by time interaction in regression analysis. Based on our sample size calculation, one hundred-fifty (150) patients were randomly chosen from each treatment arm in the replicated data, and the regression model performed on each of 10,000 simulated samples. Another 10,000 samples were also created to estimate the power of the treatment effect on

only ApoE-ε4 negative patients. The power for the overall sample was based on a type I error of 0.08, while a type I error rate of 0.01 was used for the ApoE-ε4 negative participants. An additional alpha of 0.01 was allocated for the ApoE-ε4 positive patients; however, simulations were not undertaken for this patient population, since there was no preliminary evidence of a treatment effect among the ApoE-ε4 positive patients. Based on these data simulations, we estimate a power of 92.4% to statistically detect a larger FC-SRT slope in the metformin group as compared to the FC-SRT slope among the placebo arm (e.g. 9240 out of 10,000 samples produced a significant interaction model term at the alpha = 0.08 level in the regression analysis). Similarly, we found an estimated power of 97.8% to detect a statistically larger FC-SRT slope in the metformin versus placebo groups among the ApoE-ε4 negative patients at alpha = 0.01 level. The proportion of ApoE-ε4 negative patients ranged from 67% to 79% among the 10,000 simulated samples, as compared to a proportion of 73.8% for the ApoE-ε4 negative patients (59 of 80) in the preliminary study.
Given these power analyses, the study team is confident that the reduction in sample size from 370 to 326 will not compromise the goals of the study.

9.3 POPULATIONS FOR ANALYSES

The population for analyses will be all 326 randomized participants based on an intent to treat approach.

9.4 STATISTICAL ANALYSES

9.4.1 GENERAL APPROACH

Exploratory data analysis will be performed on the entire study sample. All variables will be checked for outlying and unusual observations to detect general patterns, identify gaps and inconsistencies in the data. Visual inspection will be performed by means of box plots, scatterplots, quantile plots and others. Outliers will be checked for possible data entry errors. Reported descriptive statistics for continuous variables will include the number of participants with non-missing observations, median, mean, SD, interquartile range and range, while statistics for categorical variables will include the frequency and percentage of participants in each category.

Adjustment for covariates. In the primary analysis, all models described below will be adjusted for, ApoE-ε4 (as a binary indicator), age (continuous), self-reported sex (male or female) (categorical), baseline total recall FC-SRT score (continuous), MMSE score (continuous), BMI (continuous) and HbA1c level. Additional adjustment for site, BMI, gender, race, educational attainment, COVID-19 history, and white matter hyper intensity will be done as sensitivity analyses. Statistical tests will be two-sided.

9.4.2 ANALYSIS OF THE PRIMARY EFFICACY ENDPOINT(S)

Analytic Plan for the Primary Aim. The primary aim for this study is to compare changes from baseline to 18 months in verbal memory performance, measured with total recall in the FC-SRT, between the metformin and placebo arms. The primary null hypothesis H0 states that there is no difference between metformin and placebo arms on change in total recall in the FC-SRT from baseline to 18 months of follow-up. We will follow the ITT principle in which all randomized participants are analyzed based on the group to which they are randomized regardless of early termination or errors in randomization detected post hoc.

To estimate the average treatment effect, we will use the targeted maximum likelihood estimator (TMLE) of van der Laan and Gruber (2012). This estimator involves adjustment for baseline (i.e., pre-randomization) variables that are correlated with the outcome to improve precision in estimating the average treatment effect for FC-SRT score at 18 months. The estimator also involves adjustment aimed at reducing bias due to informative censoring, that is, participant dropout that may be correlated with the primary outcome. This adjustment for missing outcomes is done through a combination of inverse probability of censoring weights and outcome prediction models. Specifically, the weights and prediction models use the pre-specified baseline variables, study arm, and the post-randomization outcomes at 0, 6, 12, and 18 months. The motivation of this approach is that if the FC-SRT score at a given visit is correlated with the FC-SRT value at the next visit and also with dropout, then adjusting for observed differences in FC-SRT scores for those who dropout versus those who stay on study may reduce bias (under the assumption that censoring is missing at random). This adjustment is implemented by fitting models for dropout as a binary outcome using logistic regression models for the primary outcome based on FC-SRT measured at the previous visit, study arm, and baseline variables.

As the final step, average FC-SRT score under hypothetical assignment to the active treatment is estimated for each participant at 18 months (regardless of their actual assignment), given their baseline covariates. Similarly, average FC-SRT score under hypothetical assignment to the comparison arm is estimated. The primary analysis estimator of the average treatment effect as a mean difference is defined as the difference between the former and the latter. This analysis will be performed in a software that implements the above estimator: https://github.com/mrosenblum/RandomizedTrialEnhancedPrecisionEstimator.

To test the null hypothesis that the average treatment effect equals 0, a Wald statistic will be computed that is equal to the estimator of the average treatment effect divided by its estimated standard error. The standard error will be computed by the non-parametric bootstrap (resampling participants with replacement and recomputing the above estimator), and the 92% confidence interval will be computed using the bias corrected (BC) and accelerated (BCa) method.

Prior to computing the average treatment effect, missing baseline variables will be imputed using the median for continuous-valued variables (such as age and MMSE) and mode for categorical variables (such as site, APOE e4 status, gender, and education level). Intermittent missing outcomes at each time point will be imputed with fitted values from a regression model using all baseline covariates, previous outcomes, and treatment assignment as covariates.

Subpopulation defined by APOE-ε4 genotype. The primary pre-specified subpopulation analysis is in terms of the APOE-ε4 genotype. Two subpopulations will be defined based on the genotype: 1) any APOE-ε4 allele present, i.e. APOE-ε4 positive and 2) no APOE-ε4 allele present, i.e., APOE-ε4 negative). For each of the two subgroups, the above-described estimator will be computed, and the null hypothesis of no difference will be tested at 0.01 level of significance. If any of the three (one for combined population, and one for each of the two subpopulations) primary null hypotheses are rejected at their respective significance levels, the decision will be made to proceed to a phase III trial. COVID-19 history will be explored as a potential modifier of the intervention.

9.4.2.1 Additional efficacy analyses: longitudinal analysis of outcomes

The following will be used as sensitivity analyses.

Longitudinal analysis will examine the primary and secondary outcomes that are longitudinally collected at baseline and at 6, 12, and 18 months. Linear mixed effect model will be used, with FC-SRT as the primary outcome and time (in months), treatment (binary indicator) and their interaction as the primary

predictors. The model will include site and APOE-ε4 as categorical covariates and random intercept for participant to account for within-person correlation of FC-SRT scores. As noted above, additional adjustments will be included for participant pre-randomization characteristics (such as age, gender, education level, baseline MMSE or others) that show clinically meaningful differences between treatment arms. This model will be further modified to 1) use time points as four indicator variables rather than a linear term (i.e., 6, 12, and 18 months vs. baseline), 2) include random slope for time as a linear term to account for potential heterogeneity in trajectories across participants. This model will estimate two random effect variances and their covariance, and 3) account for potential correlation of residuals by specifying a covariance structure for residuals. These additional models will be informed by exploratory analysis of outcome distribution over time and will be compared using Bayesian Information Criterion (BIC). Similar longitudinal analyses will be performed for secondary outcomes.

9.4.3 ANALYSIS OF THE SECONDARY ENDPOINT(S)

Analytic plan for secondary aims. Our secondary aims compare changes in the ADCS-PACC between metformin and placebo on an ITT basis. The analytic approach will be the same as described for the Primary Aim. This will also apply to outcomes measured at all visits, including the plasma AD biomarkers. We will also compare changes from baseline to 18 months between metformin and placebo in brain cortical thickness, white matter hyperintensity volume, brain amyloid SUVR, and brain tau SUVR. Since these analyses will have only 2 time points, we will use analysis of covariance to compare the mean changes from baseline to 18 months, adjusting for study site and other prespecified covariates in the primary analysis.

9.4.4 SAFETY ANALYSES

The number of SAEs will be tabulated by type for the entire study sample as well as by treatment group. Any treatment group differences in SAEs enumerated for interim analyses will remain blinded to investigators associated with this study. Unmasked results will be made available only during the closed sessions of DSMB meetings.

9.4.5 BASELINE DESCRIPTIVE STATISTICS

Treatment arms will be compared on pre-randomization (baseline) characteristics potentially correlated with outcomes to assess for differences in distributions and, therefore, potential confounding. No inferential statistics will be used.

9.4.6 PLANNED INTERIM ANALYSES

Safety data will be looked at by the DSMB at a specified schedule, which is different from a formal interim analysis for efficacy. There will be no interim analysis for efficacy.

9.4.7 SUB-GROUP ANALYSES

Analyses of primary and secondary outcomes will be stratified by the following baseline participant characteristics: (primary) APOE e4 status and age, gender, race, site, type of MCI (early vs. late), BMI, cognition and metabolic status, as measured by Hb1Ac.

9.4.8 TABULATION OF INDIVIDUAL PARTICIPANT DATA

No individual participant data will be tabulated. Individual level data might be presented to the DSMB in a closed report, at their specific request.

9.4.9 EXPLORATORY ANALYSES

Exploratory analyses will include: sensitivity analyses including and excluding data outliers for which data entry errors were ruled out: exploratory analyses examining different metformin cumulative doses according to the doses tolerated during the study; examination of other outcomes such as functional outcomes and other neuropsychological domains such as executive function.

9.4.10 DECISION TO MOVE TO PHASE III TRIAL

The advantage of phase II/III study design is that it is an adaptive design and can decrease the overall number of participants, time and cost of making the decision about efficacy of the intervention. The decision to move to a phase III trial will be based on the analysis of the unblinded phase II trial data, made by a data monitoring committee independent of the study investigators and the DSMB, and will include at least one biostatistician.

Phase III will not be started until all phase II primary outcome data are analyzed. As noted above, if the primary hypothesis regarding the average treatment effect is rejected either for the entire sample or one of the primary subgroups based on the APOE e4 status, at their respective statistical significance levels, the decision will be made to proceed to the phase III trial.

