

A Phase II, Single Center, Randomized, Double-Blind, Placebo-Controlled Study of the Safety and the Therapeutic Effectiveness of Intranasal Glutathione in Amnesic Mild Cognitive Impairment and Probable Mild Alzheimer's disease.

Clinical Study Protocol

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Sponsor

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This study will be conducted in compliance with the protocol, IND regulations and other applicable regulatory requirements.

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PROTOCOL SIGNATURE PAGE

I have read this protocol and agree to adhere to the requirements. I will provide copies of this protocol and pertinent information to the study personnel under my supervision and my hospital ethics committee/institutional review board (EC/IRB). I will discuss this material with them and ensure they are fully informed regarding the study medication and the conduct of the study according to this protocol, applicable law, applicable regulatory requirements including 21 CFR parts, 50, 54, 56 and 812, general standards of good clinical practice and hospital EC/IRB requirements.

Principal Investigator

Date

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DEFINITIONS

Adverse event (AE) – any undesirable patient experience that may include but is not limited to an abnormal sign, symptom, illness, abnormal laboratory value, or other medical event.

ADAS-Cog 13 - an outcome measure for global cognition in clinical trials for Alzheimer's Disease.

Amnesic Mild Cognitive Impairment (aMCI) - A condition characterized by subject memory complaints and objective neuropsychological testing demonstrating abnormalities >1.0 SD affecting memory. There is preservation of instrumental activities of daily living without functional limitations. This population has a 40% chance of developing Alzheimer's Disease over a 2 year period.

Alberta Smell Test (AST) - A test to identify the olfactory abilities of an individual.

Alzheimer's disease (AD) – A chronic progressive neurodegenerative condition resulting in memory impairment, loss of function, and progressive deterioration in other cognitive domains including language, perceptual skills, attention, construction, orientation, and problem solving.

Animal Naming - a word fluency test measuring executive functioning and language ability.

Mild Alzheimer's disease - Patients meeting NINCDS-ADRDA criteria for probable AD with a CDR=1

Columbia-Suicide Severity Rating Scale (C-SSRS) - A scale designed to quantify the severity of suicidal ideation and behavior.

Controlled Oral Word Association Test (COWAT) - a short, paper/pencil measure of phonemic fluency and executive functioning from the Multilingual Aphasia Examination.

Clinical Dementia Rating (CDR) - a numeric scale used to quantify the severity of symptoms of dementia (i.e. stage).

Data Safety Monitoring Board (DSMB) - an independent group assigned to review safety data to monitor for incidence of trends that would warrant termination of the trial.

Diagnostics and Statistical Manual-IV(DSM-IV TR) – a diagnostic manual published by the American Psychiatric Association that provides a common language and standard criteria for the classification of mental disorders including Alzheimer's Disease.

Digit Span subtest from the Wechsler Adult Intelligence Scale -4th Edition (WAIS-IV) - is a brief measure of auditory attention, concentration, and working memory.

Epworth Sleepiness Scale (ESS) – A measure to identify excessive daytime sleepiness.

Geriatric Depression Scale (GDS) - a screening test for depression in older adults.

Glulisine - a rapidly absorbed insulin analogue lacking the zinc ingredient commonly found in insulin formulations and characterized by a unique amino acid sequence that distinguishes it from other commercially available insulin compounds.

The Functional Activities Questionnaires (FAQ) – a measure of instrumental activities of daily living (IADLs).

Hachinski Ischemia Score - a screening tool used to differentiate vascular causes of dementia from neurodegenerative causes. A score of ≥ 7 is suggestive of a vascular etiology.

Intranasal (IN) - a method of drug delivery that is particularly applicable to conveying centrally acting medications into the central nervous system.

Mini Mental State Exam (MMSE) - a 30 point cognitive screening tool that assesses orientation, working memory, short term memory, visuospatial construction, and language.

Montreal Cognitive Assessment (MOCA) - a 30 point screening instrument for cognitive impairment that has a 90% sensitivity for MCI and 100% sensitivity for AD.

National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's disease and Related Disorders Association (NINCDS-ADRDA) criteria - criteria used to diagnose definite, probable, possible and unlikely Alzheimer's disease for research and clinical purposes. These criteria specify eight cognitive domains that may be impaired in AD: memory, language, perceptual skills, attention, constructive abilities, orientation, problem solving and functional abilities. These require that symptoms are confirmed by neuropsychological testing or screening in probable AD.

Trail Making Test, Parts A & B - a brief paper and pencil measure of attention, processing speed, and mental flexibility (or set-shifting).

Serious adverse events (SAE) - any symptom, sign, illness or experience that develops during the study and results in a life-threatening situation, hospitalization, significant disability, and other events determined by the investigator to be significant.

WMB Logical Memory - The Wechsler Memory Scale (WMS) is a neuropsychological test designed to measure different memory functions.

PROTOCOL SUMMARY

PROTOCOL TITLE	A Phase II, Single Center, Randomized, Double-Blind, Placebo-Controlled Study of the Safety and the Therapeutic Effectiveness of Intranasal Glulisine in Amnesic Mild Cognitive Impairment and Probable Mild Alzheimer's Disease.	
SHORT TITLE	MCI-AD IN Insulin Study	
STUDY PHASE	Phase II	
STUDY OBJECTIVES AND PURPOSE		
Primary Objective: To measure the chronic effects of IN insulin glulisine on cognition and function in subjects with aMCI and probable mild AD over a 6 month period.		
Secondary Objectives:		
<ul style="list-style-type: none"> To measure the effect of IN insulin glulisine on mood in subjects with aMCI and mild AD over a 6 month period. To measure the safety and efficacy of IN glulisine in aMCI and mild AD subjects with non-insulin dependent diabetes over a 6 month period. 		
Exploratory Objectives:		
<ul style="list-style-type: none"> To measure the effect of IN delivery of insulin glulisine on parieto-temporal and posterior cingulate/precuneus glucose metabolism in subjects with aMCI and mild AD over a 6 month period. To measure the chronic effect of IN delivery of insulin glulisine on AD-specific cerebrospinal (CSF) biomarkers (Abeta42, tau, and phosphotau) in subjects with aMCI and mild AD over a 6 month period. To measure the chronic effect of IN delivery of insulin glulisine on sleep in subjects with aMCI and mild AD over a 6 month period. To measure the chronic effect of IN delivery of insulin glulisine on olfaction in subjects with aMCI and mild AD over a 6 month period. 		
STUDY DESIGN		
Study Type	Safety	
Control Type	Placebo	
Study Indication Type	Treatment	
Blinding Schema	Double-Blind	
Device	Pressurized Olfactory Delivery (POD) device	
Study Design	Randomized, double-blinded control	
Planned Duration of Subject Participation	8 months	
ENDPOINTS	Primary	<ul style="list-style-type: none"> ADAS-Cog 13 CDR-SOB FAQ
	Secondary	<ul style="list-style-type: none"> Digit Span Forward

