Therapeutic effects of intranasally-administered insulin (INI) in adults with amnestic mild cognitive impairment (aMCI) or mild Alzheimer's disease (AD)

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TABLE OF CONTENTS

1.0 **INTRODUCTION** **11**
1.1 Primary Aim 11
1.2 Secondary Aims 11
1.2.1 Secondary Aim 1 ..... *11*
1.2.2 Secondary Aim 2 ..... *11*
1.2.3 Secondary Aim 3 ..... *11*
1.2.4 Secondary Aim 4 ..... *11*
1.2.5 Secondary Aim 5 ..... *11*

2.0 **BACKGROUND AND SIGNIFICANCE** **12**
2.1 Rationale for Insulin 12
2.2 Insulin as a Therapeutic Agent 12
2.2.1 Intranasal Pathways to the CNS *13*
2.2.2 Intranasal Delivery System Device *13*

3.0 **PRELIMINARY STUDY** **15**
3.1 Participants 15
3.2 Procedures 16
3.3 Safety and Compliance 16
3.4 Statistical Analyses 16
3.5 Results: Cognitive and Functional Outcome Measures 17
3.6 Results: AD Biomarkers 18
3.7 Results: FDG-PET CMRglc 18
3.8 Safety and Compliance 19
3.9 Implications 20

4.0 **STUDY DESIGN RATIONALE** **20**
4.1 Rationale for Population Selection 20
4.2 Rationale for Dosage Selection 20
4.3 Rationale for Primary and Secondary Outcome Measures 21
4.4 Rationale for Length and Design of Trial 21
4.5 Rationale for Magnetic Resonance Imaging 21
4.6 Rationale for Biofluids 22

5.0 **STUDY OVERVIEW** **22**
5.1 Study Population 23
5.2 Diagnosis Criteria 23
5.3 Inclusion Criteria 24
5.4 Exclusion Criteria 24
5.5 Recruitment and Retention Strategies 25

6.0 **STUDY TIMELINE** **26**

7.0 **DESCRIPTION OF STUDY VISITS** **26**
7.1 Pre-Screening 26
7.2 Screening (Visit 1) 26
7.3 Baseline (Visit 2) 27
7.4 Month 3 (Visit 3) 28
7.5 Month 6 (Visit 4) 28
7.6 Month 9 (Visit 5) 28
7.7 Month 12 (Visit 6) 29

7.8 Month 15 (Visit 7, Open Label) 29
7.9 Month 18 (Visit 8, Open Label) 30

8.0 **EARLY TREATMENT/STUDY DISCONTINUATION 30**
8.1 Reasons for Early Discontinuation 30

9.0 **STUDY-SPECIFIC PROCEDURES 31**
9.1 Cognitive Evaluations 31
9.1.1 Alzheimer's Disease Assessment Scale-Cognitive (ADAS-Cog12) 31
9.1.2 Mini-Mental State Examinations (MMSE) 31
9.1.3 Memory Composite (Story Recall and Free and Cued Selective Reminding Test) 31
9.1.4 Logical Memory Test (Wechsler Memory Scale-Revised) 32
9.1.5 Trail-making Test (Part A and B) 32
9.2 Clinical and Functional Evaluations 32
9.2.1 Clinical Dementia Rating Scale – Sum of Boxes (CDR-SB) 32
9.2.2 ADCS-MCI Activities of Daily Living (ADCS-ADL-MCI) 32
9.2.3 Neuropsychiatric Inventory (NPI) 33
9.2.4 Modified Hachinski 33
9.3 Research Satisfaction Survey 33
9.4 Treatment Blinding Questionnaire 33

10.0 **STUDY METHODS 33**
10.1 Safety Assessments 33
10.2 Physical and Neurological Examination 34
10.3 Electrocardiogram (ECG) 34
10.4 Clinical Laboratory Evaluations 34

11.0 **BIOMARKER STUDIES 34**
11.1 CSF 34
11.2 Blood Collection 35
11.3 Insulin & Glucose (HgA1c Test) 35
11.4 Magnetic Resonance Imaging (MRI) 36
11.5 Genetic Samples, Storage and Future Use 36

12.0 **STATISTICAL PLAN 36**
12.1 Power Analyses 37

13.0 **POTENTIAL RISKS 38**
13.1 Safety of Intranasal Insulin 38
13.2 Risks associated with use of the POD device 39
13.3 Lumbar Puncture 39
13.4 MRI 39
13.5 Blood Draw 40

14.0 **PERSONNEL REQUIREMENTS 40**

15.0 **STUDY DRUG 41**
15.1 Humulin® R U-100 Insulin 41
15.2 Placebo (Sterile Diluent) 41
15.3 Randomization 41
15.4 Blinding 41
15.5 Study Drug Dispensing 41
15.6 Intranasal Administration 42
15.7 Compliance 42
15.8 Dose Adjustments 42

15.9 Breaking the Blind 42
15.10 Storage 42
15.11 Drug Accountability 43

16.0 **ADVERSE EVENTS 43**
16.1 Definition 43
16.2 Following Up on AEs 43

17.0 **SERIOUS ADVERSE EVENTS (SAE) 43**
17.1 Definition 43
17.2 Reporting SAEs 44

18.0 **ETHICS & REGULATORY CONSIDERATIONS 44**
18.1 Ethical Standard 44
18.2 Institutional Review Board (IRB) 44
18.3 Informed Consent & HIPAA Authorization 45
18.4 Participant Confidentiality | HIPAA 45

19.0 **GENETIC RESEARCH & STORAGE OF GENETIC MATERIAL 46**
19.1 Storage of Biospecimen Samples 46

20.0 **RISKS AND BENEFITS ASSOCIATED WITH THIS STUDY 46**
20.1 Potential Benefits of the Proposed Research to Human Subjects 46
20.2 Inclusion of Women and Minorities 47
20.3 Inclusion of Children as Participants in Research Involving Human Subjects 47

21.0 **STUDY MONITORING & DATA COLLECTION 47**
21.1 Communication Plan & Site Monitoring 48

22.0 **DATA AND SAFETY MONITORING PLAN AND BOARD 48**

23.0 **PUBLICATION POLICY 49**

24.0 **CLINICAL TRIAL REGISTRATION & SHARING OF FINAL RESEARCH DATA 49**

25.0 **TABLE 4: SCHEDULE OF PROCEDURES AND ASSESSMENTS 50**

26.0 **LITERATURE CITED 51**

STUDY GLOSSARY

3MSE	**MODIFIED MINI-MENTAL STATUS EXAM**
AB	**BETA AMYLOID**
AD	**ALZHEIMER'S DISEASE**
ADAS-COG	**ALZHEIMER'S DISEASE ASSESSMENT SCALE – COGNITIVE SUBSCALE**
ADCS	**ALZHEIMER'S DISEASE COOPERATIVE STUDY**
ADCS-ADL	**ALZHEIMER'S DISEASE COOPERATIVE STUDY - ACTIVITIES OF DAILY LIVING**
ADEAR	**ALZHEIMER'S DISEASE EDUCATION AND REFERRAL CENTER**
ADNI	**ALZHEIMER'S DISEASE NEUROIMAGING INITIATIVE**
AE	**ADVERSE EVENT**
AMCI	**AMNESTIC MILD COGNITIVE IMPAIRMENT**
ANCOVA	**ANALYSIS OF COVARIANCE**
APOE/APOE4	**APOLIPOPROTEIN (APOE) EPSILON 4 (APOE4)**
ATRI	**ALZHEIMER'S THERAPEUTIC RESEARCH INSTITUTE**
BDNF	**BRAIN-DERIVED NEUROTROPHIC FACTOR**
BID	**BIS IN DIE (TWICE A DAY)**
BUN	**BLOOD UREA NITROGEN**
CDR-SB	**CLINICAL DEMENTIA RATING – SUM OF BOXES**
CFR	**CODE OF FEDERAL REGULATIONS**
CMRGLC	**CEREBRAL METABOLIC RATE OF GLUCOSE UTILIZATION**
CNS	**CENTRAL NERVOUS SYSTEM**
CPD	**CONTROLLED PARTICLE DISPERSION**
CPK	**CREATINE PHOSPHOKINASE**
CREB	**CAMP RESPONSE ELEMENT-BINDING PROTEIN**
CRF/E-CRF	**CASE REPORT FORM/ELECTRONIC CASE REPORT FORM**
CSF	**CEREBRAL SPINAL FLUID**
DNA	**DEOXYRIBONUCLEIC ACID**
DSMB	**DATA & SAFETY MONITORING BOARD**
DSM-IV	**DIAGNOSTIC AND STATISTICAL MANUAL OF MENTAL DISORDERS, FOURTH EDITION**
DSRS	**DEMENTIA SEVERITY RATING SCALE**