The decision about the participant eligibility/study population for phase III trial will be based on the average treatment effect in the entire study sample versus the primary subgroups. If a positive treatment effect is observed (i.e., average treatment effect > 0 comparing metformin vs. placebo arm) in the study, no additional exclusions for phase III compared to phase II study population will be implemented. However, this decision might be modified by the independent committee depending on the safety profile among different participant subgroups.

Statistically, the phase II and phase III trials are distinct, i.e., no phase II data will be used in the primary analysis of the phase III trial.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

10.1.1 INFORMED CONSENT PROCESS

10.1.1.1 Overview

The investigator is responsible for following all federal, state, and local regulations regarding the obtainment of informed consent from all participants (e.g., 21 CFR 50). The investigator or designee must explain to each participant (or legally authorized representative) the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits involved and any discomfort it may entail. Each participant must be informed that participation in the study is voluntary and that he/she may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical treatment or relationship with the treating physician.

The ICFs must be submitted by the investigator for sIRB/IEC approval. The CCC will supply template ICFs that comply with regulatory requirements and are appropriate for the study.

10.1.1.2 Consent Procedures and Documentation

Informed consent will be conducted in person, electronically, or remotely by video or telephone using a hard-copy (paper) ICF.

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Consent forms will be IRB-approved and the participant will be asked to read and review the document. The consent designee will explain the research study to the participant and answer any questions that may arise. A verbal explanation will be provided in terms suited to the participant's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. Participants must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the signed ICF will be given to the participants for their records. The informed consent process must be conducted, and the form signed, before the participant undergoes any study-specific procedures. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

MAP requires the participation of a study partner who should be available by telephone or in person to answer questionnaires about the participant's cognition, mental health, and function. The study partner must provide verbal consent to participation. The consent designee will read a consent script and document that the study partner verbally consented to participation in the MAP trial. The waiver of written documentation of consent is available only to study partners, not to study participants.

Electronic consent procedure: to conduct informed consent electronically, the consent designee will perform the informed consent process as stated above but will utilize the VISION EDC instead of a paper consent form. First, the consent designee will walk the participant through creating a personal account in VISION. In their new VISION account, the participant will be taken to the electronic informed consent form (eICF), either in English or Spanish based on their preferred language and will have the opportunity to carefully review the electronic consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate, as with an in-person consent. After reviewing the document in the desired language, the participant will answer the questions in the consent form, sign and date the consent form, and save the form by using VISION's eICF functions. A link to the signed eICF will then be provided to the participant so they can access it to print or review at any time. Lastly, the VISION database will store and track audit trails for initial and re-consents.

Remote paper consent procedure: a copy of the IRB-approved ICF is provided to the participant prior to the remote consent meeting via email, fax, or mail. The consenting process may be conducted either via telephone or video. After the participant reviews the consent form, he/she is offered the opportunity to ask any questions and have those questions answered. The consent designee must verify the participant physically signed the ICF either by viewing this via video conference, obtaining a photo of the signed pages from the ICF, or obtaining verbal confirmation from the participant that he/she signed the consent form. The consent designee will sign their ICF copy after the participant has acknowledged signature on their copy. The signed pages of the ICF (optional components of the study (future contact, future use of biospecimens, undergoing MRI) and the site-specific section of the consent)) are then mailed, emailed,

photo/scanned to text, or faxed to the consent designee. Once the consent designee receives the signed pages of the ICF, as an electronic copy (emailed, photo/scanned to text, or faxed), or by mail, study-specific procedures may be commenced. The participant must return the original signed document on their first in-person visit. Once the participant's original copy is received, the consent designee will append this to their own signed copy and create a single document. No study-related procedures may occur until the consent designee is in possession of the signed ICF.

There will be three informed consent forms, one for the main study, one for the PET imaging sub-study, and one for volunteers (non-participants) undergoing MRI for calibration.

10.1.2 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to the investigator. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the sIRB, and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

- Circumstances that may warrant termination or suspension include, but are not limited to:
- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and/or FDA.

10.1.3 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their interventions. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the IRB, regulatory agencies or pharmaceutical company supplying study product may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the Johns Hopkins TIC DCC. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites and by the Johns Hopkins TIC DCC research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived at the Johns Hopkins TIC DCC.

Certificate of Confidentiality (if applicable)

To further protect the privacy of study participants, a Certificate of Confidentiality (CoC) will be issued by the NIH. This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, (CoCs) help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

10.1.4 FUTURE USE OF STORED SPECIMENS AND DATA

Data collected for this study will be analyzed and stored at the Johns Hopkins TIC DCC. After the study is completed, the de-identified, archived data will be transmitted to and stored in a NIH-approved public data repository (to be determined), for use by other researchers including those outside of the study. Permission to transmit data to the public Data Repository will be included in the informed consent.

With the participant's approval and as approved by local IRBs, de-identified biological samples will be stored at the CUMC Biospecimen Repository with the same goal as the sharing of data with the public data repository. These samples could be used to research the causes of Alzheimer's disease, its complications and other conditions for which individuals with Alzheimer's disease are at increased risk, and to improve treatment. The CUMC Biospecimen Repository will also be provided with a code-link that will allow linking the biological specimens with the phenotypic data from each participant, maintaining the blinding of the identity of the participant.

During the conduct of the study, an individual participant can choose to withdraw consent to have biological specimens stored for future research. However, withdrawal of consent with regard to bio-sample storage may not be possible after the study is completed.

When the study is completed, access to study data and/or samples will be provided through the CUMC Biospecimen Repository.

10.1.5 KEY ROLES AND STUDY GOVERNANCE

11.1.5.1 Overall Structure of the Study Team

The following is a description of the elements of the study team:

Principal Investigator: José Luchsinger, MD, MPH
Columbia University Irving Medical Center
630 West 168th Street,
New York, NY 10032

Medical Monitor: Natasha Mehta, MD, address as above

Administrative center based at CUMC. The center is led by Dr. Luchsinger who will be assisted by a full-time program manager responsible for the logistics of coordinating and communicating with all study components and clinical sites. Dr. Luchsinger will be responsible for all contacts with the funding agency, NIA.

Clinical coordinating and monitoring center (CCMC) based at CUMC. The CCMC will be led by Drs. Luchsinger and Terry Goldberg, who will be assisted by a senior coordinator. The CCMC will oversee QA and quality control (QC) related to the collection of the non-brain imaging data. The CCMC will organize remote training and certification in study procedures and will conduct QC procedures including double scoring of all neuropsychological tests and yearly site visits as needed. The PI will designate a medical monitor who will monitor adverse events in coordination with the CCMC and the clinical sites.

Data coordinating center (DCC) based at the John Hopkins TIC, led by Daniel Hanley, MD, Professor of Neurology, assisted by Lindsay M Eyzaguirre, MS, Project Administrator, Ying Wang, MS, Senior Research Data Analyst and Gayane Yenokyan, PhD, Associate Scientist, Biostatistics Consulting Center. The DCC will assist the CCMC in QC activities and will oversee QA/QC related to data upload and management. The DCC will also lead the randomization process.

Single IRB will be the Johns Hopkins Medicine IRB (JHM sIRB).

Imaging core will be located at the Johns Hopkins TIC, led by Dr. Hanley. The Imaging Core will oversee all QA/QC procedures related to acquisition, transfer, processing, and analysis of brain MRI images. The Imaging Core will conduct site visits in Year 1 of the study in order to harmonize image acquisition across sites.

PET core will be located at the University of California-Berkeley, led by William Jagust, MD.

Central research pharmacy function will be shared by the research pharmacies of the University of Rochester and University of Iowa. The University of Iowa will bottle IP received in bulk from the EMD Serono Research & Development Institute, and will send it to the University of Rochester, which will prepare kits for dispensation to clinical sites in coordination with the DCC, who will lead the randomization process.

Central laboratory function will be located at CALM at CUMC, led by Eldad Hod. CALM will prepare kits for all study sites with laboratory supplies per participant and a return package, send the kits to the clinical sites, receive them within 24 hours, process the samples and conduct laboratory assays, and return the results to the CCMC.

Data safety and monitoring board (DSMB) has four members: Anton Porsteinsson, MD (chair), Professor of Psychiatry at the University of Rochester, expert in Alzheimer's clinical trials; Steven Arnold, MD, Professor of Neurology at Harvard Medical school, expert in Alzheimer's disease with experience in metformin trials; Jonathan Purnell, MD, Professor of Medicine at Oregon Health and Science University, endocrinologist with expertise in metformin; Emilia Bagiella, PhD, Professor of Population Health Science and Policy at Mount Sinai, a statistician with expertise in clinical trials. The DCC will provide reports to the DSMB as needed. The DSMB will hold meetings or calls twice a year.

The previous elements will be coordinated by an executive committee that will hold weekly one-hour calls or convene as needed in case of contingencies. The executive committee will be led by José Luchsinger and will include Drs. Terry Goldberg, Devangere Devanand, and Daniel Hanley, representatives of the Johns Hopkins TIC, and three members from the clinical sites outside of CUMC. The executive committee will be charge of resolving any conflicts or contingencies, such as underperforming clinical sites. The executive committee and all the clinical site PIs and coordinators will convene once a year at a central meeting.

10.1.6 SAFETY OVERSIGHT

Safety oversight will be under the direction of a DSMB. Members of the DSMB should be independent from the study conduct and free of conflict of interest, or measures should be in place to minimize perceived conflict of interest. The DSMB will meet at least semiannually to assess safety and efficacy data on each arm of the study. The DSMB will operate under the rules of an approved charter that will be written and reviewed at the organizational meeting of the DSMB. At this time, each data element that the DSMB needs to assess will be clearly defined. The DSMB will provide its input to CUMC, Johns Hopkins TIC Data Coordinating Center, and NIH staff.