		<ul style="list-style-type: none"> • Trailmaking Test Parts A&B • Controlled Oral Word Association Test (COWAT) • Animal Naming • Weschler Memory Scale (WMS) • Geriatric Depression Scale (GDS)
	Exploratory	<ul style="list-style-type: none"> • CSF Abeta, tau, phosphotau • FDG-PET metabolism posterior parietotemporal, posterior cingulate, and precuneus regions • Sleep questionnaire/Epworth Sleepiness Scale (ESS) • Alberta Smell Test
INVESTIGATIONAL PRODUCTS, DOSE AND MODE OF ADMINISTRATION		
Investigational Product	Glulisine 20 IU/IN (.1ml/10 units IN in each nostril) BID	
Placebo	Sterile Normal Saline 20 IU/IN placebo (.1ml IN in each nostril) BID	
SUBJECT SELECTION		
Targeted Accrual	Approximately 90 randomized subjects. We estimate will need to consent 130 participants in order to reach this goal.	
Inclusion Criteria <ol style="list-style-type: none"> 1. Male or female subject with a clinical and research diagnosis of amnesic-mild cognitive impairment (1, 2) OR probable mild AD in accordance with NINCDS-ADRDA criteria (3) 2. Subject has a MOCA score of 18-27. 3. Subject has a Hachinski Ischemia Score ≤ 4. 4. Subject is ≥ 50 and <90 years of age. 5. Female subjects must be at least 2 years post-menopausal or surgically sterile. 6. The subject must be proficient in speaking, reading and understanding English in order to comply with procedural testing of cognitive function, memory and physiology. 7. Subject has a dedicated family member /caregiver, who will be able to attend all visits and report on subject's status. 8. Subject and family member/caregiver have both provided fully informed written consent prior to participation. In the event that subject is legally unable to provide informed written consent due to deterioration in cognitive abilities, fully informed written consent must be provided by a legally authorized representative. 9. If AD, subject must have a brain CT or MRI in the initial diagnostic work up or subsequent care that is compatible with the diagnosis of probable Alzheimer's Disease. 		
Exclusion Criteria <ol style="list-style-type: none"> 1. Subject has medical history and/or clinically determined evidence of other CNS disorders including, but not limited to brain tumor, active subdural hematoma, seizure disorder, multiple sclerosis, dementia with Lewy bodies, vascular dementia, corticobasal syndrome, progressive supranuclear palsy, Parkinson's disease, multiple system atrophy, frontotemporal dementia, normal pressure hydrocephalus, Huntington's disease, or Jakob-Creutzfeldt disease 		

presenting as dementia.

2. Subject has medical history and/or clinically determined disorders: current B12 deficiency, chronic sinusitis or any untreated thyroid disease, significant head trauma, or history of difficulty with smell and/or taste prior to AD diagnosis.
3. Subject has history of any of the following: moderate to severe pulmonary disease, poorly controlled congestive heart failure, significant cardiovascular and/or cerebrovascular events within previous 6 months, condition known to affect absorption, distribution, metabolism, or excretion of drugs such as any hepatic, renal or gastrointestinal disease or any other clinically relevant abnormality that inclusion would pose a safety risk to the subject as determined by Investigator.
4. Subject has had previous nasal and/or oto-pharyngeal surgery and severe deviated septum and/or other anomalies.
5. Subject has history of any psychiatric illness, with the exception of major depressive and anxiety disorder (according to DSM-IV TR) currently in remission or stable with treatment for ≥ 2 years, or any other psychiatric condition that inclusion would pose a safety risk to the subject as determined by investigator.
6. Subject is currently taking any medications, herbals and food supplements that are medically/clinically contraindicated as determined by investigator in order to comply with procedural testing of cognitive function as well as ensure study safety. See list of prohibited medications and compounds.
7. Subject has undergone a recent change (≤ 1 month) in their prescribed acetylcholinesterase inhibitor (e.g. donepezil, rivastigmine, galantamine) or memantine.
8. Subject has undergone a recent change (< 1 month) in their SSRI or anti-depressant medication.
9. Subject has current or recent drug or alcohol abuse or dependence as defined by DSM-IV TR.
10. Screening laboratory results that are medically relevant, in which inclusion would pose a safety risk to the subject as determined by investigator.
11. The subject has participated in any other research study at least 3 months prior to this study.
12. The subject has an insulin allergy.

1. INTRODUCTION

1.1 Background and Rationale

Alzheimer's disease (AD) is the most common cause of dementia, representing 60-80% of all cases and afflicting 5.4 million individuals within the United States. While characteristic amyloid plaques (AP) and neurofibrillary tangles (NFT) represent the pathognomic signature of AD, the AD brain is characterized by a severe impairment of insulin-signaling, including deficits of insulin, insulin-like growth factor, insulin receptors as well as central resistance to insulin action (4-8). Disruption of central nervous system (CNS) insulin signaling has been increasingly associated with AD pathogenesis, and consequently this disease has been referred to as a type III diabetes of the brain (9). Furthermore, longitudinal cohort studies have shown that diabetes doubles the risk of AD (10) and have linked high blood sugar with increased dementia risk (11). Fluorodeoxyglucose positive emission tomography (FDG-PET) studies of subjects with peripheral insulin resistance demonstrate a cerebral pattern of frontal, parietotemporal, and cingulate cortical hypometabolism that mimics AD (12).

Originally thought to exist solely in the periphery, insulin has since been determined to be instrumental in the overall health and function of the CNS (13). Central insulin and insulin receptors (IRs) have been established as differing from that of the systemically occurring counterparts that specifically regulate glucose utilization. Although central insulin does induce glucose uptake in the brain, it also functions in the modulation of various neurotransmitters and receptors involved in executive function and the long-term potentiation of memories. For example, systems with impaired insulin signaling pathways have demonstrated inhibition of acetylcholine biosynthesis and subsequently have incurred debilitating effects on neuronal plasticity (7, 14). Consistent with evidence of insulin functioning as a neuromodulator for memory-related function is the high-density of IRs in the hippocampus and cerebral cortex, brain regions integral to the formation, retention and recall of information (13, 15).

Brain deposition of beta-amyloid ($A\beta$) plaques and neurofibrillary tangles (NFTs) are both critical pathological signatures of AD, but are also modulated by CNS insulin activity. Increased insulin concentration upregulates the production of insulin-degrading enzyme (IDE); a multifunctional enzyme that removes soluble $A\beta$ and promotes intracellular $A\beta$ degradation (5). As AD progresses, the presence of soluble $A\beta$ aggregates further induces tau hyperphosphorylation as well as decreases the number of synaptic insulin receptors (IRs) (5). The downregulation of dendritic surface IRs from accumulating $A\beta$ compromises synaptic function in brain regions associated with memory and cognitive function, leading to the disruption in the insulin-signaling pathway that is characteristic of AD (16). Insulin further inhibits neurofibrillary tangle production by maintaining a phosphorylation equilibrium between kinase and phosphatase activity (8, 17-19).

Intranasal (IN) delivery offers a non-invasive route to deliver large molecules such as insulin directly to the brain while minimizing systemic exposure. Peptides, proteins, vaccines, drug treatments and ions of various sizes are able to pass along the olfactory and trigeminal nerves and are deposited directly into the CNS without having to pass through the BBB that may degrade or limit the amount arriving at the target (20-27). Increased insulin in healthy adults has been detected in CSF as soon as 10 minutes after IN delivery while not measurably increasing

systemic blood-glucose levels (22, 28). Similar effects have been achieved using IN insulin with human subjects of altered brain insulin concentrations (29, 30).