EAG	**ESTIMATED AVERAGE GLUCOSE**
ECG	**ELECTROCARDIOGRAM**
EDC	**ELECTRONIC DATA CAPTURE**
EDTA	**ETHYLENE DIAMINE TETRA ACETIC ACID**
ELISA	**ENZYME-LINKED IMMUNOSORBENT ASSAY**
FCSRT	**FREE AND CUED SELECTIVE REMINDING TEST**
FDA	**FOOD AND DRUG ADMINISTRATION**
FDG PET	**FLUORO DEOXY GLUCOSE POSITRON EMISSION TOMOGRAPHY**
GCP	**GOOD CLINICAL PRACTICE**
GEE	**GENERALIZED ESTIMATING EQUATION**
GGT	**GAMMA GLUTAMYL TRANSPEPTIDASE**
GSK3*B*	**GLYCOGEN SYNTHASE KINASE 3 BETA**
HGA1C	**HEMOGLOBIN A1C**
HC	**HOMOCYSTEINE**
HCT	**HEMATOCRIT**
HCY	**HOMOCYSTEINE**
HEENT	**HEAD | EARS | EYES | NOSE | THROAT**
HGB	**HEMOGLOBIN**
HIPAA	**HEALTH INSURANCE PORTABILITY AND ACCOUNTABILITY ACT**
HOMA-IR	**HOMEOSTATIS MODEL ASSESSMENT OF INSULIN RESISTANCE**
ICF	**INFORMED CONSENT FORM**
ICH	**INTERNATIONAL CONFERENCE ON HARMONISATION**
IDE	**INSULIN DEGRADING ENZYME**
IGF-1	**INSULIN-LIKE GROWTH FACTOR-1**
INI	**INTRANASAL INSULIN**
IRB	**INSTITUTIONAL REVIEW BOARD**
ITT	**INTENT-TO-TREAT**
IU	**INTERNATIONAL UNIT**
LDH	**LACTATE DEHYDROGENASE**
LP	**LUMBAR PUNCTURE**
LTP	**LONG TERM POTENTIATION**
MCV	**MEAN CORPUSCULAR VOLUME**
ML	**MILLILITER**

MMA	**METHYLMALONIC ACID**
MMSE	**MINI MENTAL STATE EXAMINATION**
MPRAGE	**MAGNETIZATION PREPARED RAPID GRADIENT ECHO**
MR/MRI	**MAGNETIC RESONANCE / MAGNETIC RESONANCE IMAGING**
NBAC	**NATIONAL BIOETHICS ADVISORY COMMISSION**
NIA	**NATIONAL INSTITUTE ON AGING**
NIH	**NATIONAL INSTITUTES OF HEALTH**
NINCDS/ADRDA	**NATIONAL INSTITUTE OF NEUROLOGICAL AND COMMUNICATIVE DISEASES AND STROKE / ALZHEIMER'S DISEASE AND RELATED DISORDERS ASSOCIATION**
NBDA	**N-METHYL – D-ASPARTATE**
NSAID	**NON-STEROIDAL ANTI-INFLAMMATORY DRUGS**
OHRP	**OFFICE FOR HUMAN RESEARCH PROTECTIONS**
PBMC	**PERIPHERAL BLOOD MONONUCLEAR CELL**
PD	**PROJECT DIRECTOR**
PCP	**PRIMARY CARE PHYSICIAN**
PET	**POSITRON EMISSION TOMOGRAPHY**
PHI	**PROTECTED HEALTH INFORMATION**
PI	**PRINCIPAL INVESTIGATOR**
PID	**PARTICIPANT ID**
RBC	**RED BLOOD CELL**
RE	**RANDOM EFFECTS**
ROI	**REGIONS OF INTEREST**
SAE	**SEVERE ADVERSE EVENT**
SD	**STANDARD DEVIATION**
SGOT	**SERUM GLUTAMIC OXALOACETIC TRANSAMINASE**
SGPT	**SERUM GLUTAMIC PYRUVIC TRANSAMINASE**
T	**TESLA**
TSH	**THYROID STIMULATING HORMONE**
U/A	**URINALYSIS**
WBC	**WHITE BLOOD COUNT**

SIGNATURE PAGE

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PROTOCOL SYNOPSIS

TITLE	Therapeutic effects of intranasally-administered insulin (INI) in adults with amnestic mild cognitive impairment (aMCI) or mild Alzheimer's disease (AD)
PROJECT DIRECTOR	Suzanne Craft, Ph.D.
STUDY SPONSOR	National Institute on Aging
STUDY PHASE	Phase II/III
INDICATION	Amnestic mild cognitive impairment (aMCI) or probable mild Alzheimer's disease (AD)
AIM OF STUDY	To examine the effects of intranasally-administered insulin (INI) on cognition, entorhinal cortex and hippocampal atrophy, and cerebrospinal fluid biomarkers in amnestic mild cognitive impairment (aMCI) or probable mild Alzheimer's disease (AD)
PRIMARY OBJECTIVE	To test the hypothesis that 12 months of treatment with INI (compared to placebo) in adults with aMCI and probable mild AD will improve performance on a global measure of cognition
SECONDARY OBJECTIVES	1. To test the hypothesis that 12 months of treatment with INI (compared to placebo) in adults with aMCI and probable mild AD will improve performance on a memory composite (Story Recall and FCSRT) and daily functioning. 2. To test the hypothesis that INI treatment reduces the rate of hippocampal and entorhinal atrophy as measured by MRI, and to conduct exploratory analyses of other brain regions. 3. To test the hypothesis that INI will favorably alter CSF Aβ and the CSF Aβ/tau ratio, and will modulate inflammatory markers. 4. To examine whether baseline AD biomarker profile, APOE-ε4 allele carriage and gender predict treatment response. 5. To determine whether further improvement occurs after 18 months of treatment.
PRIMARY OUTCOME MEASURE	Alzheimer's Disease Assessment Scale-Cognition 12 (ADAS-Cog12)

SECONDARY OUTCOME MEASURES	Memory composite, executive function test, CDR-SB, MMSE, ADCS-ADL-MCI, rate of hippocampal and entorhinal atrophy, CSF total tau, Aβ40, Aβ42, phospho-tau 181
STUDY DESIGN	Multi-site, double-blind, placebo-controlled study in 240-300 participants with aMCI or probable mild AD for 12 months, followed by a 6-month open-label period in which all participants will receive INI.
SAMPLE SIZE	• n=240-300 • 50% (n=120-150) assigned to active INI (20 IU bid); 50% (n=120-150) assigned to placebo
SUMMARY OF KEY ELIGIBILITY CRITERIA	• Diagnosis of aMCI or probable mild AD according to the core clinical criteria updated in the NIA and Alzheimer's Association guidelines. • Age: 55 to 85 yrs (inclusive) • MMSE $\geq$ 20 at screening • Clinical Dementia Rating 0.5-1 at screening • For aMCI only: Logical Memory ≥10 for 16 or more years of education, ≥6 for 8-15 years of education, ≥4 for 0-7 years of education (Delayed Paragraph Recall of Wechsler Memory Scale–Revised). Measured at screening • Modified Hachinski score ≥ 4
DRUG DOSAGE & FORMULATION	20 IU Humulin® R U-100 or matching placebo twice daily (bid) for a total of 40 IU daily
DURATION OF PARTICIPATION	The approximate timeline for this four-year study is projected as: 1) approximately six months for study startup activities including FDA and IND submission, site IRB and regulatory approval, and training meeting; 2) approximately 18 months for recruitment; 3) 18 months of follow up (last 6 months are open label)
PLACEBO	A matching placebo (sterile diluent) will be used
ROUTE OF ADMINISTRATION	Intranasal
PROCEDURES	Physical and neurological exam, nasal examination, optional lumbar puncture, MRI, ECG, Modified Hachinski, ADAS-Cog12, CDR-SB, ADCS-ADL-MCI, memory composite (Story Recall and FCSRT), Trail-making test, MMSE, NPI, Vitals, Clinical Labs, CSF analysis & banking, Peripheral Blood Mononuclear Cell (PBMC) isolation & banking, plasma biomarkers and genotyping.

1.0 INTRODUCTION

An urgent need exists to find effective treatments for AD that can arrest or reverse the disease at its earliest stages. The emotional and financial burden of AD to patients, family members, and society is enormous, and is predicted to grow exponentially as the median population age increases. Current FDA-approved therapies are modestly effective at best. This study will examine a novel therapeutic approach using intranasally administered insulin (INI) that has shown promise in short-term clinical trials. If successful, information gained from the study has the potential to move INI forward rapidly as a therapy for AD. The study will also provide evidence for the mechanisms through which INI may produce benefits by examining key CSF biomarkers and hippocampal/entorhinal atrophy. These results will have considerable clinical and scientific significance, and provide therapeutically-relevant knowledge about insulin's effects on AD pathophysiology.

1.1 Primary Aim

To test the hypothesis that 12 months of treatment with INI (compared to placebo) in adults with aMCI or probable mild AD will improve performance on a global measure of cognition (ADAS-Cog12).

1.2 Secondary Aims

1.2.1 Secondary Aim 1

To test the hypothesis that 12 months of treatment with INI (compared to placebo) in adults with aMCI or probable mild AD will improve performance on a memory composite (Story Recall and FCSRT) and on daily function in adults with aMCI and mild AD.

1.2.2 Secondary Aim 2

To test the hypothesis that INI treatment reduces the rate of hippocampal and entorhinal atrophy as measured by MRI, and conduct exploratory analyses of other brain regions.

1.2.3 Secondary Aim 3

To test the hypothesis that INI will favorably alter CSF Aβ and the CSF Aβ/tau ratio, and will modulate inflammatory markers.

1.2.4 Secondary Aim 4

To examine whether baseline AD biomarker profile, gender or APOE-ε4 allele carriage predict treatment response.

1.2.5 Secondary Aim 5

To determine whether further improvement occurs after 18 months of treatment.

2.0 BACKGROUND AND SIGNIFICANCE

2.1 Rationale for Insulin

The rationale for the study is derived from growing evidence that insulin carries out multiple functions in the brain, and that insulin dysregulation may contribute to AD pathogenesis (Craft and Watson 2004). Insulin receptors are densely localized in the hippocampus and in entorhinal, frontal, and other cortical areas; they are found primarily in synapses, where insulin signaling modulates synaptogenesis and synaptic remodeling (Chiu, Chen et al. 2008; Zhao and Townsend 2009). Insulin facilitates memory at optimal levels, possibly through synaptic effects and enhanced hippocampal glucose utilization (Grillo, Piroli et al. 2009).