The DSMB will be responsible for the interests of the participants and, to this end, will undertake regular reviews of the safety data. The DSMB will have access to all study data throughout the study duration. If the DSMB finds it necessary to recommend actions regarding interruption of the study or changes to the protocol based on medical rationale that would make it unethical to continue the study in its present form, those recommendations will be forwarded to the sponsor who will then take appropriate action. The sponsor will make any changes, protocol amendments, etc. necessary to meet the recommendations of the DSMB. The sponsor will then propagate those changes to the site PI's and others as necessary. Details of the DSMB's functions and early stopping rules will be delineated in a separate DSMB charter.

The study may be stopped at any time if, in the opinion of the Sponsor, the Medical Monitor, or the DSMB, continuation of the study represents a serious medical risk to the participants. This may include, but is not limited to, the presence of serious, life threatening, or fatal AEs, or AEs that are unacceptable in nature, severity, or frequency.

10.1.7 CLINICAL MONITORING

Clinical site monitoring is conducted to ensure that the rights and well-being of trial participants are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with applicable regulatory requirement(s).

- The CCC located at Columbia University Irving Medical Center will provide centralized monitoring, throughout the study, and targeted data verification of endpoint, safety and other key data variables, and the distribution of monitoring reports.
- Independent audits will be conducted by the CCC located at Columbia University Irving Medical Center to ensure monitoring practices are performed consistently across all participating sites.
- The frequency of site contact will be monthly for routine monitoring. Monitoring will take place weekly while the site is under enhanced monitoring.

Central monitoring: site and project metrics will be compiled and reviewed routinely with the study sites and formally reviewed by the Columbia University Irving Medical Center monitoring team. Appropriate documentation will be retained in the electronic Trial Master File (eTMF), to include the findings of the review, whether any thresholds have been breached, and any action items to be addressed.

Site Management: As one of the "monitoring checks," a site manager will routinely communicate with sites via weekly project meeting and Study Coordinator calls biweekly. The monitor should make a qualitative assessment of the risk-level for the site and note new risk issues in the eTMF. If the site manager or monitor has material concerns about any aspect of site performance, he or she should escalate the site to enhanced monitoring until those concerns are addressed.

Source Document Verification (SDV): Sites will be required to enter data promptly with the participant's completed informed consent with 48 hours; all other data should be entered within 7 days of the visit and to upload associated source documents. (Note: Generally, the EDC will be the primary/original source of most study data (no source to review), albeit worksheets will be provided for critical assessments and medical history may be taken from clinic charts.) Monitors will review the EDC entries against the available source documents within 15 days of upload and generate associated queries. Sites are expected to resolve queries within 15 days. A site's failure to enter data, upload source documents or answer queries promptly is a risk-elevation issue that, if not promptly resolved, should trigger enhanced monitoring. The baseline percentage (approximate) of SDV review is stipulated as:

- CRF entries vs. source documents supporting critical data & processes: ≥ 80%
- Informed Consents (properly executed): 25%
- CRF entries vs. source documents on other data: 25%

The Johns Hopkins TIC Data Coordinating Center will develop a set of data verifications that will be run in off-line mode for the purposes of data cleaning, as stipulated in the Data Management Plan. Johns Hopkins TIC Data Coordinating Center will review the EDC entries for completeness, timeliness, compliance with the protocol and general accuracy with expected medical practice. Critical Data and Processes will be reviewed at a higher percentage than other data. Critical Data Points include:

- Age
- Gender
- BMI
- Documentation of the Total Recall Score of the Free and Cued Selective Reminding Test (FC-SRT)
- Documentation of the Alzheimer's Disease Cooperative Study, Preclinical Alzheimer Cognitive Composite Score (ADCS-PACC)
- In those participants who underwent MRI, upload of MRI Images at baseline and 18 months
- APOE-ε4 genotype

10.1.8 QUALITY ASSURANCE AND QUALITY CONTROL

Each clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation, and completion. An individualized quality management plan will be developed to describe a site's quality management.]

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written standard operating procedures (SOPs), the monitors will verify that the clinical trial is conducted, data are generated, and biological specimens are collected, documented (recorded), and reported in compliance with the protocol and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

10.1.9 DATA HANDLING AND RECORD KEEPING

10.1.9.1 Data Collection and Management Responsibilities

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

Hardcopies of the study visit worksheets will be provided for use as source document worksheets for recording data for each participant enrolled in the study. Data recorded in the electronic case report form (eCRF) derived from source documents should be consistent with the data recorded on the source documents.

Clinical data (including AEs and concomitant medications) and clinical laboratory data will be entered into Prelude Dynamics VISION, a 21 CFR Part 11-compliant data capture system provided by the Johns Hopkins TIC Data Coordinating Center. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

Responsibilities of the Johns Hopkins TIC data coordinating center

The responsibilities of the Johns Hopkins TIC Data Coordinating Center (DCC) in the MAP study include: trial design development, monitoring of trial performance, data management planning and execution, review and verification of Clinical Coordinating Center (CCC) data for analysis/presentation, statistical design and analysis, and reporting of results for various aspects of trial management (e.g., DSMB reporting, safety, site performance, study performance), and data sharing (e.g., publications, resource sharing). The DCC will collaborate closely with CCC throughout the duration of the trial and will rely on study leadership and CCC to provide the appropriate scientific and clinical guidance to ensure the successful completion of the responsibilities described above.

Trial design development

The statistical core of the DCC will participate in the following processes: a) Developing study hypotheses in collaboration with study PI and study team members; b) Randomization and stratification; c) Blinding strategies; d) Defining intervention and control groups; e) Establishing study population and estimating sample size; f) Selecting and operationalizing study endpoints; g) Developing Data Safety Monitoring Board (DSMB) analysis plan, and h) Developing of Statistical Analysis Plan (SAP) in collaboration with study leadership.

Data management planning and execution

DCC will ensure that the CRFs include all variables needed to address the study hypotheses, and safety and efficacy endpoints; and that no redundant or unnecessary variables are included. DCC will develop electronic data capture screens using Prelude Dynamics, LLC VISION™ EDC system. The design and development of the electronic database system will reflect the FDA Guidance for Industry for Computerized Systems Used in Clinical Trials (April 1999) as well as the Electronic Records/Electronic Signatures rule (21 CFR part 11).

When data is entered in VISION™ EDC, a secure and time-stamped electronic record is generated with full audit trail. It will allow real-time monitoring of data through reconstruction of the course of events relating to the creation, modification, and deletion of an electronic record. Source documents will be retained to enable a reconstruction and evaluation of the trial. The system will ensure that all applicable regulatory requirements for record keeping and record retention in clinical trials are met. Additional features of the

VISION™ EDC system include randomization, multi-site access, querying, site management, inventory management, invoicing, lab processing, user management and document management. Data from the EDC system will be exported in an XML/SAS format at pre-determined intervals. DCC will provide data ETL (Extract, Transform, Load) support and make data available in most of commonly used data formats.

Audit trail

An audit trail is a record of a sequence of events from which a history may be constructed. All changes made to data in the EDC are tracked and recorded in the database. This audit trail will capture the date/time, the contents of the changes made, and the login ID used to make the change. Query resolution correspondence will be maintained in EDC as well and eCRF edits will be tracked by the system. The audit trails will be created incrementally in chronological order with prevention of overwrite and are in compliance with the 21 CFR 11.10(e). Audit trail information will be reviewed by pre-authorized personnel if the need arises to verify the quality and integrity of the data.

Individuals who maintain the EDC system as well as the audit trail will carry the responsibilities to protect authenticity, integrity, and confidentiality of electronic records. Audit trails will be available for FDA inspectors at the study site or any other location where associated electronic study records are maintained.

Data validation and checks

The electronic CRFs will be designed to contain the prompts, lookup values, data type and range validation, cross-field logic check, and warning/error flags. This process ensures high quality of data is collected. On-line help is also available to encourage consistent use of clinical terminology across the study sites.

Security and back-up

The EDC system will be hosted on high availability servers in secure data center. The server will be strictly monitored and maintained by designated administrators at Prelude Dynamics. Users at the participating sites will be made aware of system security measures and the importance of limiting access to authorized personnel. Access to the data at a clinical site will be restricted and monitored by the system through required login, security verification procedures, and audit trail. VISION™ EDC implements role-based access control. It grants user access only to the forms or data fields that they need to see and prevents them from accessing data that doesn't pertain to them.

Each user will be assigned an individual account with a unique username, password and a role (such as investigator, project manager, site coordinator). Any user will be locked out after a pre-determined number of consecutive attempts, with any unauthorized access attempt recorded in a log file. Users will be required to exit the system upon leaving a workstation. The computer will automatically log off the current session when an idle period reaches a pre-determined length. For short periods of inactivity, the automatic screensaver will be password protected to prevent unauthorized access to the system.

Records will be backed up at pre-determined intervals to prevent a catastrophic loss compromising the quality and integrity of the data. Data will be backed up onto digital media, which will be stored at an offsite location. Backup and recovery logs will be maintained to facilitate an assessment of the nature and scope of data loss in the event of a system failure.

Written procedures describing contingency plans for continuing the study by alternate means in the event of hardware or facilities failures with alternate hardware or at an alternate site will be provided to each site. It should be noted that the data management procedures will reflect the advanced use of computer and software technology; include database technology, and electronic file management principles; and

therefore, be of the highest possible standards achievable for data security and information integrity. Specifically, the data center is SAS-70 Type II compliant, HIPAA-audited and certified for maintenance of banking, credit card, and PHI).