Clinical trials of intranasal insulin in AD have demonstrated therapeutic effects involving memory, attention and cognition without significantly altering serum insulin or glucose levels. A clinical trial consisting of 26 memory impaired subjects (13 with AD and 13 with mild cognitive impairment) and 35 normal controls showed that IN insulin 20 IU or 40 IU resulted in improvements on two declarative memory tasks compared to placebo within 15 minutes of drug administration (29). IN insulin administered at 20 IU resulted in greater story recall whereas doses at 40 IU more favorably improved word list recall. There was no impact on IN insulin on serum glucose or insulin levels. Another study of 24 early AD/mild cognitive impairment subjects showed that 20 IU BID of intranasal insulin resulted in sustained benefit in over a 21 day period (30). Furthermore, IN insulin induced favorable changes in the serum amyloid-beta 40/42 ratio while having no impact on systemic glucose or insulin levels. Most recently, Craft and colleagues have shown improved memory and function as represented by the ADAS-Cog and ADCS-ADL, respectively in AD patients treated during a four month clinical trial (31). These changes in memory and function were associated with favorable changes in CSF tau and tau-Ab42 ratio. Additionally, IN insulin minimized progression in loss of cerebral hypometabolism as measured by FDG-PET.

Therapeutic response to IN insulin may be modified by both insulin type as well as ApoE4 carrier status. A study of 38 normal subjects showed that rapid-acting insulin aspart (Insulin NovoLog HM, Novo Nordisk) resulted in greater declarative memory improvements compared to regular insulin over 8 an week period (32). Additional clinical trials have suggested memory benefits associated with IN insulin are diminished among ApoE4 carriers diagnosed with AD (29).

Glulisine is a rapidly absorbed insulin analogue lacking the zinc ingredient commonly found in insulin formulations. This analogue differs from human insulin in that the amino acid asparagine at position B3 is replaced by lysine and the lysine in position B29 is replaced by glutamic acid. For this study, we have chosen glulisine due to prior speculation that zinc-containing compounds may be toxic to olfactory neurons (33, 34) as well as prior studies demonstrating improvements in declarative memory with RA compared to regular insulin.

To test the hypothesis regarding the acute safety and efficacy of glulisine, the HealthPartners Center for Memory and Aging performed a double blinded, randomized, placebo-controlled, cross-over pilot study in 9 subjects with mild-moderate AD. We further sought to address the responsiveness of ApoE4 carriers to this treatment, and so all subjects were carriers of this polymorphism. The study drug was intranasally administered using the LMA Mucosal Atomization Device (MAD) and was not associated with any serious adverse events. Treated subjects made fewer Trails B errors relative to controls. Otherwise, there were no significant difference between IN glulisine and placebo for cognitive tests of learning/memory, attention/executive function, language, or visuospatial function. Fingertick glucose was not impacted by IN glulisine, but the drug resulted in a 19% decrease in insulin levels compared to placebo. The conclusion was that ApoE4 subjects were unresponsive to acute IN glulisine, but the drug was safe and well-tolerated.

In the current study, we are investigating the chronic effects of IN glulisine 20 IU administered two times daily in adults with amnesic-mild cognitive impairment (a-MCI) and mild Alzheimer's disease (AD). The investigation will enroll n=90 subjects and follow them over a 6 month period. This study is characterized by three major changes: 1.) the IN glulisine will be administered using the Pressurized Olfactory Delivery (POD) Device; 2.) the study will include non-insulin dependent diabetics; 3.) the study will incorporate biomarkers for amyloidosis (cerebrospinal fluid Abeta42) and neurodegeneration (cerebrospinal tau/phosphotau and FDG-PET).

In the acute pilot study, we had employed the LMA MAD device in the acute setting, but for this chronic trial, there is concern about variable, user-dependent drug delivery due to dependency upon subject positioning and inconsistent volume ejection. The POD device is more practical for a 6 month clinical trial. The POD device is powered with a standard hydrofluoroalkane (HFA) meter valve to administer drug into the nasal cavity rather than the mechanical ability of the user, which results in greater inter-user consistency.

This study will also include a subsample of subjects with non-insulin dependent diabetes. Studies have shown that diabetics have twice the risk of Alzheimer's disease (35) and FDG-PET scans demonstrate a pattern of hypometabolism resembling Alzheimer's disease (12). We hypothesize that the subjects with peripheral deficits in insulin signaling will also be the ones to demonstrate deficits in central nervous system signaling. While diabetics comprise a significant portion of patients with AD, previous studies have excluded this population due to concerns regarding hypoglycemia in the setting of concomitant oral hypoglycemics or intranasal insulin. However, studies in non-diabetic subjects have failed to reveal any significant glucose lowering effects of IN insulin. There are minimal investigations addressing the effect of intranasal insulin on peripheral glucose in non-insulin dependent diabetes. One of the secondary objectives of this clinical trial is to demonstrate the safety and efficacy of IN glulisine in this population. Based on the multiple trials failing to reveal effects on peripheral glucose, we suspect that IN insulin will be well-tolerated in this population.

Finally, investigations have shown that Alzheimer's disease is characterized by the co-existence of cerebral amyloidosis with neurodegeneration, and that biomarkers of these abnormal changes can be used to support an AD diagnosis as well as longitudinally track disease progression (36). Most recent AD clinical trials have included biomarkers from CSF and FDG-PET to provide evidence for biological drug actions related to amyloidosis and neurodegeneration (37, 38).

2. Summary of Device Description

2.1. Pressurized Olfactory Delivery (POD) Device

The Pressurized Olfactory Delivery (POD) device is intended to be used with a variety of known and yet-to-be-known drugs. The POD device is designed specifically to deliver centrally acting drugs via the olfactory and trigeminal neural pathways in the roof of the nasal cavity and olfactory nasal epithelium.

Commercially available aerosol nasal devices such as the LMA MAD device are not specifically engineered to facilitate nose-brain delivery, and consequently deposit most of the drug within the lower nasal cavity, resulting in suboptimal CNS penetration. Other problems associated with

traditional nasal sprays include variable aerosolized product, dependence on user position, and high frequency of device non-compliance/misuse. The POD device has specifically been developed to effectively and consistently deliver CNS therapeutics to the brain via the nasal/olfactory pathways. The device is not currently FDA approved, but numerous studies support its role in intranasal brain delivery of radiolabeled and therapeutic compounds.

3.0. OBJECTIVES

3.1. Primary Objective

- To measure the chronic effects of IN insulin glulisine on cognition and function in subjects with aMCI and probable mild AD over a 6 month period.

3.2. Secondary Objectives

- To measure the effect of IN insulin glulisine on mood in subjects with aMCI and mild AD over a 6 month period.
- To measure the safety and efficacy of IN glulisine in aMCI and mild AD subjects with non-insulin dependent diabetes over a 6 month period.

3.3 Exploratory Objectives

- To measure the effect of IN delivery of insulin glulisine on posterior parietotemporal and cingulate/precuneus glucose metabolism in subjects with aMCI and mild AD over a 6 month period.
- To measure the chronic effect of IN delivery of insulin glulisine on AD-specific cerebrospinal (CSF) biomarkers (A β 42, tau, and phosphotau) in subjects with aMCI and mild AD over a 6 month period.
- To measure the chronic effect of IN delivery of insulin glulisine on sleep (Sleep questionnaire and ESS) in subjects with aMCI and mild AD over a 6 month period
- To measure the chronic effect of IN delivery of insulin glulisine on olfaction in subjects with aMCI and mild AD over a 6 month period.