The importance of insulin in normal brain function is underscored by evidence that insulin dysregulation contributes to the pathophysiology of AD, a disorder characterized in its earliest stages by synaptic loss and memory impairment. Hoyer and colleagues first identified a reduction in insulin receptors and signaling markers in the AD brain (Frolich, Blum-Degen et al. 1998). This initial finding has been confirmed and extended by other investigators, who have demonstrated reduced CSF insulin in patients with AD and MCI (Craft, Peskind et al. 1998; Gil-Bea, Solas et al. 2010), and reduced insulin and IGF-I messaging with increasing AD pathology and cholinergic deficit (Rivera, Goldin et al. 2005). Insulin has a close relationship with β-amyloid, the toxic peptide produced by cleavage of the amyloid precursor protein (Zhao and Townsend 2009). In AD, insoluble Aβ peptides deposit in brain parenchyma and vasculature. Soluble Aβ species, particularly oligomers of the 42 amino acid specie (Aβ42), have synaptotoxic effects, possibly resulting in synapse loss, which is the earliest structural defect observed in AD (Selkoe 2008). Insulin reduces oligomer formation and protects against Aβ-induced synaptotoxicty and LTP disruption (Gasparini, Gouras et al. 2001; De Felice, Vieira et al. 2009; Lee, Kuo et al. 2009). Interestingly, Aβ also regulates brain insulin signaling. Soluble Aβ binds to the insulin receptor and disrupts insulin signaling and LTP induction in mouse hippocampal slice preparations (Townsend, Mehta et al. 2007). These effects could be prevented by exposing tissue to insulin prior to Aβ exposure. Insulin pre-treatment also prevented synthetic soluble Aβ oligomers from downregulating plasma membrane insulin receptors and reducing dendritic spines in primary hippocampal neurons (De Felice, Vieira et al. 2009). Insulin may also modulate Aβ degradation by regulating expression of insulin degrading enzyme (IDE), a metalloprotease that catabolizes insulin (Zhao, Teter et al. 2004). Collectively, these findings suggest that soluble Aβ may induce central nervous system (CNS) insulin resistance and synapse loss, and that treatment with insulin may prevent these pathological processes**.**

A role for insulin has also been suggested for other AD-related mechanisms. Insulin inhibits phosphorylation of tau, through its regulation of glycogen synthase kinase 3β, a downstream target in the insulin signaling pathway (Hong and Lee 1997). Insulin dysregulation is also associated with oxidative stress, inflammation, and impaired neurogenesis (Craft and Watson 2004). Thus, insulin has been implicated in numerous processes related to AD pathophysiology, suggesting that correction of insulin dysregulation may be a therapeutic strategy with considerable clinical and scientific significance.

2.2 Insulin as a Therapeutic Agent

The study uses insulin as a therapeutic agent and intranasal administration focusing on nose to brain transport as a mode of delivery. As reviewed above, insulin has pleiotropic effects on

pathways implicated in AD pathogenesis. As such, augmenting CNS insulin is an alternative approach to AD therapy, in contrast to the majority of therapeutic approaches that focus on narrowly defined mechanisms such as acetylcholine modulation or amyloid accumulation. Restoring normal brain insulin levels in persons with AD may improve cognition and AD pathologic processes. Such an approach is possible with an intranasal administration technique.

2.2.1 **Intranasal Pathways to the CNS**

Olfactory sensory neurons are directly exposed to the external environment in the upper nasal cavity while their axons extend through the cribriform plate to the olfactory bulb. Following intranasal administration, drugs can be directly transported to the CNS, bypassing the periphery. Several extraneuronal and intraneuronal pathways from the nasal cavity to the CNS are possible. The extraneuronal pathways appear to rely on bulk flow transport through perineural channels to the brain or CSF. In recent studies, labeled INI or a closely related peptide, insulin-like growth factor-I (IGF-I), were administered to rodents (Thorne, Pronk et al. 2004; Francis, Martinez et al. 2008). Within 30 minutes, signal was detected along olfactory and trigeminal channels, as well as in the hippocampus, amygdala and rostral and caudal cortex. An additional extracellular pathway was identified with quick access to the CSF after absorption into the submucosa along the olfactory nerve and cribriform plate (Born, Lange et al. 2002; Frey 2002; Thorne, Pronk et al. 2004). These extracellular pathways provide direct access to the CNS within minutes of intranasal administration. Additionally, an intraneuronal pathway delivers drugs to the CNS hours or days later (Broadwell and Balin 1985; Shipley 1985; Baker and Spencer 1986; Balin, Broadwell et al. 1986). Viruses and microorganisms (Fairbrother and Hurst 1930; Faber 1938; Bodian and Howe 1941), amino acids (Weiss and Holland 1967), and proteins (Kristensson and Olsson 1971; Shipley 1985; Thorne, Emory et al. 1995) can also enter the CNS via nasal routes. In particular, substances with lower molecular weights are more likely to be transported to the CNS along intranasal pathways (Sakane, Akizuki et al. 1995). Insulin's molecular weight of about 5800 g/mol makes it a good candidate for intranasal delivery. Animal studies show labeled uptake to hippocampus and rostral and caudal cortex following INI administration (Francis, Martinez et al. 2008). In a murine diabetes model, INI reduced brain atrophy, while increasing synaptic markers and activation of Akt, CREB, and GSK3β. Memory enhancement was also observed on Water Maze and radial arm tasks (Francis, Martinez et al. 2008). Human functional and cognitive studies of INI also support insulin's transport to the CNS. INI treatment increases CSF insulin levels and induces changes in auditory-evoked brain potentials compared to placebo (Kern, Born et al. 1999). INI improves verbal memory acutely in persons with AD and aMCI without affecting plasma insulin or glucose levels at the dose included in the study (Reger, Watson et al. 2006). Regarding chronic effects, several studies reported that 2 months of daily insulin administration (160 IU/day) significantly improves verbal memory in young healthy adults (Benedict, Hallschmid et al. 2004; Stockhorst, de Fries et al. 2004; Benedict, Kern et al. 2008; Hallschmid, Benedict et al. 2008; Stockhorst, de Fries et al. Submitted for publication). Finally, Section 3.0 presents results in a preliminary study in which insulin was administered to adults with AD or aMCI for 4 months.

2.2.2 **Intranasal Delivery System Devices**

The Precision Olfactory Delivery (POD) Device developed by Impel NeuroPharma (Seattle, WA) will be provided to study participants. Typical spray bottle administration results in large droplets that penetrate only within the first 20% of the lower nasal cavity. Due to gravity and insufficient airflow, approximately 90% of the droplets end up in the stomach. The POD device delivers a substance throughout the nasal cavity to the olfactory region and paranasal sinuses, thereby

maximizing access to nose-to-brain channels.

The POD device reliably delivers drug to the upper nasal cavity by employing a commercially available metered dose inhaler canister, an actuator containing a diffuser and a nasal tip. The canister contains liquid hydrofluoralkane (HFA), a propellant used whose use in metered dose inhalers was required by the FDA in 2012 as a replacement to chlorofluorocarbons. HFA propels the study drug to the nasal cavity. The HFA travels through the POD actuator body and enters the POD tip, where it mixes with the study drug formulation. The study drug formulation and HFA are then sprayed from the device.

A drug-device compatibility and functionality verification of the Impel NeuroPharma clinical Precision Olfactory Delivery device and the Novolog® insulin formulation was conducted (Impel NeuroPharma, 2015) to identify the optimal dose volume of Novolog® insulin required to achieve the target dose and confirm that the POD device consistently delivered the desired amount of both insulin and placebo. A qualitative assessment using an anatomically representative human nasal cavity model (derived from Liu, Johnson et al, 2008) confirmed that the insulin deposition was concentrated in the upper nasal cavity region.

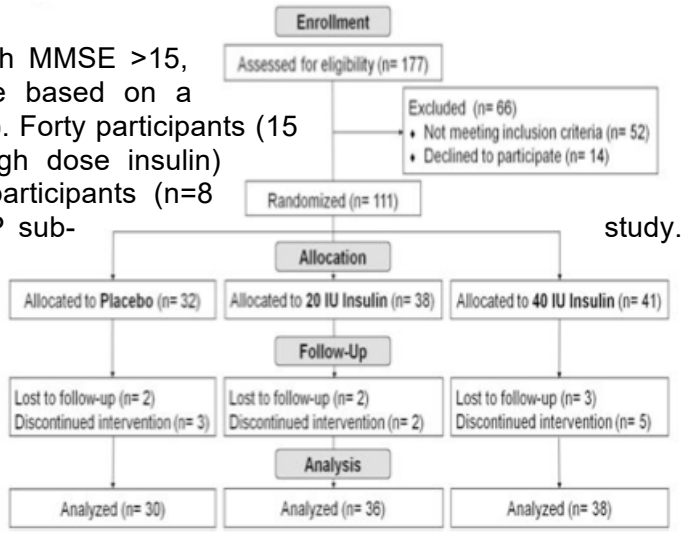
3.0 PRELIMINARY STUDY

A preliminary study using the Kurve ViaNase™ device – a device similar to the Impel POD device in terms of drug delivery action -- examined the impact of 4-month INI administration (10 or 20 IU bid vs. placebo) on the primary outcome measures of delayed story recall and the Dementia Severity Rating Scale (DSRS) as well as on measures of global cognition and function used in traditional AD clinical trials. In a subset of participants, effects on CSF AD biomarkers (Aβ42 and tau/Aβ42 ratio), and on cerebral metabolic rate of glucose utilization (CMRglc) assessed by F18FDG PET were also examined (Craft, Baker et al. 2012). Given the similar drug delivery action and equivalent dosing between the Kurve ViaNase™ and Impel NeuroPharma POD devices, the preliminary study is applicable to the Impel POD device used in this study.