Organization of Study Data: All study data related documents will be organized as follows:

- Clinical Data: All data collected in the EDC system
- External files: Any data uploaded to the EDC system including PDF versions of consent forms, participant source docs, video files, and scans
- Site Management Data: All study site related data and documents collected in the EDC system
- Structural metadata: Information about forms, tables, and visit schedule
- Coding dictionary: Data dictionary indicating the variable names and types, labels and value labels for categorical variables
- Lab ranges: Reference ranges for lab values with version control if different versions are used
- Audit trail: Study audit trail maintained in a tamper-proof format
- Listing of edit checks and derived variables: Program files used for edit checks and for creating derived variables
- Discrepancy management logs: Listing of records with failed edit checks and information on how they were resolved
- Queries: Electronic copies of all queries and query correspondence
- Program code: Syntax code for data edit checks, data derivation and statistical analyses (interim and final) performed on the trial data with version control, if applicable
- Data management plan: Electronic copy of SOPs for data management with version control, if applicable
- Database lock: Procedures for database lock; final locked, de-identified databases and final analytic database(s) used for manuscript generation

Review and verification of data

Data quality assurance: Data entry error checks will be programmed and processed at predetermined intervals during the course of the study. The resulting queries will be sent to the site coordinators for resolution. Resolutions will be documented in the EDC system. A list of all queries that cannot be resolved will be maintained.

Weekly/Monthly Reports: DCC will prepare weekly/monthly screening, enrollment, and study monitoring reports for each site at pre-determined intervals during the course of the study. These reports are intended to provide input to each site on their performance and areas of improvement.

Statistical design and analysis

The statistical core of DCC in collaboration of study leadership and CCC, will develop a study monitoring plan and DSMB analysis plan to measure safety, site performance, and study performance and monitoring. Reports will be generated at pre-determined intervals by DCC and shared with sites, study leadership and DSMB members as appropriate.

A Statistical Analysis Plan (SAP) will be developed by the statistical core of DCC in collaboration with study leadership with detailed information on statistical methods being used for primary, secondary and tertiary outcomes, if applicable.

Reporting of results

DSMB analysis plan: Interim safety analyses will be prepared for the external DSMB on a pre-determined schedule (such as semi-annually or after enrollment of a fixed number of participants) to evaluate efficacy and safety. Prior to DSMB report generation, the CCC will work with the enrolling sites to finalize the case forms and complete the resolution of all pending data queries. DCC will ensure that all the variables needed for the DSMB are reviewed and prepare an analytic database. DCC will then conduct all requested analyses, and compile suitable reports, tables and graphs on blinded and unblinded treatment assignment data, if applicable.

SAP: DCC will run the entire database through the edit check programs and deem the database clean and locked. DCC will then create analytic programs with syntax for derived variables and statistical procedures along with comments to indicate the purpose of each analysis. DCC will also generate tables and graphs required for the manuscript.

Data sharing

Final data archiving: At the conclusion of the study when the database has been locked, all entered participant data and uploaded documents in the EDC system will be archived and provided to the site on flash drives. Regulations require that study documents (including the archive CDs and any study documents not uploaded to the EDC) must be retained in the files of the responsible investigator for potential review by regulatory agencies.

ClinicalTrials.gov: DCC will share the final de-identified analytic database, study protocol, SAP, manual of operations, and published manuscript with NIA through a secure NIH dropbox/FTP site for public access. The final study results published in the peer-reviewed journal manuscript will be entered into the Protocol Registration and Results (PRS) system on the ClinicalTrials.gov website. After completion of review, ClinicalTrials.gov will release study data to the public.

10.1.9.2 Study Records Retention

Health Insurance Portability and Accountability Act Under the HIPAA Privacy Rule, participants have the right to ask for an accounting of certain disclosures of their identifiable health information for a period dating 6 years from the date of the last covered disclosure. To ensure that sites can meet this accounting requirement, investigators must retain study records, along with records of all disclosures of study information, for at least 7 years after either of the following (whichever is later):

- The last participant has completed his or her participation in the study; or,
- The date of the last disclosure of identifiable health information from study records if disclosures continue after all participants have completed the study. [45 CFR 164.528]

This requirement to retain study records and to account for disclosures also applies to research that involves the secondary use of medical records or other identifiable health information.

Federally funded research and FDA-regulated research

DHHS regulations require that, "records relating to research which is conducted shall be retained for at least 3 years after completion of the research." [45 CFR 46.115(b)]

10.1.10 PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the clinical trial protocol, or Manual of Procedures (MOP) requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

It is the responsibility of the site investigator to use continuous vigilance to identify and report deviations within the required number of working days of identification of the protocol deviation. All deviations must be addressed in study source documents, reported to the NIA Program Official and Johns Hopkins TIC Data Coordinating Center. Protocol deviations must be sent to the reviewing sIRB per their policies. The site investigator is responsible for knowing and adhering to the reviewing sIRB requirements. Further details about the handling of protocol deviations will be included in the MOP.

Emergency deviations require reporting to the sIRB promptly after they occur

Emergency deviations are those occurring in an emergency situation, such as when a departure from the protocol is required immediately to protect the life or physical well-being of a participant. In such cases there is no time to prospectively seek the approval of the IRB. The sponsor and the sIRB must be notified as soon as possible, but not later than 5 days after the emergency situation occurred (21 CFR 812.150(a)(4)). Deviations of this nature are always considered to be unanticipated problems involving risks to participants or others (see JHM IRB Policy No. 103.6(b)).

Major, non-emergent deviations require approval by the sIRB before they occur

Major, non-emergent deviations are planned deviations that are non-emergent and represent a major change in the approved protocol. These deviations are changes the IRB must approve before the proposed change is implemented. Examples include exceptions to eligibility criteria, exceptions to the form and manner of obtaining informed consent, and exceptions to the schedule of administration of an investigational product. If a planned major, non-emergent deviation occurs without prior IRB approval, the event is non-compliance, which must be reported promptly to the sIRB. A PI's failure to report promptly any major, non-emergent deviation for which the PI did not obtain prior approval is itself an incident of non-compliance. Incidents of non-compliance will be managed in accordance with the JHM IRB Organization Policy on Investigator Non-Compliance Policy No. 103.7.

Minor or administrative protocol deviations require reporting to the sIRB at continuing review

Minor or administrative deviations are those which do not "affect the scientific soundness of the research plan or the rights, safety, or welfare of human subjects." If a protocol deviation occurs which meets this definition, the deviation should be reported to the JHM IRB at the time the continuing review application is submitted in eIRB using the JHM Protocol Deviation Summary Sheet. Examples of minor or administrative deviations include follow-up visits occurring outside the protocol required time frame because of the participant's schedule, or blood samples being obtained at times close to but not precisely at the time points specified in the protocol.

10.1.11 PUBLICATION AND DATA SHARING POLICY

This study will be conducted in accordance with the following publication and data sharing policies and regulations:

National Institutes of Health (NIH) Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

This study will comply with the NIH Data Sharing Policy and Policy on the Dissemination of NIH-Funded Clinical Trial Information and the Clinical Trials Registration and Results Information Submission rule. As such, this trial will be registered at ClinicalTrials.gov, and results information from this trial will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed

journals. Data from this study may be requested from other researchers 7 years after the completion of the primary endpoint by contacting the PI, José Luchsinger, MD.

In addition, this study will comply with the NIH Genomic Data Sharing Policy, which applies to all NIH-funded research that generates large-scale human or non-human genomic data, as well as the use of these data for subsequent research. Large-scale data include genome-wide association studies (GWAS) and SNP arrays, genome sequence, transcriptomic, epigenomic, and gene expression data.

10.1.12 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the NIA has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

10.2 ABBREVIATIONS

AD	Alzheimer's disease
ADAS-cog	Alzheimer's Disease Assessment Scale-cognitive subscale
ADCS-PACC	Alzheimer's Disease Cooperative Study, Preclinical Alzheimer Cognitive Composite
ADDF	Alzheimer's disease Drug Discovery Foundation
ADNI	Alzheimer's Disease Neuroimaging Initiative
AGE	advanced glycation end products
AE	adverse event
aMCI	amnestic mild cognitive impairment
ANCOVA	analysis of covariance
APA	Active Place Avoidance
BMI	body mass index
BMP	basic metabolic panel
CALM	Center for Advanced Laboratory Medicine
CAPI	computer-assisted personal interviews
CBC	complete blood count
CCMC	clinical coordinating and monitoring center
CDR	clinical dementia rating
CFR	Code of Federal Regulations
CGIC-MCI	clinical global impression of change for mild cognitive impairment
CHF	congestive heart failure
CMSU	Clinical Materials Services Unit (at the University of Rochester)
CoC	certificate of confidentiality
CONSORT	Consolidated Standards of Reporting Trials
COVID-19	coronavirus disease 2019
CRC	clinical research coordinator
CRF	case report form

CTSA	Clinical Translational Science Award
CUMC	Columbia University Medical Center
DCC	data coordinating center
DPP	Diabetes Prevention Program
DPPOS	Diabetes Prevention Program Outcomes Study
DSMB	data safety monitoring board
EC	ethics committee
eCRF	electronic case report forms
EDC	electronic data capture
ETL	Extract, Transform, Load
FC-SRT	Free and Cued Selective Reminding Test
FDA	Food and Drug Administration
FDG	F-labeled 2-deoxy-2-fluoro-D-glucose
GCP	good clinical practice
GFR	glomerular filtration rates
GLP	good laboratory practices
GLP1	Glucagon like peptide-1
GMP	good manufacturing practices
GPA	glucagon-like peptide agonists
GWAS	genome-wide association studies
HbA1c	hemoglobin A1c
HIPAA	Health Insurance Portability and Accountability Act
HOMA	homeostatic model assessment
IADL	Instrumental Activities of Daily Living
IB	investigator's brochure
ICF	informed consent form
ICH	International Conference on Harmonization
IDE	insulin degrading enzyme
IND	investigational new drug application
IP	Investigational product
IRB	institutional review board
ITT	intention-to-treat
JHM	Johns Hopkins Medicine
JHU	Johns Hopkins University
MAC-Q	Memory Complaint Questionnaire
MAP	Metformin in Alzheimer's dementia Prevention
MCI	mild cognitive impairment
MetMCI	Phase II trial of metformin in aMCI
MI	myocardial infarction
MOP	manual of procedures
MMSE	Mini-Mental Status Exam
MRI	magnetic resonance imaging
NACC	National Alzheimer's Coordinating Centers
NCATS	National Center for Advancing Translational Sciences
NCT	national clinical trial

NHLBI	National Heart, Lung, and Blood Institute
NIA	National Institute on Aging
NIH	National Institutes of Health
OHRP	Office for Human Research Protections
PET	positron emission tomography
PHI	protected health information
PI	principal investigator
QA	quality assurance
QC	quality control
RIC	recruitment innovation center
RPR	rapid plasma reagin
SAE	serious adverse event
SAP	statistical analysis plan
SDV	source document verification
SOP	standard operating procedure
SNP	single nucleotide polymorphisms
SRT	Selective Reminding Test
SUSAR	suspected unexpected serious adverse reaction
SUVR	standardized uptake value ratio
TIC	trial innovation center
TICS	telephone interview for cognitive status
TSH	thyroid stimulating hormone
UBACC	University of California, San Diego Brief Assessment of Capacity to Consent
UDS	uniform data set
UIP	University of Iowa Pharmaceuticals
UP	unanticipated problem
US	United States

10.3 PROTOCOL AMENDMENT HISTORY

The table below is intended to capture changes of IRB-approved versions of the protocol, including a description of the change and rationale. A comprehensive Summary of Changes table for the current amendment is provided separately.