4. ENDPOINT(S)

4.1. Primary Endpoints

- Change in global measure of cognition as measured by the Alzheimer's disease Assessment Scale-Cognitive (ADAS-Cog-13) in subjects with aMCI and mild AD between baseline and visit 7 and between baseline and visit 10.
- Change in Clinical Dementia Rating-Sum of Boxes (CDR-SOB) between screen/baseline and final assessment visit.

- Change in daily functioning as measured by the Functional Assessment Questionnaire (FAQ) in subjects with aMCI and mild AD between baseline and final assessment visit.

4.2. Safety

- Incidence and severity of serious adverse events (SAEs) and adverse events (AEs).
- Frequency of change in clinically-significant vital signs, physical exam or neurological exam.
- Change from baseline in 12-lead ECG.
- Plasma glucose level <70mg/dl.

5. STUDY DESIGN

This study is a single center, phase II randomized, double-blind, placebo-controlled clinical trial designed to assess the efficacy of intranasally (IN) administered glulisine versus placebo in patients with aMCI and probable mild AD.

After written informed consent has been obtained from the subject and family member/caregiver, subjects will be screened to assess study eligibility based on study inclusion/exclusion criteria. Subjects who are eligible at the end of the screening visit (Visit 1) will be scheduled for a baseline visit (visit 2). During the baseline visit, subjects will undergo neuropsychological testing with the ADAS-Cog 13 battery and supplemental tests including the Trailmaking Test Parts A & B, Digit Span, Controlled Oral Word Association Test, Animal Naming, and WMS logical memory to establish a baseline. Repeat cognitive assessments will be performed at 12 and 24 weeks. Patients will also be assessed on two additional measures for olfaction (Alberta smell test) and sleep measures (Sleep Questionnaire and ESS) at baseline, 12 weeks, and 24 weeks. Patients enrolled in the AD biomarker substudy will undergo a baseline FDG-PET scan and CSF biomarkers (Abeta42, tau, phosphotau) at baseline and 24 weeks.

A total of n=90 subjects will be randomized to receiving either IN glulisine (n=45) or placebo (n=45) two times daily (morning and evening, separate by at least 8 hours). In addition, subjects will be block randomized based on ApoE4 status, presence of non-insulin dependent diabetes, and MOCA score (18-22 vs. 23-27). Subjects will receive either 0.2 ml IN insulin (20 IU) (0.1ml/10 units IN in each nostril) or placebo administered two times daily over a 6 month period.

5.1. Outcome Measures

Neuropsychological, functional, and depression batteries will be performed at screen/baseline, Visit 7 and at Visit 10 (refer to section 7.4 for full description) to assess primary endpoint. A subset of subjects will undergo testing for CSF biomarkers (Abeta42, tau, phosphotau) and FDG-PET imaging at baseline and Visit 10 to address exploratory endpoints.

5.2. Study Duration

Study participation will last approximately 8 months, consisting of a minimum of 7 days but no more than 4 week screening phase and baseline visit, and a total of 9 treatment/safety visits over 6 months. If study visit window exceeds 28 days, study drug will be resupplied which may require additional study drug pickup visits. Patients who discontinue treatment will continue follow-up measures as possible and will be analyzed as randomized per the intent to treat principal.

6. STUDY POPULATION

6.1. Eligibility Criteria

6.1.1. Inclusion Criteria

A subject will be included for consideration in this study only if all of the following criteria are met:

1. Male or female subject with a clinical and research diagnosis of amnesic-mild cognitive impairment (1, 2) OR probable mild AD in accordance with NINCDS-ADRDA criteria (3).
2. Subject has a MOCA score of 18-27.
3. Subject has a Hachinski Ischemia Score ≤ 4 .
4. Subject is ≥ 50 and <90 years of age.
5. Female subjects must be at least 2 years post-menopausal or surgically sterile.
6. The subject must be proficient in speaking, reading and understanding English in order to comply with procedural testing of cognitive function, memory and physiology.
7. Subject has a dedicated family member/study partner, who will be able to attend all visits and report on subject's status.
8. Subject and family member/study partner have both provided fully informed written consent prior to participation. In the event that subject is legally unable to provide informed written consent due to deterioration in cognitive abilities, fully informed written consent must be provided by a legally authorized representative.
9. If AD, subject must have a brain CT or MRI in initial diagnostic workup or subsequent care that is compatible with the diagnosis of probable Alzheimer's disease.

6.1.2. Exclusion Criteria

A subject will *not* be included for consideration in this study if any of the following criteria are met:

1. Subject has medical history and/or clinically determined evidence of other CNS disorders including, but not limited to brain tumor, active subdural hematoma, seizure disorder, multiple sclerosis, dementia with Lewy bodies, vascular dementia, corticobasal syndrome, progressive supranuclear palsy, Parkinson's disease, multiple system atrophy, frontotemporal dementia, normal pressure hydrocephalus, Huntington's disease, or Jakob-Creutzfeldt disease presenting as dementia.
2. Subject has medical history and/or clinically determined disorders: current B12 deficiency, chronic sinusitis or any untreated thyroid disease, significant head trauma, or history of difficulty with smell and/or taste prior to AD diagnosis.
3. Subject has history of any of the following: moderate to severe pulmonary disease, poorly

controlled congestive heart failure, significant cardiovascular and/or cerebrovascular events within previous 6 months, condition known to affect absorption, distribution, metabolism, or excretion of drugs such as any hepatic, renal or gastrointestinal disease or any other clinically relevant abnormality that inclusion, would pose a safety risk to the subject as determined by investigator.

4. Subject has had previous nasal and/or oto-pharyngeal surgery and severe deviated septum and/or other anomalies.
5. Subject has history of any psychiatric illness, with the exception of major depressive and anxiety disorder (according to DSM-IV TR) currently in remission or stable with treatment for ≥ 2 years, or any other psychiatric condition that inclusion would pose a safety risk to the subject as determined by investigator.
6. Subject is currently taking any medications, herbals and food supplements that are medically/clinically contraindicated as determined by investigator in order to comply with procedural testing of cognitive function as well as ensure study safety. See list of prohibited medications and compounds.
7. Subject has undergone a recent change (≤ 1 month) in their prescribed acetylcholinesterase inhibitor (e.g. donepezil, rivastigmine, galantamine) or memantine.
8. Subject has undergone a recent change (< 1 month) in their SSRI or anti-depressant medication.
9. Subject has current or recent drug or alcohol abuse or dependence as defined by DSM-IV TR.
10. Screening laboratory results that are medically relevant, in which inclusion would pose a safety risk to the subject as determined by investigator.
11. The subject has participated in any other research study at least 3 months prior to
12. The subject has an insulin allergy.

1. Study assessments and procedures

A summary of study events and procedures is outlined in the Study Visit Table. Demographic, Screening, and Baseline Assessments are described in Sections 7.1.1-7.1.2, the Treatment Phase is outlined in Sections 7.1.3-7.1.10, and the Safety monitoring are detailed in Section 7.1.11; details of neuropsychological assessments are outlined in Section 7.4. Note that all visits are non-fasting unless otherwise noted.

7.1. Demographic and Baseline Assessments

7.1.1. Visit 1; Screening Visit Fasting

The following procedures will be performed at this visit:

- Obtain written informed consent from study partner and subject (or subject's legally authorized representative) prior to any study related procedures.
- Collect laboratory samples for screening assessment, for specific tests refer to Table 1.
- Administer MOCA.
- Obtain Hachinski Score.
- Administer Geriatric Depression Scale (GDS).
- Review Inclusion/Exclusion Criteria.