3.1 Participants

Figure 1 | Trial Enrollment Flow

A total of 111 older adults were randomized in the study (Fig. 1). Data from 104 participants were analyzed.



Sample composition (aMCI, n=64; AD with MMSE >15, n=40), size, and diagnostic criteria were based on a previous study (Reger, Watson et al. 2008). Forty participants (15 placebo, 13 low dose insulin and 12 high dose insulin) completed the PET sub-study, and 23 participants (n=8 placebo and 15 insulin) completed the LP sub-study. Diagnoses were determined by expert physician and neuropsychologist consensus. Participants, caregivers, and all personnel involved in data collection were blinded to treatment assignment. Groups did not differ in education, body mass index, MMSE, gender, diagnosis, cholinesterase inhibitor treatment, or apolipoprotein E (APOE) ε4 allele carriage (Table 1).

Table 1 | Participant Characteristics

	Placebo (n=30)	Low Insulin (n=36)	High Insulin (n = 38)
Age (mean yrs, SEM)	74.9 (1.6)	72.8 (1.5)	69.9 (1.4)*
Education (mean yrs, SEM)	15.3 (0.6)	15.5 (0.5)	16.2 (0.5)
3MSE (mean, SEM)	84.2 (2.7)	83.7 (2.5)	84.3 (2.4)
BMI (mean kg/m^2, SEM)	27.4 (0.8)	26.7 (0.8)	26.9 (0.7)
Gender (%F/M)	43.3%/56.7%	38.9%/61.1%	47.4%/52.6%
AChEI Treatment (%No/Yes)	60%/40%	72.2%/27.8%	65.8%/34.2%
APOE-ε4 Carriers (%No/Yes)	55.2%/44.8%	50%/50%	57.9%/42.1%
Diagnosis (%MCI/AD)	70%/30%	55.6%/44.4%	60.5%/39.5%

*High dose<placebo, p<0.05

Participants in the high dose insulin group were younger than placebo-assigned participants (p=0.02), whereas no differences were observed between placebo and low dose insulin groups. Age was included as a covariate in all analyses.

3.2 **Procedures**

Participants were randomized to receive 10 IU INI bid for a total daily dose of 20 IU INI (n=36), 20 IU INI bid for a total dose of 40 IU (n=38) or placebo (saline bid, n=30) for 4 months. Participants were stratified by APOE-ε4 carriage. Saline or insulin (Novolin R, Novo) was administered after breakfast and dinner with ViaNase™, an intranasal delivery system (Craft, Baker et al. 2012). Parallel versions of the cognitive protocol were administered at baseline, and months 2 and 4 of treatment. Co-primary outcome measures were delayed story recall and the DSRS which had previously demonstrated beneficial effects of insulin (Reger, Watson et al. 2008). Secondary measures included the AD Assessment Scale for Cognition (ADAS-Cog) (Reger, Watson et al. 2008), a test comprised of measures of memory, orientation, and language, with higher scores reflecting impairment ranging from 0 (best) to 70 (worst), and the ADCS-Activities of Daily Living scale (ADCS-ADL) (Galasko, Bennett et al. 1997). Baseline and post-treatment fasting CSF was analyzed for Aβ42 and tau with multi-parameter bead-based immunoassay INNO-BIA AlzBio3 (Innogenetics NV). Resting PET images were obtained using a GE Advance PET scanner (GE Medical Systems, Milwaukee, WI) using a previously described protocol (Baker, Cross et al. 2011).

3.3 **Safety and Compliance**

Support persons supervised participants' intranasal administration. Blood glucose was measured daily for the first week and then weekly; no group changes were observed over the course of the study (Craft, Baker et al. 2012). Compliance was monitored by quantifying unused drug. Safety data were reviewed semi-annually by a Data and Safety Monitoring Board. Adverse event reporting followed standard guidelines.

3.4 **Statistical Analyses**

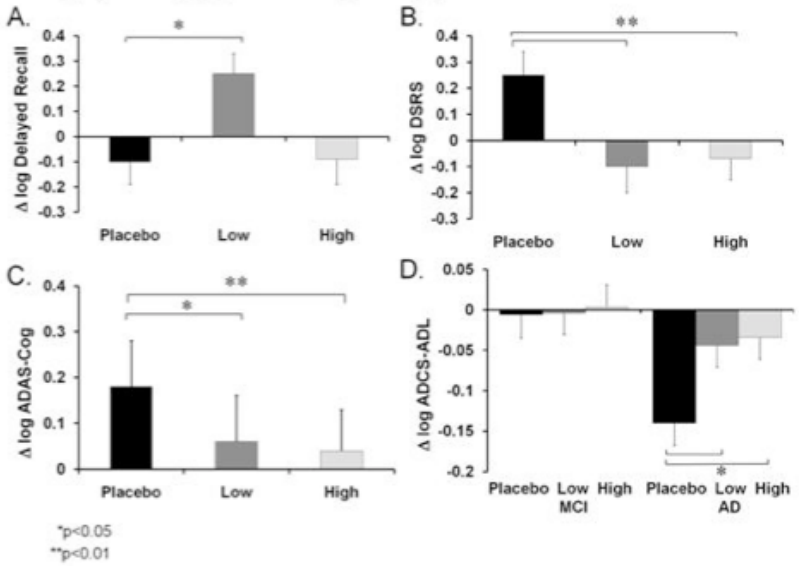
For the intent-to-treat (ITT) sample, co-primary (delayed story recall and DSRS) and secondary (ADAS-Cog and ADAS-ADL) scores were log transformed. Scores were first subjected to mixed model repeated ANCOVA including all treatment groups (placebo, low dose insulin or high dose insulin) as the between subjects factor, and time (baseline, month 2, month 4) as the repeated factor using the SAS v9.2 General Linear Models procedure. After a significant ($p<0.05$) time by treatment group interaction reflecting a different pattern of change, each of the two insulin groups was compared separately with the placebo group using repeated measures ANCOVAs. Effect sizes (Cohen's f2) were calculated for all significant effects. Age was included as a covariate in all analyses. Diagnosis (aMCI or AD), gender, APOE-ε4 carriage status (yes or no), baseline 3MSE score, and years of education were also included as covariates. Non-significant covariates were dropped from the model. Significant relationships with covariates were explored with Pearson correlation (continuous variables) or follow-up ANOVAs (class variables). Missing values were treated with multiple imputation (Rubin 1987). For exploratory CSF biomarker analyses, because only a subset of participants elected to undergo LP and no differences were observed between the two insulin dose arms, the groups were combined into a single insulin-treated group to maximize power. Biomarkers were analyzed with the repeated ANCOVA strategy described above and, due to the small sample size, exploratory Spearman correlations were conducted to examine relationships among changes in biomarkers and outcome measures. Only study completers underwent post-treatment FDG-PET. Pre and post treatment scans were co-registered within subject and anatomically standardized to Talairach and Tournoux stereotactic coordinates (Talairach and Tournoux 1988; Minoshima, Koeppe et al. 1994). Pixel intensity was normalized to pontine values (Minoshima, Frey et al. 1995). Interval

regional CMRglc changes within groups were assessed using voxel-wise one-sample t statistics (pre-/post-treatment pair) and probability integral conversion to z scores (Worsley, Evans et al. 1992). Interval changes in regional CMRglc were then compared between 1) low insulin vs. placebo groups, and 2) high insulin vs. placebo groups. Based on the number of voxels and smoothness of the statistical map, a Type I error rate was controlled at 0.05 to account for multiple comparisons (Worsley, Evans et al. 1992). The resulting statistical maps were visualized in three-dimensional stereotactic surface projections.

3.5 **Results: Cognitive and Functional Outcome Measures**

The three groups did not differ at baseline on any outcome measure; change from baseline is represented in figures for ease of interpretation. A significant overall treatment group by time interaction was observed for primary outcome delayed story recall ($p=0.005$). Compared to placebo, the low dose group had improved delayed recall (Fig. 2A; treatment by time $p=0.02$, Cohen's $f2=0.36$), whereas no effect was observed for the high dose group. Exploratory post-hoc analyses were then conducted to more closely examine the relationship of insulin dose to story recall, as this was a primary goal of this pilot clinical trial. Given findings that delayed recall may not be a sensitive measure for AD subjects due to increased variability and floor effects (Sano, Raman et al. 2011) we constructed a total story recall score (immediate and delayed), which showed improvement for the high-dose group (time by treatment interaction $p<0.05$, mean log total story recall change score with SEM = -.15(.1) for placebo vs.12 (.09) for the high dose group). A significant overall treatment by time interaction was observed for the other primary outcome measure, the DSRS ($p=0.008$). Compared with placebo, DSRS scores were preserved for both low and high dose groups (Fig.2B; treatment by time ps=0.01 and 0.01, Cohen's $f2=0.38$ and 0.41). For secondary measures, significant effects were observed for the ADAS-Cog (overall treatment by time interaction $p=0.004$). Both low and high insulin groups had less decline in cognition compared with placebo (Fig. 2C; treatment by time ps=0.04 and $p=0.002$, Cohen's $f2=0.27$ and .40). Treatment effects on the ADAS-Cog interacted with age; for the high dose insulin group, greater improvement (lowered score) tended to be associated with younger age ($r=.31$, $p=0.06$). For the ADCS-ADL, no overall effects of treatment on daily function were observed. However, a significant interaction with diagnosis was observed for this measure (overall treatment by time by diagnosis interaction $p=0.02$). Participants with AD receiving either dose of insulin had preserved function compared with placebo-assigned participants with AD who showed slight decline, whereas participants with aMCI showed no change regardless of treatment assignment (interactions for the participants with AD in low and high dose groups compared with placebo, ps=0.01 and 0.02, Cohen's $f=0.45$ and 0.43; Fig. 2D). Adjustment for APOE-ε4 status, baseline MMSE score, cholinesterase inhibitor treatment, gender, and education did not affect the pattern of any result.