Version	Date	Description of Change	Brief Rationale
1.1	14 Feb 2020	See Protocol v1.1 SOC	Major changes in protocol activities and schedule
1.2	20 May 2020	See Protocol v1.2 SOC	Minor changes and clarifications
1.3	29 Sep 2020	See Protocol v1.3 SOC	Added PET sub-study
1.4	22 Dec 2020	See Protocol v1.4 SOC	Minor changes and clarifications
1.6	10 Aug 2021	See Protocol v1.6 SOC	Updated inclusion/exclusion criteria and minor changes
1.7	10 May 2022	See Protocol v1.7 SOC	Updated inclusion/exclusion criteria, verbal consent of study partner, two-month period from randomization to start of study drug to allow for imaging.
1.8	30 Jun 2022	1. Change of study drug kits to non-site-subject- and visit-specific. 2. Addition of option for remote consenting by paper. 3. Defining physical exam as brief neurological exam and anthropometric measures.	1. Facilitate drug supply; 2. Facilitate consenting process. 3. Clarify that full physical exam is not performed.
1.9	24 April 2023	See Protocol v1.9 SOC	Two major changes: decrease in sample size to 326 from 370 and decrease in follow-up duration to 18 months from 24 months. Other changes are relatively minor clarifications.
2.0	21 May 2024	See Protocol v2.0 SOC	Clarified three exclusion criteria and added one additional exclusion criterion. Also updated information about plasma AD biomarkers and changed them from exploratory to secondary outcomes.

Table 8. History of Major Protocol Changes and Rationale

11 REFERENCES

1. Ritchie K, Lovestone S. The dementias. *Lancet*. Nov 30 2002;360(9347):1759-66.
2. Evans DA, Funkenstein HH, Albert MS, et al. Prevalence of Alzheimer's disease in a community population of older persons. Higher than previously reported. *Jama*. 1989;262(18):2551-6.
3. Brookmeyer R, Gray S, Kawas C. Projections of Alzheimer's disease in the United States and the public health impact of delaying disease onset. *Am J Public Health*. 1998;88(9):1337-42.
4. Petersen RC, Roberts RO, Knopman DS, et al. Mild Cognitive Impairment: Ten Years Later. *Arch Neurol*. December 1, 2009 2009;66(12):1447-1455. doi:10.1001/archneurol.2009.266
5. Cummings JL. Alzheimer's Disease. *N Engl J Med*. July 1, 2004 2004;351(1):56-67.
6. Plassman BL, Langa KM, Fisher GG, et al. Prevalence of Cognitive Impairment without Dementia in the United States. *Ann Intern Med*. March 18, 2008 2008;148(6):427-434.
7. Small SA, Mayeux R. A clinical approach to memory decline. *J Pract Psychiatry Behav Health*. 1999;5:87-94.
8. Selkoe DJ. The origins of Alzheimer disease: a is for amyloid. *Jama*. 2000;283(12):1615-7.
9. Honig LS, Vellas B, Woodward M, et al. Trial of Solanezumab for Mild Dementia Due to Alzheimer's Disease. *New England Journal of Medicine*. 2018/01/25 2018;378(4):321-330. doi:10.1056/NEJMoa1705971
10. Doody RS, Thomas RG, Farlow M, et al. Phase 3 Trials of Solanezumab for Mild-to-Moderate Alzheimer's Disease. *New England Journal of Medicine*. 2014/01/23 2014;370(4):311-321. doi:10.1056/NEJMoa1312889
11. Salloway S, Sperling R, Fox NC, et al. Two Phase 3 Trials of Bapineuzumab in Mild-to-Moderate Alzheimer's Disease. *New England Journal of Medicine*. 2014/01/23 2014;370(4):322-333. doi:10.1056/NEJMoa1304839
12. Egan MF, Kost J, Tariot PN, et al. Randomized Trial of Verubecestat for Mild-to-Moderate Alzheimer's Disease. *New England Journal of Medicine*. 2018/05/03 2018;378(18):1691-1703. doi:10.1056/NEJMoa1706441
13. Doody RS, Raman R, Farlow M, et al. A Phase 3 Trial of Semagacestat for Treatment of Alzheimer's Disease. *New England Journal of Medicine*. 2013/07/25 2013;369(4):341-350. doi:10.1056/NEJMoa1210951
14. Karran E, Hardy J. Antiamyloid therapy for Alzheimer's disease--are we on the right road? Comment Editorial. *N Engl J Med*. Jan 23 2014;370(4):377-8. doi:10.1056/NEJMe1313943
15. Luchsinger JA. Type 2 diabetes, related conditions, in relation and dementia: an opportunity for prevention? *J Alzheimers Dis*. 2010;20(3):723-36. doi:10.3233/JAD-2010-091687
16. Biessels GJ, Strachan MWJ, Visseren FLJ, Kappelle LJ, Whitmer RA. Dementia and cognitive decline in type 2 diabetes and prediabetic stages: towards targeted interventions. *The Lancet Diabetes & Endocrinology*. 3// 2014;2(3):246-255. doi:*http://dx.doi.org/10.1016/S2213-8587(13)70088-3*
17. Festa A, Williams K, D'Agostino R, Jr., Wagenknecht LE, Haffner SM. The Natural Course of Beta-Cell Function in Nondiabetic and Diabetic Individuals: The Insulin Resistance Atherosclerosis Study. *Diabetes*. April 1, 2006 2006;55(4):1114-1120.
18. Grundy SM, Cleeman JI, Daniels SR, et al. Diagnosis and Management of the Metabolic Syndrome: An American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation*. October 25, 2005 2005;112(17):2735-2752.
19. Boden-Albala B, Cammack S, Chong J, et al. Diabetes, fasting glucose levels, and risk of ischemic stroke and vascular events: findings from the Northern Manhattan Study (NOMAS). *Diabetes Care*. Jun 2008;31(6):1132-7. doi:dc07-0797 [pii]
10.2337/dc07-0797
20. Snowdon DA, Greiner LH, Mortimer JA, Riley KP, Greiner PA, Markesbery WR. Brain infarction and the clinical expression of Alzheimer disease. The Nun Study. *Jama*. 1997;277(10):813-7.
21. Schneider JA, Wilson RS, Cochran EJ, et al. Relation of cerebral infarctions to dementia and cognitive function in older persons. *Neurology*. April 8, 2003 2003;60(7):1082-1088.
22. Schneider JA, Wilson RS, Bienias JL, Evans DA, Bennett DA. Cerebral infarctions and the likelihood of dementia from Alzheimer disease pathology. *Neurology*. April 13, 2004 2004;62(7):1148-1155.
23. Schneider JA, Arvanitakis Z, Bang W, Bennett DA. Mixed brain pathologies account for most dementia cases in community-dwelling older persons. *Neurology*. December 11, 2007 2007;69(24):2197-2204. doi:10.1212/01.wnl.0000271090.28148.24
24. Craft S, Watson GS. Insulin and neurodegenerative disease: shared and specific mechanisms. *Lancet Neurol*. Mar 2004;3(3):169-78. doi:10.1016/S1474-4422(04)00681-7