- Review medical history, as it pertains to inclusion/exclusion criteria, such as research diagnosis, disease severity, and course of AD.
- Obtain subject's demographic information (date of birth, gender, race, education, etc.).
- Obtain details of medications taken over the course of the last 30 days.
- Complete physical exam, including neurological exam. Collect vital signs, height and weight prior to ECG and blood draw.
- Perform a standard 12-lead ECG.

7.1.2. Visit 2; Baseline Visit

The following procedures will be performed at this visit:

- Review Inclusion/Exclusion Criteria.
- Collect vital signs and weight.
- Collect concomitant medication information and record AEs/SAEs.
- Neuropsychological testing with ADAS-Cog 13 and supplemental cognitive assessments.
- Administer CDR
- Administer Columbia-Suicide Severity Rating Scale (C-SSRS).
- Administer Functional Assessment Questionnaire (FAQ).
- Administer Alberta Smell Test.
- Administer sleep measures (Sleep questionnaire and ESS)
- Study Drug administration training.
- Hypoglycemia rescue training.
- Sub-study PET scan. For participating subjects, scan will be obtained within 1 week (\pm 3 days).
- Sub-study LP/ CSF. For participating subjects, procedure will be performed within 1 week (\pm 3 days).

7.1.3. Visit 3; Initial Treatment Visit; (Week 0)

The following procedures will be performed at this visit:

- Review Inclusion/Exclusion Criteria.
- Collect vital signs and weight.
- Collect concomitant medication information and record AEs/SAEs.
- Study Drug administration training.
- Study drug dispensing /diary.
- Administer study dose in clinic with subject/study partner.
- Obtain fingerstick blood glucose pre study dose and post study dose at 15 min, 30 min and 1 hour.
- Visit 4 scheduled within 1 week (\pm 3 days).

7.1.4. Visit 4; Follow-Up Safety Visit; (Week 1)

The following procedures will be performed at this visit:

- Collect concomitant medication information and record AEs/SAEs.
- Collect vital signs and weight
- Obtain laboratory studies.
- Administer study dose in clinic by subject/ study partner.
- Obtain fingerstick blood glucose pre study dose and post study dose at 15min, 30 min and 1 hour.
- Study drug administration training.
- Study drug dispensing/diary.
- Visit 5 scheduled within 3 weeks (\pm 3 days).

7.1.5. Visit 5; Treatment and Adherence; (Week 4)

The following procedures will be performed at this visit:

- Collect vital signs, and weight.
- Collect concomitant medication information and record AEs/SAEs.
- Review training of study drug administration.
- Study drug dispensing/diary.
- Visit 6 will be scheduled within 1 month (\pm 3 days).

7.1.6. Visit 6; Treatment and Adherence; (Week 8)

Following procedures will be performed at this visit.

- Collect vital signs and weight
- Collect concomitant medication information and record AEs/SAEs.
- Review training of study drug administration.
- Study drug dispensing /diary.
- Visit 7 scheduled within 1 month (\pm 7 days).

7.1.7. Visit 7; Treatment and Adherence; (Week 12)

Following procedures will be performed at this visit.

- Collect vital signs and weight.
- Collect concomitant medication information and record AEs/SAEs.
- Neuropsychological testing with ADAS-Cog 13 and supplemental cognitive assessments.
- Administer CDR.
- Administer FAQ.
- Administer GDS.
- Administer C-SSRS.
- Administer sleep measures (Sleep questionnaire and ESS)
- Administer Alberta smell test
- Review training of study drug administration.
- Study drug dispensing/diary.

- Visit 8 will be scheduled within 1 month (± 3 days).

7.1.8. Visit 8; Treatment and Adherence (Week 16)

Following procedures will be performed at this visit.

- Collect vital signs and weight.
- Collect concomitant medication information and record AEs/SAEs.
- Review training of study drug administration.
- Study drug dispensing/diary.
- Visit 9 will be scheduled within 1 month (± 3 days).

7.1.9. Visit 9; 5-month check-in (Week 20)

Following procedures will be performed at this visit.

- Collect vital signs and weight.
- Collect concomitant medication information and record AEs/SAEs.
- Review training of study drug administration.
- Study drug dispensing/diary.
- Visit 10 will be scheduled within 1 month (± 7 days).

7.1.10. Visit 10; 6 month follow-up (Week 24)

Following procedures will be performed at this visit.

- Collect vital signs and weight.
- Collect concomitant medication information and record AEs/SAEs.
- Neuropsychological testing with ADAS-Cog 13 and supplemental cognitive assessments.
- Administer CDR.
- Administer FAQ.
- Administer GDS.
- Administer Alberta smell test
- Administer sleep measures (Sleep questionnaire and ESS)
- Obtain repeat FDG-PET study in subgroup. Scan will be obtained within 1 week (± 3 days).
- Obtain repeat spinal fluid analysis in subgroup. Procedure will be performed within 1 week (± 3 days).
- Visit 11 scheduled within 1 month (± 7 days).

7.1.11. Visit 11; Final Safety and Discharge; Fasting (Week 28)

Following procedures will be performed at this visit.

- Collect vital signs and weight.
- Complete physical and neurological exam.

- Collect concomitant medication information and record AEs/SAEs.
- Collect laboratory samples, for specific tests refer to in Table 1.
- Perform a standard 12-lead ECG.
- Administer C-SSRS.

7.2. Early Withdrawal

If subject withdraws from the study after the screening visit, but before visit 3, no further evaluations are necessary. If subject withdraws from the study after visit 3, all safety assessments will be performed (see section 7.3).

7.3. Safety

For all safety assessments described below, any clinically significant change will be recorded as an AE or SAE.

7.3.1. Physical Examination

Complete physical examination will be performed at visits, 1 and 11, or if the subject withdraws or is withdrawn from the study early. Any abnormalities noted at Visit 1, will be documented as part of the subject's medical history.

7.3.2. Neurological Examination

Neurological examination will be performed at visits, 1 and 11, or if the subject withdraws early. Any abnormalities noted at Visit 1, will be documented as part of the subject's medical history.

7.3.3. Vital Signs

Vital signs will be recorded at all visits. For within subject consistency, brachial artery pressure will be obtained in the routine fashion, the same arm should be used for all study measurements.

Blood pressure and heart rate to be measured after subject has been sitting quietly for a minimum of 5 minutes. Diastolic blood pressure will be measured at the disappearance of Korotkoff sounds. Vitals sign will be monitored by clinical staff during each visit of the study.

In addition, vital signs will be measured pre and post study dose on Visits 3 and 4. The investigator will be notified for any baseline changes in blood pressure >20 mmHg systolic and >10 mmHg diastolic

A pre-dose fingerstick blood glucose will be measured followed by measurements post- study drug at 15 minute, 30 minute and 1 hour during visit 3 and 4. The investigator will be notified for any baseline changes. A blood glucose < 70 mg/dL will be considered clinically significant.

Vital signs will be taken prior to ECG and blood draw.

7.3.4. Weight

Body weight will be measured at all visits.

7.3.5. ECG

A standard 12-lead ECG will be performed on subjects at visit 1 and 11

7.3.6. Laboratory Samples

APOE genotyping procedure is discussed in Section 7.5.