Figure 2 | Change (Month 4-baseline) in log scores for (A) delayed story recall, (B) DSRS, (C) ADAS-Cog and (D) ADCS-ADL.



3.6 **Results: AD Biomarkers**

CSF Aβ42, Aβ40 and tau did not change for the placebo or insulin-treated groups as a whole. In exploratory analyses, however, for insulin-treated participants, increased CSF Aβ42 concentrations were associated with improved delayed story recall and ADCS-ADL scores, whereas decreased Aβ42 was associated with worse performance (Spearman rhos=.59, p=0.02 and .60, p=0.02). Similarly, decreased tau/Aβ42 ratios over the 4-month study period correlated with improved delayed story recall and better daily function on both ADAS-ADL and DSRS for insulin-treated participants (Spearman rhos=-.52, p=0.05, -.50, p=0.07, and .53, p=0.05). No significant correlations were observed for the placebo group.

3.7 **Results: FDG-PET CMRglc**

Compared with placebo-assigned participants, the lower dose insulin group showed reduced progression of hypometabolism in bilateral frontal, right temporal, bilateral occipital, and right precuneus and cuneus regions over the 4-month treatment period (Fig. 3 and Table 2). The higher dose insulin group showed even greater treatment effects (higher Z scores) indicating less hypometabolism progression in most regions and in left parietal cortex.

Figure 3 | Areas of hypometabolism at baseline (scan 1) and month 4 (scan 2), along with changes in hypometabolism (time 2-time 1) within each group and differences in change between placebo and low or high insulin groups (nasal insulin-placebo). Hotter floors indicate areas of greater hypometabolism from time 1 to time 2, and from placebo to insulin groups.

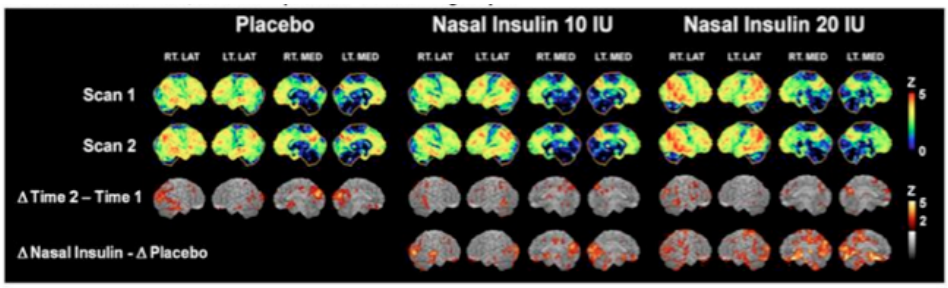


Table 2 | Z scores and stereotactic coordinates for areas of reduced progression for low and high does insulin groups compared to placebo

	Z	Stereo Coordinates x	y	z
Low Dose Insulin – Placebo				
Inferior occipital cortex (L)	4.3	19	-62	-7
Lateral temporo-occipital cortex (R)	3.9	-39	-80	2
Precuneus (R)	3.8	-3	-73	23
Superior temporal cortex (R)	3.7	-53	-24	2
Lateral occipital cortex (L)	3.5	6	-87	9
Orbital frontal cortex	3.2	-1	48	-16
High Dose Insulin - Placebo				
Orbital frontal cortex	5.8	1	23	-18
Inferior occipital cortex (L)	5.3	21	-64	-9
Inferior parietal cortex (L)	4.1	35	-40	47
Precuneus/Cuneus (R)	4.1	3	-80	18
Lateral occipital cortex (L)	3.7	26	-85	11
Medial fronto-parietal cortex (L)	3.7	10	-19	41
Caudate (R)	3.6	-12	3	20

Positive value on the x coordinate indicates the left hemisphere.
Positive value on the y coordinate indicates anterior brain.
Positive value on the z coordinate indicates superior brain.

3.8 Safety and Compliance

No treatment-related serious adverse events (SAEs) occurred during the study; most adverse events (AEs) were minor, such as mild rhinitis. AEs with an occurrence of >5% in any group are listed in Table 3. The total AE mean was higher for the low dose group compared with placebo (low dose mean total AEs with standard error=1.44±0.20, placebo =0.80±.22, p=0.04), with a similar trend for the high dose and placebo group comparison (high dose mean total AEs =1.21±0.16, placebo =0.80±.22, p=0.10). Mean compliance (number of completed doses) ranged from 95-97% and did not differ across groups.

Table 3 | Total number of adverse events and percent of sample for all events occurring for at least 5% of the participants in any treatment group.

	Placebo	Low Insulin	High Insulin
Total AEs	27/56.7%	55*/72.2%	51+/68.4%
Dizziness	3/10%	3/8.3%	5/13.2%
Headache	1/3.3%	4/8.3%	2/5.3%
Nose bleed	0/0.0%	6/8.3%	3/2.6%
Rhinitis	1/3.3%	8/16.7%	4/7.9%
URI	2/6.7%	2/5.6%	1/2.6%
Fall	2/6.7%	1/2.8%	1/2.6%
Rash	2/6.7%	1/2.8%	2/2.6%
Other	16/46.7%	30/58.3%	33/60.5%

* Low Insulin Total AEs > Placebo, p<0.05
+High Insulin Total AEs > Placebo, p<0.10

3.9 Implications

These results suggest that adults with aMCI or AD may benefit from INI treatment. Compared with placebo, the lower dose of insulin improved delayed memory, and both insulin doses preserved caregiver-rated ability to carry out daily functions. General cognition as assessed with the ADAS-Cog, the primary outcome measure for the current trial, was also preserved by both doses of INI. In exploratory analyses, changes in CSF Aβ42 and tau/Aβ42 ratios were associated with cognitive and functional changes for insulin-treated participants. Placebo-assigned participants showed decreased CMRglc values in frontal, temporal, and parietal cortices as well as precuneus and cuneus over the 4-month period, whereas insulin-treated participants showed no decline. The longstanding FDG PET finding of posterior cingulate, precuneus and cuneus hypometabolism in AD has been hypothesized to be due to functional disconnection of the hippocampal formation, so enhanced metabolism and memory with INI may reflect enhanced hippocampal input to this region. Similarly there are strong connections between the posterior cingulate, precuneus, cuneus and prefrontal and superior temporal cortex (Cavanna and Trimble 2006), which may also be affected by INI. Finally, no treatment-related SAEs occurred. These promising results provide a strong rationale for the longer, larger, multi-site trial proposed in this application.

4.0 STUDY DESIGN RATIONALE

4.1 Rationale for Population Selection

Similar to previous pilot trials that yielded positive results (Craft, Baker et al. 2012), participants with aMCI or probable mild AD will be enrolled in the current study. The rationale for this approach is based on the lack of clear clinical or neuropathologic borders between the two conditions, such that recent reviews on trial design have suggested a combined group approach (Aisen, Andrieu et al. 2011). Enrollment will not be restricted to biomarker-positive patients. The trial will collect CSF biomarkers and MRI measures of atrophy. Thus, secondary analyses will explore the relationship of biomarkers to treatment response. Inclusionary criteria parallel the Alzheimer's Disease Neuroimaging Initiative (ADNI) criteria, which will allow comparisons of imaging and biomarker data to the large ADNI cohort.

4.2 Rationale for Dosage Selection

The dosage selected (20 IU INI bid for a total of 40 IU INI daily) showed greater effect sizes for

the ADAS-Cog and greater ability to preserve cerebral glucose metabolism with PET with a good safety profile than did a lower 10 IU dose (Craft, Baker et al. 2012). Although the delayed story recall did not change with 20 IU INI bid, the trial used different inclusion criteria than the current study (MMSE 15 compared with MMSE 20) and did not sum immediate and delayed story recall. Exploratory analyses were conducted comparing the 20 IU bid dose with placebo in only participants with MMSE 20, and summed immediate and delayed recall. A significant time by treatment interaction was observed ($p<0.05$), reflecting improved performance for the 20 IU bid INI-treated group and decline for the placebo group (mean logical memory total story recall change score with standard error = .15 (.1) for placebo vs .12 (.09) for the 20 bid IU INI group) (Craft, Baker et al. 2012).