25. Farris W, Mansourian S, Chang Y, et al. Insulin-degrading enzyme regulates the levels of insulin, amyloid beta-protein, and the beta-amyloid precursor protein intracellular domain in vivo. *Proc Natl Acad Sci U S A*. Apr 1 2003;100(7):4162-7.
26. Vekrellis K, Ye Z, Qiu WQ, et al. Neurons regulate extracellular levels of amyloid beta-protein via proteolysis by insulin-degrading enzyme. *J Neurosci*. 2000;20(5):1657-65.
27. Qiu WQ, Walsh DM, Ye Z, et al. Insulin-degrading enzyme regulates extracellular levels of amyloid beta-protein by degradation. *J Biol Chem*. 1998;273(49):32730-8.
28. Farris W, Mansourian S, Leissring MA, et al. Partial Loss-of-Function Mutations in Insulin-Degrading Enzyme that Induce Diabetes also Impair Degradation of Amyloid {beta}-Protein. *Am J Pathol*. April 1, 2004 2004;164(4):1425-1434.
29. de la Monte SM. Insulin resistance and Alzheimer's disease. *BMB Rep*. Aug 31 2009;42(8):475-81.
30. de la Monte SM, Wands JR. Alzheimer's disease is type 3 diabetes-evidence reviewed. *J Diabetes Sci Technol*. Nov 2008;2(6):1101-13.
31. Luchsinger JA, Tang M-X, Shea S, Mayeux R. Hyperinsulinemia and risk of Alzheimer disease. *Neurology*. October 12, 2004 2004;63(7):1187-1192.
32. Peila R, Rodriguez BL, White LR, Launer LJ. Fasting insulin and incident dementia in an elderly population of Japanese-American men. *Neurology*. July 27, 2004 2004;63(2):228-233.
33. Wu W, Brickman AM, Luchsinger J, et al. The brain in the age of old: the hippocampal formation is targeted differentially by diseases of late life. *Ann Neurol*. Dec 2008;64(6):698-706. doi:10.1002/ana.21557
34. Baker LD, Cross DJ, Minoshima S, Belongia DA, Watson GS, Craft S. INSULIN RESISTANCE IS ASSOCIATED WITH ALZHEIMER-LIKE REDUCTIONS 2 IN REGIONAL CEREBRAL GLUCOSE METABOLISM FOR COGNITIVELY NORMAL ADULTS 3 WITH PRE-DIABETES OR EARLY TYPE 2 DIABETES. *Arch Neurol*. 2010;
35. Matsuzaki T, Sasaki K, Tanizaki Y, et al. Insulin resistance is associated with the pathology of Alzheimer's disease: the Hisayama Study. *Neurology*. 2010;
36. Craft S, Asthana S, Schellenberg G, et al. Insulin effects on glucose metabolism, memory, and plasma amyloid precursor protein in Alzheimer's disease differ according to apolipoprotein-E genotype. *Ann N Y Acad Sci*. Apr 2000;903:222-8.
37. Watson GS, Craft S. Insulin resistance, inflammation, and cognition in Alzheimer's Disease: lessons for multiple sclerosis. *J Neurol Sci*. Jun 15 2006;245(1-2):21-33. doi:10.1016/j.jns.2005.08.017
38. Greenberg AS, Obin MS. Obesity and the role of adipose tissue in inflammation and metabolism. *Am J Clin Nutr*. Feb 2006;83(2):461S-465S.
39. Yan SD, Bierhaus A, Nawroth PP, Stern DM. RAGE and Alzheimer's disease: a progression factor for amyloid-beta-induced cellular perturbation? *J Alzheimers Dis*. Apr 2009;16(4):833-43. doi:D6621608N32478R2 [pii]
10.3233/JAD-2009-1030
40. Luchsinger JA. Type 2 diabetes, related conditions, in relation and dementia: an opportunity for prevention? *J Alzheimers Dis*. 20(3):723-36. doi:J718863P17M14978 [pii]
10.3233/JAD-2010-091687
41. The Diabetes Prevention Program Research G. Role of Insulin Secretion and Sensitivity in the Evolution of Type 2 Diabetes in the Diabetes Prevention Program: Effects of Lifestyle Intervention and Metformin. *Diabetes*. August 1, 2005 2005;54(8):2404-2414.
42. The-DREAM-Investigators. Effect of rosiglitazone on the frequency of diabetes in patients with impaired glucose tolerance or impaired fasting glucose: a randomised controlled trial. *The Lancet*. 2006;368(9541):1096-1105.
43. Knowler WC, Barrett-Connor E, Fowler SE, et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med*. Feb 7 2002;346(6):393-403.
44. Reger MA, Watson GS, Green PS, et al. Intranasal insulin improves cognition and modulates {beta}-amyloid in early AD. *Neurology*. February 5, 2008 2008;70(6):440-448. doi:10.1212/01.wnl.0000265401.62434.36
45. DeFronzo RA. Pharmacologic therapy for type 2 diabetes mellitus. *Annals of Internal Medicine*. 2000;133(1):73-4.
46. Friedrich MJ. New research on Alzheimer treatments ventures beyond plaques and tangles. News. *JAMA*. Dec 26 2012;308(24):2553-5. doi:10.1001/jama.2012.93816
47. Yki-Jarvinen H. Thiazolidinediones. *N Engl J Med*. September 9, 2004 2004;351(11):1106-1118.
48. Kahn SE, Haffner SM, Heise MA, et al. Glycemic durability of rosiglitazone, metformin, or glyburide monotherapy. *N Engl J Med*. Dec 7 2006;355(23):2427-43.

49. Nathan DM, Berkwits M. Trials that matter: rosiglitazone, ramipril, and the prevention of type 2 diabetes. *Ann Intern Med*. Mar 20 2007;146(6):461-3.
50. Nathan DM. Rosiglitazone and Cardiotoxicity -- Weighing the Evidence. *N Engl J Med*. June 5, 2007 2007:NEJMe078117. doi:10.1056/NEJMe078117
51. Risner ME, Saunders AM, Altman JF, et al. Efficacy of rosiglitazone in a genetically defined population with mild-to-moderate Alzheimer's disease. *Pharmacogenomics J*. Jul-Aug 2006;6(4):246-54.
52. Watson GS, Cholerton BA, Reger MA, et al. Preserved Cognition in Patients With Early Alzheimer Disease and Amnestic Mild Cognitive Impairment During Treatment With Rosiglitazone: A Preliminary Study. *Am J Geriatr Psychiatry*. November 1, 2005 2005;13(11):950-958. doi:10.1176/appi.ajgp.13.11.950
53. Lincoff AM, Wolski K, Nicholls SJ, Nissen SE. Pioglitazone and Risk of Cardiovascular Events in Patients With Type 2 Diabetes Mellitus: A Meta-analysis of Randomized Trials. *JAMA*. September 12, 2007 2007;298(10):1180-1188. doi:10.1001/jama.298.10.1180
54. Alzforum. There's No Tomorrow for TOMMORROW. Updated 01/25/2018. Accessed 06/01/2018, 2018.
55. Luchsinger JA. Type 2 Diabetes and Related Conditions in Relation to Dementia: An Opportunity for Prevention? *J Alzheimers Dis*. doi:J718863P17M14978 [pii]
10.3233/JAD-2010-091687 Accessed Apr 22. *http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=20413862*
56. Bailey CJ, Turner RC. Metformin. *N Engl J Med*. February 29, 1996 1996;334(9):574-579.
57. Dunn CJ, Peters DH. Metformin. A review of its pharmacological properties and therapeutic use in non-insulin-dependent diabetes mellitus.721-49, 1995 May.
58. Tian J, Shi J, Bailey K, Lendon CL, Pickering-Brown SM, Mann DMA. Association between apolipoprotein E e4 allele and arteriosclerosis, cerebral amyloid angiopathy, and cerebral white matter damage in Alzheimer's disease. *J Neurol Neurosurg Psychiatry*. May 1, 2004 2004;75(5):696-699.
59. The Diabetes Prevention Program Research Group. Intensive Lifestyle Intervention or Metformin on Inflammation and Coagulation in Participants With Impaired Glucose Tolerance. *Diabetes*. May 1, 2005 2005;54(5):1566-1572.
60. Orchard TJ, Temprosa M, Goldberg R, et al. The Effect of Metformin and Intensive Lifestyle Intervention on the Metabolic Syndrome: The Diabetes Prevention Program Randomized Trial. *Ann Intern Med*. April 19, 2005 2005;142(8):611-619.
61. Diabetes Prevention Program Research Group. Reduction in the Incidence of Type 2 Diabetes with Lifestyle Intervention or Metformin. *N Engl J Med*. February 7, 2002 2002;346(6):393-403.
62. Nathan DM. Initial Management of Glycemia in Type 2 Diabetes Mellitus. *N Engl J Med*. October 24, 2002 2002;347(17):1342-1349.
63. Knowler WC, Fowler SE, Hamman RF, et al. 10-year follow-up of diabetes incidence and weight loss in the Diabetes Prevention Program Outcomes Study. *Lancet*. Nov 14 2009;374(9702):1677-86. doi:S0140-6736(09)61457-4 [pii]
10.1016/S0140-6736(09)61457-4
64. Koenig AM, Mechanic-Hamilton D, Xie SX, et al. Effects of the Insulin Sensitizer Metformin in Alzheimer's Disease: Pilot Data from a Randomized Placebo-Controlled Crossover Study. *Alzheimer disease and associated disorders*. Apr-Jun 2017;31(2):107-113. doi:10.1097/WAD.0000000000000202
65. Park CR. Cognitive effects of insulin in the central nervous system. *Neurosci Biobehav Rev*. 2001;25(4):311-23.
66. Watson GS, Craft S. Modulation of memory by insulin and glucose: neuropsychological observations in Alzheimer's disease. *European Journal of Pharmacology*. 2004/4/19 2004;490(1-3):97-113.
67. Ishibashi Y, Matsui T, Takeuchi M, Yamagishi S. Beneficial effects of metformin and irbesartan on advanced glycation end products (AGEs)-RAGE-induced proximal tubular cell injury. Research Support, Non-U.S. Gov't. *Pharmacological research : the official journal of the Italian Pharmacological Society*. Mar 2012;65(3):297-302. doi:10.1016/j.phrs.2011.11.001
68. Ishibashi Y, Matsui T, Takeuchi M, Yamagishi S. Metformin Inhibits Advanced Glycation End Products (AGEs)-induced Renal Tubular Cell Injury by Suppressing Reactive Oxygen Species Generation via Reducing Receptor for AGEs (RAGE) Expression. *Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme*. Nov 2012;44(12):891-5. doi:10.1055/s-0032-1321878