All subjects will be required to fast for a minimum of 12 hours prior to collection of blood sampling on designated fasting visits.

7.3.6.1 HOMA-IR – Measure for Insulin Resistance

Calculation of HOMA-IR with Blood glucose and insulin level during Visit 1 using the following formula(45, 46);

$$\text{HOMA-IR} = [\text{glucose (nmol/L)} * \text{insulin } (\mu\text{U/mL})/22.5]$$

See **Table 1** for specific list of laboratory assessments.

Table 1 Laboratory Assessments

Procedure	Visit 1;	*Visit 3;	*Visit 4;	Visit 11;	Early Withdrawal
Basic Metabolic Panel (Na, K, CO ₂ , Cl, BUN, Creatinine, Glucose and Ca)	X		X	X	X
Insulin Level	X			X	X
CBC w/ Diff	X			X	X
Fingerstick Blood Glucose Baseline, post study dose, 15 min, 30 min and 1hour		X	X		
HbA1c	X				
APOE status	X				
Thyroid Stimulating Hormone (TSH)	X				
Vitamin B12	X				

***Blood samples to be collected after study dose is administered.**

7.4. Neuropsychological Assessment

See Table 2 for specific list of neuropsychological Assessment.

Table 2 Neuropsychological Assessment

Procedure	Screen	Baseline Phase	Treatment Phase		Follow-up	Early Withdrawal
	Visit 1	Visit 2	Visit 7	Visit 10	Visit 11	
FAQ		X	X	X		
GDS	X		X	X		X
ADAS-Cog 13		X	X	X		
CDR		X	X	X		
MOCA	X					
C-SSRS		X	X		X	X
Digit span forward/backward		X	X	X		
Trailmaking Parts A&B		X	X	X		
COWAT		X	X	X		
Animal naming		X	X	X		
WMS Logical Memory		X	X	X		
AST		X	X	X		
Sleep Questionnaire		X	X	X		
ESS		X	X	X		

7.4.1. ADAS-Cog 13 Summary

The ADAS-Cog was developed as an outcome measure for global cognition in clinical trials for Alzheimer’s disease. The ADAS-Cog assesses multiple cognitive domains including memory, language, praxis, and orientation (47). Overall, the ADAS-Cog has proven successful for its intended purpose with higher scores indicating greater cognitive impairment. The modified ADAS-Cog 13-item scale (48) includes all original ADAS-Cog items with the addition of a number cancellation task and a delayed free recall task, for a total of 85 points. The purpose of these additional items increases the number of cognitive domains and range of symptom severity to best assess subjects affected by the earlier stages of Alzheimer’s disease (e.g. amnesic MCI and mild AD).

7.4.2. Digit Span subtest from the Wechsler Adult Intelligence Scale -4th Edition (WAIS-IV)

The WAIS-IV Digit Span test is a brief measure of auditory attention, concentration, and working memory. Subjects are read a random list of numbers at a rate of one per second and immediately asked to repeat them in a forward sequence, a backward sequence, or to rearrange them in numeric order starting with the lowest number. Digit strings gradually expand and become lengthier until the subject makes consistent errors, at which point the test is discontinued. The test yields numerous scores for interpretation.

7.4.3. Controlled Oral Word Association Test (COWAT)

The COWAT is a short, paper/pencil measure of phonemic fluency and executive functioning from the Multilingual Aphasia Examination.

7.4.4. Trail Making Test, Parts A & B

The Trail Making Test is a brief paper and pencil measure of attention, processing speed, and mental flexibility (or set-shifting). The test has been well validated for use with a wide range of subject populations, is easy to administer, and has been shown to be sensitive in detecting subtle changes in central nervous system compromise and specifically changes in frontal lobe or “executive” functions. Part A requires subjects to connect a series of numbered dots in order as quickly as possible. Part B requires subjects to connect numbered and lettered dots as quickly as possible by alternating back and forth between the two without making mistakes. Scores include both time to completion and various error types.

7.4.5. Montreal Cognitive Assessment (MOCA)

The Montreal Cognitive Assessment (MoCA) was designed as a rapid screening instrument for mild cognitive dysfunction. It assesses different cognitive domains: attention and concentration, executive functions, memory, language, visuoconstructional skills, conceptual thinking, calculations, and orientation. The time to administer the MoCA is approximately 10 minutes. The total possible score is 30 points, and scores of 26 or above are considered normal.

7.4.6. Clinical Dementia Rating (CDR) Scale

The CDR is a 5-point scale used to characterize six domains of cognitive and functional performance applicable to Alzheimer disease and related dementias: Memory, Orientation, Judgment & Problem Solving, Community Affairs, Home & Hobbies, and Personal Care <http://alzheimer.wustl.edu/cdr/aboutcdr.htm>. The necessary information to make each rating is obtained through a semi-structured interview of the patient and a reliable informant or collateral source (e.g., family member).

The CDR table (http://knightadrc.wustl.edu/cdr/PDFs/CDR_Table.pdf) provides descriptive anchors that guide the clinician in making appropriate ratings based on interview data and clinical judgment. In addition to ratings for each domain, an overall CDR score may be calculated through the use of an [algorithm](#). This score is useful for characterizing and tracking a patient's level of impairment/dementia:

CDR-0.5 = very mild dementia

CDR-1 = mild

CDR-2 = moderate

CDR-3 = severe

In addition, the total CDR ratings for each of the six cognitive/functional domains can be added to create a CDR sum of boxes (SOB).

7.4.7. Columbia-Suicide Severity Rating Scale (C-SSRS)

The Columbia–Suicide Severity Rating Scale (C-SSRS) was designed to quantify the severity of suicidal ideation and behavior (49). In this study, suicidal ideation and behavior will be prospectively assessed using the C-SSRS. The C-SSRS will be administered by trained raters at specified time points, as indicated in the Visit Flow Chart, as well as at unscheduled visits as clinically indicated. Any subjects demonstrating evidence of suicidality will prompt immediate consultation with the site’s on-call psychiatrist for assistance with decision-making and potential referral to behavioral health services.

7.4.8. 15-Item Geriatric Depression Scale (GDS)

The 15-item GDS, a paper, interview-based depression screening tool will be administered to the subject and score by a qualified, trained rater.

7.4.9. The Functional Activities Questionnaires (FAQ)

The Functional Activities Questionnaire (FAQ) measures instrumental activities of daily living (IADLs), such as preparing balanced meals and managing personal finances. Since functional changes are noted earlier in the dementia process with IADLs that require a higher cognitive ability compared to basic activities of daily living (ADLs) this tool is useful to monitor these functional changes over time.

7.4.10. Animal Naming

Animal Naming is a word fluency test measuring executive functioning and language ability. The subject is asked to list all of the animals he/she can think of in the next 60 seconds.

7.4.11. Wechsler Memory Scale Logical Memory

The Wechsler Memory Scale (WMS) is a neuropsychological test designed to measure different memory functions in a person. Logical memory represents one of 7 memory subtests that are included within the WMS-IV. Of all the subtests, logical memory is the largest discriminator between the normal aging population and mild dementia (50). Subjects are read two thematically independent stories and later asked to recall each story immediately and later thirty minutes after the reading. Patients struggling with recall of story contents are provided cues to assist with recognition.