4.3 Rationale for Primary and Secondary Outcome Measures

Recent results from ADNI have shown that the ADAS-Cog changes reliably over 12 months in both aMCI and AD groups; these results served as the basis for the study power analyses. A version of the ADAS-Cog that includes a delayed list recall will be used as primary outcome measure. Although adding delayed recall to ADAS-Cog does not improve ability to detect change over time in AD patients because of increased variability, it does provide added sensitivity to detect change in aMCI (Petersen, Aisen et al. 2010). ADAS-Cog12 will be administered at 3 month intervals to increase power and optimize imputation of missing values. Several secondary outcome measures in cognitive and functional domains were chosen as they have previously shown to be sensitive to INI and have demonstrated significant decline over a 12-month period for aMCI and mild AD (Petersen, Aisen et al. 2010). In particular, a memory composite that combines immediate and delayed story recall scores and the Free and Cued Selective Reminding Test (FCSRT) will be used. The two measures will be combined with equal weighting in order to be sensitive to improved memory for both aMCI and AD. With respect to MRI measures, both hippocampal and entorhinal volume changes occurred over 12 months in ADNI (Petersen, Aisen et al. 2010).

4.4 Rationale for Length and Design of Trial

In the pilot study (Craft, Baker et al. 2012), beneficial effects of insulin on cognition, function and cerebral glucose metabolism were detected in a 4-month period. The current study design will extend the treatment period to a year for primary analyses, which should be sufficient to detect clear changes in memory and imaging endpoints for both aMCI and AD groups. The additional 6–month, open-label extension used in this semi-crossover design may incentivize participants to enroll and remain in the trial, and also provide added power for safety analyses (Jarjoura 2003). Exploratory efficacy analyses may also provide useful information, although these results will need to be interpreted with caution in light of their unblinded status.

4.5 Rationale for Magnetic Resonance Imaging

Recent data from ADNI demonstrate that structural MRI can be a credible predictor of future cognitive and functional decline, and it is sensitive to volume reductions over a 12-month period (Vemuri, Wiste et al. 2009). Jack, *et al,* examined the role of structural MRI as an endpoint for disease progression in therapeutic trials for AD and reported that multi-site consistency is feasible (Jack, Slomkowski et al. 2003). They also report hippocampal atrophy as one of the most reliable correlates of AD progression, such that mitigation of progression would be detectable over a year, given adequate power. Recent studies have also shown that entorhinal cortex has the greatest atrophy rate over a 12-month period compared with other regions

(Holland, Brewer et al. 2009). Given this finding, we will evaluate INI effects on both hippocampal and entorhinal volumes as our primary MRI outcome measures. However, we will also conduct exploratory analyses on other regions such as the precuneus and cuneus regions that have shown INI effects with FDG PET in a previous study (Craft, Baker et al. 2012).

Each site's scanner will have an identifying number, which will be appended to the image header of all MRI data. Each scanner will be subjected to a qualifying process that includes an evaluation for excessive vibration or other image artifact revealed through submitted MR images obtained through a standard protocol of an American College of Radiology phantom. In addition, 3D T1 MPRAGE or IR-FSPGR images of a human brain obtained through a standardized imaging protocol of a volunteer at each site will be submitted for automated segmentation and the resulting segmentations will be examined centrally for anatomical accuracy. Once the phantom and volunteer scan pass quality control, the scanner will be considered certified for the INI study. Because longitudinal analysis of brain volumetry will be used in this study, sites will be required to use the same scanner for both scans of a particular participant. Each site will be required to procure a clinical read of each participant's MRI data by a board certified radiologist. In addition, the image-derived inclusion and exclusion criteria will be evaluated centrally. All imaging data will be de-identified through a numerical coding system.

4.6 **Rationale for Biofluids**

CSF biomarkers such as CSF Aβ42, CSF Aβ40 and CSF total tau will be measured in participants agreeing to the optional lumbar puncture. Plasma biomarkers including Aβ42, and Aβ40 will be also assessed. Plasma and CSF samples obtained in this study will be banked so that other putative biomarkers (such as CSF insulin, isoprostane 8,12-iso-iPF2alpha-VI) may be measured by qualified investigators in the future given adequate rationale and feasibility. These putative biomarkers are among the leading candidates for AD biomarker development (Turner 2003; Grossman, Farmer et al. 2005). For example, Gilman et al, report a highly significant ($p < 0.001$) effect of Aβ immunization (with AN1792) on CSF tau with a total sample size of only 21 (N = 11 antibody responders and 10 placebo control participants; Gilman, Koller et al. 2005).

Several previous studies suggest that response to insulin may differ according to APOE genotype. In dose response studies that acutely elevated insulin through intravenous or intranasal administration, the greatest cognitive benefit was observed for adults with AD who were not ε4 carriers (Reger, Watson et al. 2008). The current study will examine ε4 carriage as a treatment response predictor.

Although Alzheimer's disease (AD) is a degenerative brain disorder, it may also affect peripheral tissue such as peripheral blood mononuclear cells (PBMCs). It has been suggested that PBMC-associated biomarkers may provide insights into the pathogenesis of AD and be used to monitor disease diagnosis and progression (Maes, Schipper et al. 2009). Thus biochemical analysis of the effects of intranasal insulin on PBMCs may provide some insights into therapeutic mechanisms. In addition, we will be able to compare treatment-related biomarker profiles in CSF and PBMCs.

5.0 **STUDY OVERVIEW**

The study will consist of a multisite, randomized, double-blind trial comparing the effects of INI (20 IU bid for total daily dose of 40 IU) and placebo for 12 months, followed by a 6-month open-label period in which all participants will receive INI. Participants with aMCI or mild AD (n=240-

300) will be enrolled. The primary outcome measure will consist of the ADAS-Cog12. Secondary measures will include a memory composite, an executive function test, CDR Sum of Boxes (CDR-SB), and the ADCS-ADL-MCI. The ADAS-Cog12 will be administered at 3 month intervals to optimize imputation of missing data. Other cognitive and functional measures will be administered at 6 month intervals. MRI measures of entorhinal cortex and hippocampal atrophy will be obtained at screening and 12 months. CSF and plasma biomarkers, as well as PBMCs and APOE-ε4 allele carriage will also be assessed. The trial will be conducted in collaboration with the Alzheimer Therapeutic Research Institute (ATRI).

5.1 **Study Population**

A total of 240-300 adults diagnosed with aMCI or probable mild AD will be enrolled in this trial. We expect to enroll no more than 50% of participants with probable mild AD and no more than 60% participants with aMCI diagnosis. To determine eligibility, all participants will undergo cognitive assessment, physical and neurological examination, ECG, clinical/safety laboratory assessment, and interviews of the participant and study partner.

5.2 **Diagnosis Criteria**

Diagnoses will be assigned by an expert clinician, which will be ensured by the fact that the majority of sites are affiliated with NIH-funded Alzheimer's Disease Centers and all sites are affiliated with reknowned Alzheimer's programs; thus all sites are staffed by clinicians expert in the diagnosis of AD and aMCI.

Participants with a MCI or probable mild AD will be diagnosed according to core clinical criteria updated in the NIA and Alzheimer's Association.

Diagnosis of aMCI requires:

a) Evidence of a decline in episodic memory
b) General preservation of independence in functional abilities
c) Absence of dementia

Biomarker evidence consistent with AD will not be required for entry for either aMCI or probable mild AD, given that knowledge in this area is evolving. Also, recent evidence indicates that requiring an AD-positive biomarker does not uniformly improve the efficiency of clinical trials (Schneider, Kennedy et al. 2010).

5.3 **Inclusion Criteria**

The following inclusion criteria* will be used:

1. Age 55 to 85 (inclusive)
2. Fluent in English or Spanish
3. Diagnosis of aMCI or probable mild AD according to the core clinical criteria outlined in the NIA and Alzheimer's Association Guidelines.
4. MMSE ≥ 20 at screening. Approval for exceptions can be requested to the Project Director for minority or low education participants with MMSE scores of 18 or 19
5. CDR 0.5-1 (inclusive) at screening
6. For aMCI group only: Logical Memory ≥10 for 16 or more years of education, ≥6 for 8-15 years of education, ≥4 for 0-7 years of education. Scores measured at screening on Delayed Paragraph Recall from the Wechsler Memory Scale–Revised
7. Modified Hachinski score ≤ 4
8. A study partner able to accompany the participant to most visits and answer questions about the participant. The study partner must have direct contact with the participant > 2 days/week (minimum of 10 hrs a week)
9. A study partner able to participate in study drug administration or able to assure that another person (such a family member or friend or other) will be able to assist the participant in the study drug administration
10. Stable medical condition for 3 months prior to screening visit
11. Stable medications for 4 weeks prior to the screening and baseline visits. However, cholinesterase inhibitors and memantine are allowable if stable for 12 weeks prior to screening and baseline visits.
12. Able to complete baseline assessments
13. At least six years of education or a work history sufficient to exclude mental retardation
14. Clinical laboratory values must be within normal limits or, if abnormal, must be judged to be not clinically significant by the investigator
15. Visual and auditory acuity adequate for neuropsychological testing

*Exceptions to these criteria may be considered on a case-by-case basis at the discretion of the Project Director.