69. Mallik R, Chowdhury TA. Metformin in cancer. *Diabetes Research and Clinical Practice*. doi:*https://doi.org/10.1016/j.diabres.2018.05.023*
70. Barzilai N, Crandall JP, Kritchevsky SB, Espeland MA. Metformin as a Tool to Target Aging. *Cell Metabolism*. 2016/06/14/ 2016;23(6):1060-1065. doi:*https://doi.org/10.1016/j.cmet.2016.05.011*
71. McCreight LJ, Bailey CJ, Pearson ER. Metformin and the gastrointestinal tract. *Diabetologia*. 2016;59:426-435. doi:10.1007/s00125-015-3844-9
72. DeFronzo R, Fleming GA, Chen K, Bicsak TA. Metformin-associated lactic acidosis: Current perspectives on causes and risk. *Metabolism*. 2016/02/01/ 2016;65(2):20-29. doi:*https://doi.org/10.1016/j.metabol.2015.10.014*
73. Reinstatler L, Qi YP, Williamson RS, Garn JV, Oakley GP. Association of Biochemical B12 Deficiency With Metformin Therapy and Vitamin B12 Supplements. 10.2337/dc11-1582. *Diabetes Care*. 2012;35(2):327.
74. Smith AD, Refsum H. Homocysteine, B Vitamins, and Cognitive Impairment. *Annual Review of Nutrition*. 2016/07/17 2016;36(1):211-239. doi:10.1146/annurev-nutr-071715-050947
75. Chen Y, Zhou K, Wang R, et al. Antidiabetic drug metformin (GlucophageR) increases biogenesis of Alzheimer's amyloid peptides via up-regulating BACE1 transcription. *Proc Natl Acad Sci U S A*. 2009;106(10):3907-12.
76. Son SM, Shin HJ, Byun J, et al. Metformin Facilitates Amyloid-beta Generation by beta- and gamma-Secretases via Autophagy Activation. *J Alzheimers Dis*. 2016;51(4):1197-208. doi:10.3233/JAD-151200
77. Chung M-M, Chen Y-L, Pei D, et al. The neuroprotective role of metformin in advanced glycation end product treated human neural stem cells is AMPK-dependent. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*. 2015/05/01/ 2015;1852(5):720-731. doi:*https://doi.org/10.1016/j.bbadis.2015.01.006*
78. Qi B, Hu L, Zhu L, et al. Metformin Attenuates Cognitive Impairments in Hypoxia–Ischemia Neonatal Rats via Improving Remyelination. *Cellular and Molecular Neurobiology*. 2017/10/01 2017;37(7):1269-1278. doi:10.1007/s10571-016-0459-8
79. Ou Z, Kong X, Sun X, et al. Metformin treatment prevents amyloid plaque deposition and memory impairment in APP/PS1 mice. *Brain, Behavior, and Immunity*. 2018/03/01/ 2018;69:351-363. doi:*https://doi.org/10.1016/j.bbi.2017.12.009*
80. Imfeld P, Bodmer M, Jick SS, Meier CR. Metformin, other antidiabetic drugs, and risk of Alzheimer's disease: a population-based case-control study. Research Support, Non-U.S. Gov't. *Journal of the American Geriatrics Society*. May 2012;60(5):916-21. doi:10.1111/j.1532-5415.2012.03916.x
81. Moore EM, Mander AG, Ames D, et al. Increased Risk of Cognitive Impairment in Patients With Diabetes Is Associated With Metformin. *Diabetes Care*. 2013;36(10):2981-2987.
82. Luchsinger JA, Ma Y, Christophi CA, et al. Metformin, Lifestyle Intervention, and Cognition in the Diabetes Prevention Program Outcomes Study. 10.2337/dc16-2376. *Diabetes Care*. 2017;
83. Orkaby AR, Cho K, Cormack J, Gagnon DR, Driver JA. Metformin vs sulfonylurea use and risk of dementia in US veterans aged ≥65 years with diabetes. *Neurology*. 2017;89(18):1877. doi:10.1212/WNL.0000000000004586
84. Austin PC. An Introduction to Propensity Score Methods for Reducing the Effects of Confounding in Observational Studies. *Multivariate Behavioral Research*. 06/08 2011;46(3):399-424. doi:10.1080/00273171.2011.568786
85. Ali MS, Groenwold Rolf HH, Pestman Wiebe R, et al. Propensity score balance measures in pharmacoepidemiology: a simulation study. *Pharmacoepidemiology and Drug Safety*. 2014/08/01 2014;23(8):802-811. doi:10.1002/pds.3574
86. Jack CR, Jr., Knopman DS, Jagust WJ, et al. Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers. *Lancet Neurol*. Feb 2013;12(2):207-16. doi:10.1016/S1474-4422(12)70291-0
87. Jack CR, Jr., Knopman DS, Jagust WJ, et al. Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. *Lancet Neurol*. Jan 2010;9(1):119-28.
88. Jack CR, Jr., Bennett DA, Blennow K, et al. NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. *Alzheimer's & dementia : the journal of the Alzheimer's Association*. 2018;14(4):535-562. doi:10.1016/j.jalz.2018.02.018
89. Olsson B, Lautner R, Andreasson U, et al. CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis. *The Lancet Neurology*. 2016/06/01/ 2016;15(7):673-684. doi:*https://doi.org/10.1016/S1474-4422(16)00070-3*

90. Ossenkoppele R, Jansen WJ, Rabinovici GD, et al. Prevalence of amyloid PET positivity in dementia syndromes: a meta-analysis. *JAMA*. 2015;313(19):1939-1949. doi:10.1001/jama.2015.4669
91. James OG, Doraiswamy PM, Borges-Neto S. PET Imaging of Tau Pathology in Alzheimer's Disease and Tauopathies. Review. *Frontiers in neurology*. 2015;6:38. doi:10.3389/fneur.2015.00038
92. Schwarz CG, Gunter JL, Wiste HJ, et al. A large-scale comparison of cortical thickness and volume methods for measuring Alzheimer's disease severity. *NeuroImage : Clinical*. 2016;11:802-812. doi:10.1016/j.nicl.2016.05.017
93. Viggars AP, Wharton SB, Simpson JE, et al. Alterations in the blood brain barrier in ageing cerebral cortex in relationship to Alzheimer-type pathology: a study in the MRC-CFAS population neuropathology cohort. *Neurosci Lett*. 2011// 2011;505
94. Montagne A, Barnes SR, Sweeney MD, et al. Blood-Brain Barrier Breakdown in the Aging Human Hippocampus. *Neuron*. 2015;85(2):296-302. doi:10.1016/j.neuron.2014.12.032
95. Baird AL, Westwood S, Lovestone S. Blood-Based Proteomic Biomarkers of Alzheimer's Disease Pathology. *Frontiers in neurology*. 2015;6:236. doi:10.3389/fneur.2015.00236
96. Andreasson U, Blennow K, Zetterberg H. Update on ultrasensitive technologies to facilitate research on blood biomarkers for central nervous system disorders. *Alzheimer's & Dementia : Diagnosis, Assessment & Disease Monitoring*. 06/25 2016;3:98-102. doi:10.1016/j.dadm.2016.05.005
97. Zetterberg H, Mörtberg E, Song L, et al. Hypoxia Due to Cardiac Arrest Induces a Time-Dependent Increase in Serum Amyloid β Levels in Humans. *PLoS ONE*. 2011;6(12):e28263. doi:10.1371/journal.pone.0028263
98. Verberk IMW, Slot RE, Verfaillie SCJ, et al. Plasma Amyloid as Prescreener for the Earliest Alzheimer Pathological Changes. *Ann Neurol*. Sep 9 2018;doi:10.1002/ana.25334
99. Palmqvist S, Janelidze S, Stomrud E, et al. Performance of Fully Automated Plasma Assays as Screening Tests for Alzheimer Disease-Related beta-Amyloid Status. *JAMA Neurol*. Jun 24 2019;doi:10.1001/jamaneurol.2019.1632
100. Vergallo A, Megret L, Lista S, et al. Plasma amyloid beta 40/42 ratio predicts cerebral amyloidosis in cognitively normal individuals at risk for Alzheimer's disease. *Alzheimers Dement*. Jun 2019;15(6):764-775. doi:10.1016/j.jalz.2019.03.009
101. Dage JL, Wennberg AMV, Airey DC, et al. Levels of tau protein in plasma are associated with neurodegeneration and cognitive function in a population based elderly cohort. *Alzheimer's & dementia : the journal of the Alzheimer's Association*. 07/18 2016;12(12):1226-1234. doi:10.1016/j.jalz.2016.06.001
102. Mattsson N, Zetterberg H, Janelidze S, et al. Plasma tau in Alzheimer disease. *Neurology*. 10/16/received 07/12/accepted 2016;87(17):1827-1835. doi:10.1212/WNL.0000000000003246
103. Pase MP, Beiser AS, Himali JJ, et al. Assessment of Plasma Total Tau Level as a Predictive Biomarker for Dementia and Related Endophenotypes. *JAMA Neurol*. Mar 4 2019;doi:10.1001/jamaneurol.2018.4666
104. Park JC, Han SH, Yi D, et al. Plasma tau/amyloid-beta1-42 ratio predicts brain tau deposition and neurodegeneration in Alzheimer's disease. *Brain*. Mar 1 2019;142(3):771-786. doi:10.1093/brain/awy347
105. Gisslén M, Price RW, Andreasson U, et al. Plasma Concentration of the Neurofilament Light Protein (NFL) is a Biomarker of CNS Injury in HIV Infection: A Cross-Sectional Study. *EBioMedicine*. 2016;3:135-140. doi:10.1016/j.ebiom.2015.11.036
106. Mattsson N, Andreasson U, Zetterberg H, Blennow K. Association of Plasma Neurofilament Light With Neurodegeneration in Patients With Alzheimer Disease. *JAMA Neurology*. 2017;74(5):557-566. doi:10.1001/jamaneurol.2016.6117
107. Mattsson N, Cullen NC, Andreasson U, Zetterberg H, Blennow K. Association Between Longitudinal Plasma Neurofilament Light and Neurodegeneration in Patients With Alzheimer Disease. *JAMA Neurol*. 2019;doi:10.1001/jamaneurol.2019.0765
108. Luchsinger JA, Zetterberg H. Tracking the potential involvement of metabolic disease in Alzheimer's disease-Biomarkers and beyond. *Int Rev Neurobiol*. 2020;154:51-77. doi:10.1016/bs.irn.2020.03.014
109. Ashton NJ, Brum WS, Di Molfetta G, et al. Diagnostic Accuracy of a Plasma Phosphorylated Tau 217 Immunoassay for Alzheimer Disease Pathology. *JAMA Neurol*. Mar 1 2024;81(3):255-263. doi:10.1001/jamaneurol.2023.5319
110. Pereira JB, Janelidze S, Smith R, et al. Plasma GFAP is an early marker of amyloid-β but not tau pathology in Alzheimer's disease. *Brain*. Dec 16 2021;144(11):3505-3516. doi:10.1093/brain/awab223
111. Simrén J, Elmgren A, Blennow K, Zetterberg H. Fluid biomarkers in Alzheimer's disease. *Adv Clin Chem*. 2023;112:249-281. doi:10.1016/bs.acc.2022.09.006