7.4.12 Alberta Smell Test

The Alberta Smell Test (AST) is a quick, easy to use method to test the ability to identify smells presented to one nostril at a time (51). Over the course of the test, the subject is presented with 8 different odors of the Mr. Sketch scented markers namely, licorice, cinnamon, mint, orange, lemon, grape, melon, and raspberry. In 20 trials, the different scented markers are held up to the participants’ right or left nostril with their eyes closed. The subjects then select the scent of the marker from a list of the 8 scents. Each smell correctly identified will be recorded as a correct response with a maximum score of 20. A score of 2/10 or less in either nostril suggests impairment.

7.4.13 Sleep Questions

A set of 4 questions will be asked to the caregiver/spouse that looks at areas of sleep quality including sleep duration, sleep latency and sleep efficiency for the past month. The questions address the patient's usual bedtime and wake-up time, as well as how long it takes to fall asleep and how many hours of sleep are obtained per night. The caregiver will answer these questions for the subject.

7.4.14 Epworth Sleepiness Scale (ESS)

Epworth sleepiness scale (ESS) is a simple self-administered questionnaire that is widely used to measure the daytime sleepiness of an individual (52). It has 8 standardized questions that assess the likelihood of a subject dozing off or falling asleep (on a scale of 0-3) in daily living situations. A total of 0-24 score can be obtained. Excessive daytime sleepiness is assumed if a subject reaches a score of 10 and above. The caregiver will answer these questions for the subject.

7.5. Pharmacogenetics

APOE genotyping will be performed at Visit 1, following informed consent and will be used as part of the randomization. Sample will be tested at an external genetic processing facility, and results will be returned to testing site. The identity of study participants will be withheld from outsourced company completing testing. Samples will be identified by participant initials and a number identifier specific to this study.

8. INVESTIGATIONAL PRODUCT(S)

8.1. Description of Investigational Product

The Center for Memory and Aging will utilize the following investigational products:

- Intranasal delivery
- Insulin glulisine
- Placebo saline
- POD Device

8.2. Handling and Storage

The study drug (glulisine/saline) will be given to participant/care partner after training for administration.

The study drug on site will be kept per label recommendations and institutional Standard Operational Policy, specifically, but not limited to temperature controlled secure area.

Participants will be instructed to keep study drug at room temperature.

At investigational site, the study drug will be stored according to manufacturer recommendations. To ensure that a stable temperature and/or conditions are maintained, refrigerated study drug is electronically monitored for temperature control. . A log will be securely stored at the HealthPartners Center for Memory and Aging. Study staff will be responsible for safeguarding and maintaining the master log. In the event of a medical emergency requiring a blind break, unblinded study staff will be contacted by a member of the investigative team and the appropriate information will be relayed only to those responsible for patient's immediate medical care (e.g. ED physician).

8.3. Treatment Assignment

Randomization will be stratified by MOCA score (high = 23-27; low = 18-22), presence of non-insulin dependent diabetes, and APOE4 status *a priori* by the permuted block method. The trial is a double blinded study so that the subject, the investigator, and the trial coordinator will be unaware to which treatment group the subject has been randomized. To facilitate subject blinding, all subjects will undergo the same follow-up procedures. One investigative team member at the site will be unblinded to randomization and prepare the study dose for each subject. This person will not have responsibility for obtaining any study data. An electronic database will be used for automated randomization of participants using a table generated by the statistician.

8.4. Packaging and Labeling

Actual pharmacy-product indicator will be decided by pharmacy in order to maintain investigator objectivity.

All study drug and placebo will be labeled according to the following specifications:

- Protocol identifier/IRB approval/account/study number.
- Participant ID #.
- "Store at room temperature."
- "Last day to use" date."
- "Caution: New Drug--Limited by Federal (or United States) law to investigational use".
- Study contact # 651-254-0769.

8.5. Occupational Safety

No known significant safety risks exist to site personnel in direct or indirect contact with the study drug.

9. CONCOMITANT MEDICATIONS AND NON-DRUG THERAPIES

9.1. Permitted Medications

Any medication not listed in list of Prohibited/Conditional Medications will be permitted during this study. A record will be kept by site staff detailing doses and indication of any concomitant medications used by subjects.

Table 2: Prohibited Medications

Anticholinergics
Antihistamines (centrally-acting)
Barbiturates
Benzodiazepines
Insulin
Muscle relaxants
Neuroleptics
Opiates

9.2. Prohibited Medications

Subjects taking prohibited medications at time of screening will not be allowed to participate in study, unless such treatment is discontinued within 30 days. These medications include anticholinergics, and centrally acting antihistamines (e.g. Benadryl), opiates, benzodiazepines, barbiturates, neuroleptics, muscle relaxants, and insulin. For randomized subjects who take a prohibited med (episodic or PRN) will be considered for study continuation on an individual basis. Randomized subjects who take a prohibited med (long-term or for >7 days per concomitant illness/episode) will be withdrawn from study. Randomized subjects needing to initiate a conditional medication or alter dose of conditional medication during study will be considered on an individual basis.

10. SUBJECT COMPLETION AND WITHDRAWAL

10.1. Subject Completion

Subjects completing all 11 study visits will be considered to have completed study.

10.2. Subject Withdrawal

Subject may withdraw from study at any time for any reason without penalty or be terminated from the study by the clinical investigator (see provisions for termination by study team.) Investigational team will document the reason(s) for withdrawal. In the event a subject chooses to withdraw from study before Visit 11, the safety procedures described in Section 7.3 will be performed ideally within 3 days following subject's decision to withdraw. For all subjects who withdraw, all final safety assessments will be collected regardless of time elapsed since previous visit. In addition to the termination visit, subjects who withdraw early will be contacted within 7 days by study staff via telephone to assess development of new and/or ongoing AEs and concomitant medications. All subjects randomized to the study will be included in the analysis as part of their assigned study group regardless of treatment completion, as is consistent with the intent-to-treat principle. .

Subject's participation may be terminated at the discretion of the investigator. Individuals may be withdrawn for the following reasons:

- Clinically significant adverse events.
- Lost to follow-up.
- Protocol violations.
- Inability to tolerate study medication.

- Other.

11. ADVERSE EVENTS (AE) AND SERIOUS ADVERSE EVENTS (SAE)

11.1. Definition of AE

An adverse event is any symptom, sign, illness or experience which develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

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

11.2. Definition of SAE

Adverse events are classified as either serious or non-serious. A serious adverse event is any event that results in:

- Death.
- Life-threatening situation.
- Hospitalization or prolongation of hospitalization.
- Disability or incapacitation.
- Other events determined by investigator to be medically significant in which subject's well-being is jeopardized (e.g. events that have high likelihood of escalating to the point of meeting criteria outlined above)

11.2.1. Clinical Laboratory Abnormalities & Other Abnormal Assessments as AEs & SAEs

Any new abnormal, vital, examination, or laboratory finding judged clinically significant by the investigator will be documented as an AE or SAE, if meeting the definitions for such. Abnormal lab findings or other abnormal assessments associated with the disease under study will not be considered AEs or SAEs unless more severe than expected, as judged by the investigator.

11.2.2. Time Period and Frequency of Detecting AEs and SAEs

Upon consenting, a subject is considered to be a participant in the study, and until that person either withdraws or completes study, AEs and SAEs will be recorded. The investigational team will promptly report any AE/SAE as required per federal guidelines.