5.4 **Exclusion Criteria**

The following exclusion criteria* will be used:

1. A diagnosis of dementia other than probable AD
2. Probable AD with Down syndrome
3. History of a clinically significant stroke
4. Current evidence or history in past two years of epilepsy, focal brain lesion, head injury

with loss of consciousness or DSM IV criteria for any major psychiatric disorder including psychosis, major depression, bipolar disorder, alcohol or substance abuse

5. Sensory impairment that would preclude the participant from participating in or cooperating with the protocol
6. Diabetes (type I or type II) requiring pharmacologic treatment (including both insulin dependent and non-insulin dependent diabetes mellitus)
7. Current or past use of insulin or any other anti-diabetic medication within one (1) year of Screening visit..
8. Evidence of any significant clinical disorder or laboratory finding that renders the participant unsuitable for receiving an investigational drug including clinically significant or unstable hematologic, hepatic, cardiovascular, pulmonary, gastrointestinal, endocrine, metabolic, renal or other systemic disease or laboratory abnormality
9. Active neoplastic disease, history of cancer within five years prior to screening (history of stable prostate cancer or non-melanoma skin cancers are not exclusionary)
10. History of seizure within past five years
11. Pregnancy or possible pregnancy. Participant is not pregnant, lactating, or of childbearing potential (i.e. women must be two years post-menopausal or surgically sterile)
12. For participants undergoing optional lumbar puncture, contraindications to LP: prior lumbosacral spine surgery, severe degenerative joint disease or deformity of the spine, platelets <100,000 or history of a bleeding disorder
13. For participants undergoing optional lumbar puncture, use of anticoagulants warfarin (Coumadin) and dabigatran (Pradaxa)
14. Contraindications for MRI (claustrophobia, craniofacial metal implants of any kind, pacemakers)
15. Residence in a skilled nursing facility at screening
16. Use of an investigational agent within two months of screening visit
17. Regular use of narcotics, anticonvulsants, medications with significant anticholinergic activity, antiparkinsonian medications, or any other exclusionary medications

*Exceptions to these criteria may be considered on a case-by-case basis at the discretion of the Project Director.

5.5 **Recruitment and Retention Strategies**

Recruitment will occur through a variety of mechanisms. The trial design includes a 6-month, open-label extension period in which all participants will receive active drug, which will facilitate recruitment and retention. Recruitment efforts will also be overseen by the Coordinating Center. The Coordinating Center has developed a coordinated recruitment plan to ensure that enrollment occurs in a timely fashion. The overall goals of the plan are to raise awareness of this trial among targeted populations to ensure adequate enrollment. The Coordinating Center partners with NIA and coordinates with its ADEAR Center to leverage existing resources. The Coordinating Center will oversee the development of materials specific to the INI trial for use by the sites, provide ongoing recruitment assistance and support, and develop tracking procedures

to monitor effectiveness of recruitment efforts. The recruitment plan for the INI trial will include both the utilization of participants existing at clinical trial centers and the recruitment of additional populations by methods of outreach.

6.0 STUDY TIMELINE

The approximate timeline for this four-year study is projected as follows: 1) approximately six months for study startup activities including FDA/IND submission, site IRB and regulatory approvals and the Investigator training meeting; 2) recruitment will last for approximately 18 months (for a total of 240-300 participants across about 30 sites); and 3) 18 months of follow up (last 6 months are open label)

7.0 DESCRIPTION OF STUDY VISITS

The "Schedule of Study Procedures and Assessments" in Table 4 (section 21.0) provides an overview of study visit activities. The primary outcome measure (ADAS-Cog12) will be measured at Baseline and all subsequent visits at three month intervals to optimize imputation of missing data. Secondary outcome measures, plasma biomarkers, and PBMCs will be measured every six months: Baseline (with the exception of MMSE and CDR conducted at Screening), Month 6, Month 12 and Month 18. MRI measures of entorhinal cortex and hippocampal atrophy will be obtained at Screening and Month 12. CSF biomarkers will be obtained at Baseline and Month 12. Genotyping will occur at Screening.

7.1 Pre-Screening

Prior to in-person screening evaluation, the investigator through routine clinical contact or through a pre-screening process will identify potential participants. Prospective participants will have a diagnosis of aMCI or probable mild AD and will be known to meet as many of the inclusion/exclusion criteria as possible. Potential participants and study partners will not be considered study participants until they sign the consent form.

7.2 Screening (Visit 1)

The purpose of this visit is to determine study eligibility and may be conducted over multiple days. Potential participants and their study partners must sign an informed consent form and HIPAA Authorization prior to administration of any study-related procedures. After consent is obtained, participants will be given the MMSE, the CDR and Logical Memory (Delayed Paragraph Recall from the Wechsler Memory Scale–Revised) to determine study eligibility.

In addition, information regarding demographics, concurrent medications, medical history and adverse events will be gathered from the participant and study partner. Vital signs, height and weight will be measured. A brief physical and neurological examination (which include a nasal examination), Modified Hachinski scale and a standard 12-lead resting ECG will be performed. The ECG report will be reviewed, signed, and dated by the investigator or a medically qualified staff member as delegated by the Principal Investigator. Those with clinically significant ECG findings will be referred for follow-up as deemed appropriate by the investigator.

Blood will be drawn and a urine sample obtained for routine clinical laboratory evaluations. If values are outside of the laboratory's normal range and determined clinically significant by the investigator, lab tests may need to be repeated and may be considered exclusionary for

participation in the study. Blood samples will also be collected for ApoE genotyping and optional DNA storage.

A screening MRI scan will be conducted for participants who met eligibility criteria (as determined by the site investigator). A local clinical read of the MRI will be reviewed by the site principal investigator (or appropriately delegated staff member) for eligibility. If the participant continues to meet all inclusion and exclusion criteria and no safety concerns are identified from the MRI, the participant can proceed to the baseline visit after approval by a clinical monitor.

Prior to randomization, the Project Director must approve any exceptions or questions regarding possible exclusionary medications, medical conditions, or laboratory tests. Re-screen is allowed, should the original screen be a failure. The re-screen should typically occur more than 3 months after the original screen failure.

7.3 **Baseline (Visit 2)**

Results from all screening procedures must be reviewed and all inclusion/exclusion criteria must be met prior to proceeding to baseline. Baseline visit will be scheduled 2-4 weeks after the screening visit. If the baseline visit is scheduled out-of-window (more than 4 weeks from screening), approval to proceed to the baseline visit should be obtained from the project director and a rescreen may be required. The baseline procedures may be completed over multiple days, but all assessments should be completed within seven days.

Vital signs and weight will be obtained, a nasal examination will be performed and concurrent medications and adverse events will be documented.

Blood for plasma biomarkers will be collected immediately before participants undergo the optional lumbar puncture. Plasma insulin, glucose, Aβ40 and Aβ42 should be measured in the morning after an overnight fast, if possible. Additional plasma, along with serum and peripheral blood mononuclear cells (PBMCs) will be collected and banked.

Cognitive and behavioral assessments will be performed and study procedures will be standardized such that the ADAS-Cog12 will be given first. Additional assessments include a memory composite (Story Recall and FCSRT), an executive function test (Trail-making Test), the ADCS-MCI-ADL and the Neuropsychiatric Inventory (NPI). A Research Satisfaction Survey will also be administered. Cognitive and behavioral assessments should not be administered while participants are fasting or immediately after the LP procedure. At study visits during which fasting samples are collected on the same day that cognitive assessments are administered, a break and food should be given to participants in between the fasting procedure and cognitive and behavioral assessments.

The optional lumbar puncture should be performed in the morning after an overnight fast, if possible. CSF samples will be used to measure levels of Abeta42, Abeta40, total tau, phospho-tau181, insulin, F2-Isoprostanes, cytokines, and BDNF. Additionally, CSF sample will be banked for future exploratory analysis. CSF will be collected and immediately frozen upright on dry ice for at least 20 minutes before being packaged along with the frozen plasma to be shipped overnight frozen on dry ice to the central biomarker laboratory.. Site staff will call the participant within approximately 24 hours from the lumbar puncture procedure to inquire about the participant’s well being and possible adverse events.

Participants who continue to meet all protocol inclusion and exclusion criteria will be randomized to receive either insulin or placebo for the next 12 months, plus 6 month of open-label. All Baseline study procedures should occur before the start of the administration of any study drug.

Participants and study partners will be trained on how to appropriately use the intranasal device utilizing a test dose of placebo and shown how to record information in the study diary. The participant and study partner will be instructed to bring any unused study drug and their study diary to the clinic at each study visit (Month 3, 6, 9, 12, 15, 18) so that study staff can assess study drug compliance.

7.4 **Month 3 (Visit 3)**

The Month 3 visit is scheduled three months (+/- 2 weeks) from day one of the Baseline visit. At the Month 3 visit, the ADAS-Cog12 will be administered. The Research Satisfaction Survey will also be administered Additionally, vital signs and weight will be obtained, a nasal examination will be conducted, adverse events and concurrent medications will be recorded. Participants will bring their device, study diary and any used and unused study drug vials to the site. Study drug compliance will be assessed. POD components (actuators, vial adapters, canisters and POD tips) will be dispensed..
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7.5 **Month 6 (Visit 4)**

The Month 6 visit is scheduled six months (+/- 2 weeks) from day one of the Baseline visit. At the Month 6 visit, blood for plasma biomarkers will be collected. Plasma insulin, glucose, Aβ40 and Aβ42 should be measured in the morning after an overnight fast, if possible.. Additional plasma, along with serum and peripheral blood mononuclear cells (PBMCs) will be collected and banked. Samples will be shipped frozen to the central biomarker laboratory where the samples will be analyzed and banked. Additional blood for clinical laboratory evaluations will be conducted to monitor safety and tolerability.

Cognitive and behavioral assessments will be performed (during non fasting session). The study procedures will be standardized such that the ADAS-Cog12 will be the first cognitive assessment administered. Additional assessments include the MMSE, memory composite (Story Recall and FCSRT), an executive function test (Trail-making Test), CDR-SB, the ADCS-ADL-MCI and NPI. The Research Satisfaction Survey will also be administered.

In addition, vital signs and weight will be obtained, physical, neurological and nasal examinations will be conducted, adverse events and concurrent medications will be recorded.. Participants will bring their device, study diary and any used and unused study drug vials to the site. Study drug compliance will be assessed. POD components (actuators, vial adapters, canisters and POD tips) will be dispensed.