112. Zhou H, Lu S, Chen J, et al. The landscape of cognitive function in recovered COVID-19 patients. *J Psychiatr Res*. 2020;129:98-102. doi:10.1016/j.jpsychires.2020.06.022
113. Yamamoto V, Bolanos JF, Fiallos J, et al. COVID-19: Review of a 21st Century Pandemic from Etiology to Neuro-psychiatric Implications. *Journal of Alzheimer's disease : JAD*. 2020;77(2):459-504. doi:10.3233/JAD-200831
114. Petersen RC. Mild cognitive impairment as a diagnostic entity. *J Intern Med*. Sep 2004;256(3):183-94.
115. Expert Panel on the Identification EaToOaOiA. Executive Summary of the Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults. *Arch Intern Med*. September 28, 1998 1998;158(17):1855-1867. doi:10.1001/archinte.158.17.1855
116. Lipton RB, Katz MJ, Kuslansky G, et al. Screening for Dementia by Telephone Using the Memory Impairment Screen. *Journal of the American Geriatrics Society*. October 01, 2003 2003;51(10):1382-1390.
117. Buschke H, Fuld PA. Evaluating storage, retention, and retrieval in disordered memory and learning. *Neurology*. Nov 1974;24(11):1019-25.
118. Raghavan N, Samtani MN, Farnum M, et al. The ADAS-Cog revisited: Novel composite scales based on ADAS-Cog to improve efficiency in MCI and early AD trials. *Alzheimers Dement*. Nov 2 2012;doi:10.1016/j.jalz.2012.05.2187
119. The Diabetes Prevention Program Research G. Effects of Withdrawal From Metformin on the Development of Diabetes in the Diabetes Prevention Program. *Diabetes Care*. April 1, 2003 2003;26(4):977-980.
120. Ridker PM. Inflammatory biomarkers and risks of myocardial infarction, stroke, diabetes, and total mortality: implications for longevity. Review. *Nutrition reviews*. Dec 2007;65(12 Pt 2):S253-9.
121. Noble JM, Manly JJ, Schupf N, Tang MX, Mayeux R, Luchsinger JA. Association of C-reactive protein with cognitive impairment. *Arch Neurol*. Jan 67(1):87-92. doi:67/1/87 [pii]
10.1001/archneurol.2009.308
122. Schneider LS, Olin JT, Doody RS, et al. Validity and reliability of the Alzheimer's Disease Cooperative Study-Clinical Global Impression of Change. The Alzheimer's Disease Cooperative Study. *Alzheimer Dis Assoc Disord*. 1997;11 Suppl 2:S22-32.
123. Nathan DM, Buse JB, Kahn SE, et al. Rationale and Design of the Glycemia Reduction Approaches in Diabetes: A Comparative Effectiveness Study (GRADE). 10.2337/dc13-0356. *Diabetes Care*. 2013;36(8):2254.
124. Mayeux R, Honig LS, Tang MX, et al. Plasma Aβ40 and Aβ42 and Alzheimer's disease. 10.1212/01.WNL.0000091890.32140.8F. *Neurology*. 2003;61(9):1185.
125. Schwarz CG, Gunter JL, Wiste HJ, et al. A large-scale comparison of cortical thickness and volume methods for measuring Alzheimer's disease severity. *NeuroImage Clinical*. 2016;11:802-812. doi:10.1016/j.nicl.2016.05.017
126. Rasmussen JM, Lakatos A, van Erp TG, et al. Empirical derivation of the reference region for computing diagnostic sensitive (1)(8)fluorodeoxyglucose ratios in Alzheimer's disease based on the ADNI sample. *Biochimica et biophysica acta*. Mar 2012;1822(3):457-66. doi:10.1016/j.bbadis.2011.09.008
127. Kubík Š, Fenton AA. Behavioral Evidence That Segregation and Representation Are Dissociable Hippocampal Functions. 10.1523/JNEUROSCI.1707-05.2005. *The Journal of Neuroscience*. 2005;25(40):9205.
128. Weintraub S, Wicklund AH, Salmon DP. The Neuropsychological Profile of Alzheimer Disease. *Cold Spring Harbor Perspectives in Medicine*. 2012;2(4):a006171. doi:10.1101/cshperspect.a006171
129. Korn EL, Freidlin B, Abrams JS, Halabi S. Design Issues in Randomized Phase II/III Trials. *Journal of Clinical Oncology*. 2012;30(6):667-671. doi:10.1200/JCO.2011.38.5732
130. Jessen F, Wolfsgruber S, Wiese B, et al. AD dementia risk in late MCI, in early MCI, and in subjective memory impairment. *Alzheimers Dement*. Jan 2014;10(1):76-83. doi:10.1016/j.jalz.2012.09.017
131. Duara R, Loewenstein DA, Greig MT, et al. Pre-MCI and MCI: neuropsychological, clinical, and imaging features and progression rates. *The American journal of geriatric psychiatry : official journal of the American Association for Geriatric Psychiatry*. 2011;19(11):951-60. doi:10.1097/JGP.0b013e3182107c69
132. Grober E, Veroff AE, Lipton RB. Temporal unfolding of declining episodic memory on the Free and Cued Selective Reminding Test in the predementia phase of Alzheimer's disease: Implications for clinical trials. *Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring*. 2018;10:161-171. doi:10.1016/j.dadm.2017.12.004
133. Albert MS, DeKosky ST, Dickson D, 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. *Alzheimers Dement*. May 2011;7(3):270-9. doi:10.1016/j.jalz.2011.03.008
134. ElSayed NA, Aleppo G, Aroda VR, et al. 9. Pharmacologic Approaches to Glycemic Treatment: Standards of Care in Diabetes-2023. *Diabetes Care*. Jan 1 2023;46(Suppl 1):S140-s157. doi:10.2337/dc23-S009

135. Jeste DV, Palmer BW, Appelbaum PS, et al. A New Brief Instrument for Assessing Decisional Capacity for Clinical Research. *Archives of General Psychiatry*. 2007;64(8):966-974. doi:10.1001/archpsyc.64.8.966
136. Pascoal TA, Shin M, Kang MS, et al. In vivo quantification of neurofibrillary tangles with [(18)F]MK-6240. *Alzheimers Res Ther*. 2018;10(1):74-74. doi:10.1186/s13195-018-0402-y
137. Wilson DH, Rissin DM, Kan CW, et al. The Simoa HD-1 Analyzer: A Novel Fully Automated Digital Immunoassay Analyzer with Single-Molecule Sensitivity and Multiplexing. *Journal of Laboratory Automation*. 2016/08/01 2015;21(4):533-547. doi:10.1177/2211068215589580
138. Kuhle J, Barro C, Andreasson U, et al. Comparison of three analytical platforms for quantification of the neurofilament light chain in blood samples: ELISA, electrochemiluminescence immunoassay and Simoa. Clinical Chemistry and Laboratory Medicine (CCLM)2016. p. 1655.
139. Zetterberg H, Wilson D, Andreasson U, et al. Plasma tau levels in Alzheimer's disease. *Alzheimers Res Ther*. 2013;5(2):9. doi:10.1186/alzrt163
140. Blennow K, Zetterberg H. The Past and the Future of Alzheimer's Disease Fluid Biomarkers. *Journal of Alzheimer's Disease*. 03/13
10/20/accepted 2018;62(3):1125-1140. doi:10.3233/JAD-170773
141. Janelidze S, Stomrud E, Palmqvist S, et al. Plasma beta-amyloid in Alzheimer's disease and vascular disease. *Sci Rep*. May 31 2016;6:26801. doi:10.1038/srep26801
142. Lue LF, Guerra A, Walker DG. Amyloid Beta and Tau as Alzheimer's Disease Blood Biomarkers: Promise From New Technologies. *Neurol Ther*. Jul 2017;6(Suppl 1):25-36. doi:10.1007/s40120-017-0074-8
143. Rohrer JD, Woollacott IO, Dick KM, et al. Serum neurofilament light chain protein is a measure of disease intensity in frontotemporal dementia. *Neurology*. Sep 27 2016;87(13):1329-36. doi:10.1212/WNL.0000000000003154
144. Humes KR, Jones NA, Ramirez RR. *Overview of Race adn Hispanic Origin: 2010*. Administration EaS; 2011. *2010 Census Briefs*.
145. Yesavage JA, Brink TL, Rose TL, et al. Development and validation of a geriatric depression screening scale: a preliminary report. *J Psychiatr Res*. 1982;17(1):37-49.
146. Introduction: Standards of Medical Care in Diabetes—2018. 10.2337/dc18-Sint01. *Diabetes Care*. 2018;41(Supplement 1):S1.
147. Wallace TM, Levy JC, Matthews DR. Use and Abuse of HOMA Modeling. 10.2337/diacare.27.6.1487. *Diabetes Care*. 2004;27(6):1487.
148. Weintraub S, Salmon D, Mercaldo N, et al. The Alzheimer's Disease Centers' Uniform Data Set (UDS): The Neuropsychological Test Battery. *Alzheimer disease and associated disorders*. Apr–Jun 2009;23(2):91-101. doi:10.1097/WAD.0b013e318191c7dd
149. Aisen PS, Petersen RC, Donohue M, et al. Clinical core of the Alzheimer's Disease Neuroimaging Initiative: progress and plans. *Alzheimer's & dementia : the journal of the Alzheimer's Association*. 2010;6(3):239-246. doi:10.1016/j.jalz.2010.03.006