12. DATA ANALYSIS AND STATISTICAL CONSIDERATIONS

12.1. Analysis Overview

Measures of central tendency and spread will be computed for the study population and by treatment group for each outcome variable. These summaries will describe patient characteristics, confirm that randomization produced a balanced design, and confirm that the planned analyses are appropriate for the distribution of the outcome variables.

Missing data are expected to be minimal. Therefore, the primary analyses will follow an intent-to-treat principal and include all randomized subjects regardless of intervention compliance and missing data. We will use linear mixed models, an analytic approach that can accommodate unbalanced data and use all available observations in the analysis by assuming data are missing at random. Sensitivity analyses will include evaluation of missing assumptions using pattern mixture models and multiple imputation and a per-protocol approach to accommodate subjects who do not adhere to the intervention protocol.

All statistical tests will be conducted in SAS Version 9.4 and will be evaluated using a two-sided 0.05 level of significance.

12.2. Statistical Analysis of Efficacy Outcomes

Previous research has shown that the primary outcomes (ADAS-Cog 13, CDR-SOB, and FAQ) to be highly correlated. Therefore, to increase power and account for this correlation, the primary analysis will be a multivariate, linear mixed-effects regression. Specifically, the change in cognition and function measures between baseline and week 12 and between baseline and week 24 will serve as the primary outcome variables. We will include intervention arm, intervention week, intervention arm*week, age, and baseline test scores as fixed effects. The efficacy of intranasal (IN) insulin on cognition and function will be tested on the intervention arm*week interaction. Age and baseline test scores are included because of the possibility of unequal baseline values. Random intercept effects will be included to account for the nested structure of the data, multiple outcomes and observation points.

First, the assumptions of such a model will be verified. That is, the dependent variables will be tested for: (1) multivariate normality, (2) homoscedasticity, (3) equality of covariance matrices, and (4) independent observations. These outcome variables have been analyzed after log-transformation in previous studies because they have a distribution skewed to the right. We will confirm that this transformation is suitable for our study. If outcome variables are log-transformed, efficacy measures will correspond to percent change of cognitive outcomes.

To protect against type I error, efficacy of IN insulin will be considered if an overall cognitive decline is observed relative to placebo. Efficacy measures for composite cognitive and functional decline with corresponding 95% confidence intervals and p-values will be reported.

For the analysis of secondary cognition and function measures (Digit Span, Trailmaking, COWAT, Animal Naming, and WMS), secondary mood measure (GDS), and exploratory smell measure (AST) we will follow the same approach as for the primary outcomes. We have not included these tests as primary endpoints because they measure more specific effects of AD language, memory, mood, or olfaction impairment and, therefore, may be less sensitive to overall cognitive change in MCI/AD patients. Each outcome measure will be tested in its own linear mixed-effects regression model with measures from baseline to week 12 and baseline to week 24 as the outcome variables. The efficacy of IN

insulin on each measure will be tested on the intervention arm*week interaction. Efficacy measures with corresponding 95% confidence intervals and p-values will be reported.

Exploratory measures of sleep (Sleep Questionnaire and ESS) and biomarkers (CSF Abeta, tau, and phosphotau) will follow the same approach as for the primary outcomes. Two multivariate linear mixed-effects regression models will be fit with change in either sleep or biomarker measures from baseline to visit 7 and baseline to visit 10 as the outcome variables. The efficacy of IN insulin on sleep will be tested on the intervention arm*week interaction. Efficacy measures for composite sleep decline with corresponding 95% confidence intervals and p-values will be reported.

Exploratory PET metabolism will be measured in four cortical ROIs primarily affected by AD: precuneus, posterior cingulate, and bilateral parietotemporal. A multivariate regression model will again be applied to the imaging outcome measures. Specifically, standardized change in posterior cerebral metabolism in the four ROIs from baseline to 6 months will serve as the outcomes. Intervention arm, age, and baseline test scores will be used as fixed effects. Tests of assumptions and interpretation will be similar to that described for primary outcomes. Overall cerebral metabolism and metabolism at each of the four cortical ROIs efficacy measures with corresponding 95% confidence intervals and p-values will be reported.

12.3. Statistical Analysis of Safety Outcomes

Adverse event rates will be modeled using Poisson regression with a natural logarithm link function. If over dispersion is identified, a negative binomial model will be used instead. Incidence rate ratios and 95% confidence intervals will be reported. Differences in baseline to 3-month and 6-month change in vital signs and 12-lead ECG will be assessed using Student’s two-sample t-test.

12.4. Study Power

A total of n=90 subjects will be randomized to receiving either IN glulisine (n=45) or placebo (n=45) two times daily. The study is powered on the three primary outcomes, ADAS-Cog 13, CDR-SOB, and FAQ.

	Control Group Mean	Standard Deviation	Minimum Detectable Difference	Power
ADAS-Cog 13	19.2	4.6	3	88.426
CDR-SOB	7.9	1.3	1.5	99.972
FAQ	11.56	3.6	2	74.100

13. STUDY CONDUCT CONSIDERATIONS

13.1. Regulatory and Ethical Considerations, Including the Informed Consent Process

The study will be conducted in accordance with GCP. Subject privacy requirements will also be observed as well as the fundamental concepts of the Declaration of Helsinki (e.g. IRB approval of the study, obtaining informed consent from all subjects, and meeting reporting requirements).

13.2. Data Safety Monitoring Board

The DSMB will be an independent group who are not participating in the trial and have no direct affiliation with HealthPartners Center for Memory and Aging. They will serve as an advisory panel to HealthPartners Center for Memory and Aging. The DSMB will be comprised of an independent behavioral neurologist, endocrinologist, and intranasal insulin researcher. The DSMB Charter will be established prior to initiation of the study. The DSMB responsibilities include but are not limited to the following:

- Monitoring the study for compliance to the protocol.
- Stopping the study if the rate of SAE's raises safety concerns. The details will be specified in the DSMB charter.

During the course of the trial, the DSMB will review accumulating safety data to monitor for incidence of trends that would warrant termination of the trial. The frequency of the DSMB meetings, responsibilities, membership, and procedures will be documented in the DSMB charter.

13.3. Quality Assurance

In the event of a regulatory agency audit or inspection, site will allow the auditor/inspector access to all records documented and facilities utilized in conducting the study. Site will also make accommodations (e.g. time, schedule) to discuss findings, concerns, and questions with auditor/inspector.

13.4. Study Closure

Upon completion of all subject visits, data entry and analysis, investigator will inform local IRB of study closure.

13.5. Records Retention

All site records will be maintained and stored in a safe and secure location for a minimum of 15 years post study completion.

13.6. Provision of Study Results and Information to Investigators

Study results will be made available by the study statistician once analysis (interim analysis) is complete. Study staff will not be unblinded in regards to individual subject's randomization status until after database lock.

13.7. Data Management

Data collection/reporting tools will be developed internally (i.e. CRFs and source documents). Data collected and stored electronically will remain confidential and secure (e.g. secured server, encrypted data, password protected file).

13.8. Device Accountability

A Device Tracking Log will be maintained at the investigational site. POD Device will be recorded on the log upon delivery to the investigational site and will be stored in a secured area. The Device Tracking Log will be updated as each device is delivered, dispensed, returned and the reason for the return. Serial numbers, expiration date and model number of devices delivered to the site will also be recorded.

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