7.6 **Month 9 (Visit 5)**

The Month 9 visit is scheduled nine months (+/- 2 weeks) from day one of the Baseline visit. At the Month 9 visit, the ADAS-Cog12 will be administered. In addition, vital signs and weight will be obtained, a nasal examination will be conducted, adverse events and concurrent medications will be recorded. Participants will bring their device, study diary and any used and unused study

drug vials to the site . Study drug compliance will be assessed. POD components (actuators, vial adapters, canisters and POD tips) will be dispensed.

7.7 **Month 12 (Visit 6)**

The Month 12 visit is scheduled 12 months (+/- 2 weeks) from day one of the Baseline visit. Month 12 visit is the last visit before the 6-month open-label period and the visit may occur over multiple days. The MRI should be conducted prior to the LP.

Blood for plasma biomarkers will be collected immediately before participants undergo the optional lumbar puncture. Plasma insulin, glucose, Aβ40 and Aβ42 should be measured in the morning after an overnight fast, if possible. Additional plasma, along with serum and peripheral blood mononuclear cells (PBMCs) will be collected and banked. Clinical laboratory evaluations will be conducted to monitor safety and tolerability.

The optional lumbar puncture should be performed in the morning after an overnight fast, if possible. Additionally, CSF sample will be banked for future exploratory analysis. CSF will be collected and immediately frozen upright on dry ice for at least 20 minutes before being packaged along with the frozen plasma to be shipped overnight frozen on dry ice to the central biomarker laboratory. Site staff will call the participant within approximately 24 hours from the LP procedure to inquire about the participant's well being and possible adverse events.

Cognitive and behavioral assessments will be performed. The study procedures will be standardized such that the ADAS-Cog12 will be the first cognitive test administered. Additional assessments include the MMSE, a memory composite (Story Recall and FCSRT), Trail-making Test A and B, the CDR-SB, the ADCS-ADL-MCI and the NPI. The Treatment Blinding Questionnaire and the Research Satisfaction Survey will also be administered. Cognitive and behavioral assessments should not be administered while participants are fasting or immediately after the LP procedure.

In addition, vital signs and weight will be obtained, physical, neurological and nasal examinations will be conducted, adverse events and concurrent medications will be recorded. Participants will bring their device components, study diary and any used and unused study drug vials to the site. Study drug compliance will be assessed. POD components (actuators, vial adapters, canisters and POD tips) will be dispensed.
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Participants who were previously taking placebo will cross over to active study drug. All participants will receive the active study drug during the six-month open-label period.

7.8 **Month 15 (Visit 7, Open Label)**

The Month 15 visit is scheduled 15 months (+/- 2 weeks) from day one of the Baseline visit. At the Month 15 visit, the ADAS-Cog12 will be administered. In addition, vital signs and weight will be obtained, a nasal examination will be conducted, adverse events and concurrent medications will be recorded. Participants will bring their device, study diary and any used and unused study drug vials to the site. Study drug compliance will be assessed. POD components (actuators, vial adapters, canisters and POD tips) will be dispensed.

7.9 **Month 18 (Visit 8, Open Label)**

The Month 18 visit is scheduled 18 months (+/- 2 weeks) from day one of the Baseline visit. At the final Month 18 visit, plasma insulin, glucose, Aβ40 and Aβ42 should be measured in the morning after an overnight fast, if possible. Additional plasma, along with serum and peripheral blood mononuclear cells (PBMCs) will be collected and banked. Clinical laboratory evaluations will be conducted to monitor safety and tolerability.

Cognitive and behavioral assessments will be performed (during a non- fasting session). The study procedures will be standardized such that the ADAS-Cog12 will be the first cognitive test administered. Additional assessments include the MMSE, memory composite (Story Recall and FCSRT), Trail-making Test part A and B, CDR-SB, the ADCS-ADL-MCI and NPI. The Research Satisfaction Survey will also be administered.

In addition, vital signs and weight will be obtained, physical, neurological and nasal examinations will be conducted, adverse events and concurrent medications will be recorded and study drug compliance will be assessed. Participants will return their study diary, their Impel POD device components, and any used and unused study drug vials to the clinic. Study drug compliance will be assessed during this final visit.

8.0 **EARLY TREATMENT/STUDY DISCONTINUATION**

The investigators at each site will make every reasonable effort to maximize participant retention. However, if an investigator removes a participant from treatment or study, or if a participant declines further treatment or study participation, an Early Discontinuation Visit will be completed as soon as possible following discontinuation of the study drug and or at time of study discontinuation. The Early Discontinuation Visit will contain the same assessments as the Month 12 visit, to allow collection of the main outcome measures. Depending on when the last LP or MRI was conducted, certain procedures may not be required at the Early Discontinuation Visit. If an in-person visit is not possible, site personnel will complete as much of the Early Discontinuation Visit as possible by telephone. All early treatment discontinuation participants will be strongly encouraged to complete all remaining visits through Month 12 visit.

8.1 **Reasons for Early Discontinuation**

All reason(s) for treatment/study discontinuation will be collected using categories such as:

- **Adverse experience**: The participant has experienced an adverse event that, in the opinion of the investigator, requires early termination. This may include abnormal laboratory values.
- **Death.**
- **Safety risk**: Any participant who becomes a safety risk during the trial will be withdrawn.
- **Protocol violation**: The participant fails to meet protocol entry criteria or did not adhere to protocol requirements.
- **Non-compliance**: The participant is non-compliant with completion of study-related evaluations and or intake of study drugs.

- In the **investigator's judgment**, it is in the participant's best interest to discontinue participation in the treatment/study.
- **Consent is withdrawn**. The participant wishes to withdraw from the study, or the legally authorized representative wishes the participant to be withdrawn.
- The **study is terminated** by the Coordinating Center alone or at the recommendation of the Data Safety Monitoring Board.
- **Lost to follow up.** Participant could not be recalled back to conduct follow up visits.
- **Loss of informed study partner**. The participant no longer has a responsible study partner to oversee participant visits and administration of study drug.
- **Coordinating Center Request**. The Coordinating Center determines it is in the participant's best interest to discontinue participation from the treatment and or study.

9.0 STUDY-SPECIFIC PROCEDURES

9.1 Cognitive Evaluations

9.1.1 Alzheimer's Disease Assessment Scale-Cognitive (ADAS-Cog12)

The ADAS-Cog (Rosen, Mohs et al. 1984) is a psychometric instrument that evaluates memory, attention, reasoning, language, orientation, and praxis. A higher score indicates more impairment. Scores from the original portion of the test range from 0 (best) to 70 (worse) and then number of items not recalled ranging from 0-10 is added for a maximum score of 80. A positive change indicates cognitive worsening. An ADAS-Cog12 version will be used in the current study which includes Delayed Word Recall - a measure of episodic memory (Mohs, Knopman et al. 1997). The ADAS-Cog has been the primary cognitive instrument in previous and ongoing Alzheimer's Disease therapeutic trials. Neuropsychometrists at each site are required to complete an ADAScog training and certification process. The person administering this test should not also administer the CDR.

9.1.2 Mini-Mental State Examinations (MMSE)

The MMSE (Folstein, Folstein et al. 1975) is a brief, frequently used screening instrument for AD drug studies. The MMSE evaluates orientation, memory, attention, concentration, naming, repetition, comprehension, and ability to create a sentence and to copy two overlapping pentagons. A lower score indicates more cognitive impairment. The highest score is 30.

9.1.3 Memory Composite (Story Recall and Free and Cued Selective Reminding Test)

A memory composite measure combines two episodic memory measures, immediate and delayed recall. Story Recall is a test of contextual verbal recall, in which participants listen to a story containing 44 informational bits that is read once. Participants will be asked to recall the story immediately after the reading and after approximately 20-minute delay. Credit is awarded for each bit recalled verbatim or accurately paraphrased. The previous results demonstrate the sensitivity of this task to changes in plasma insulin levels and intranasal insulin administration in adults with AD/MCI (Craft, Newcomer et al. 1996; Craft, Asthana et al. 1999; Craft, Asthana et al. 1999).

The FCSRT (Grober et al. 1988, 2008; Ferris et al. 2006) is a 16-item word list with visual and auditory presentation that uses semantic cueing to facilitate encoding and retrieval. Sixteen items, from 16 different semantic categories, are learned by identifying and naming a picture of each item when its category cue is provided. After the learning phase, a 20-second interference period is introduced to prevent rehearsal Retrieval from long-term memory is then tested by three trials of Free and Cued Recall, each preceded by a period of interference.

9.1.4 **Logical Memory Test (Wechsler Memory Scale-Revised)**

Logical Memory Test I and II (Delayed Paragraph Recall) used in this study is a modification of the episodic memory measure from the Wechsler Memory Scale-Revised (WMS-R) (Wechsler 1987). In this modified version, 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 a thirty-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. A retention or "savings" score can be computed by dividing the score achieved during delayed recall by the score achieved during immediate recall.

9.1.5 **Trail-making Test (Part A and B)**

Part A of the Trail-making tests (Reitan 1958) consists of 25 circles numbered 1 through 25 distributed over a sheet paper. The participant draws a line to connect the circles in order as quickly as possible (150 second maximum). Part B consists of 25 circles, but these circles are either numbered (1 through 13) or contain letters (A through L). Now the participant must alternate between numbers and letters in an ascending order (e.g., 1 to A; 2 to B). The time to complete Part B (300 second maximum), adjusted for the time taken to complete Part A to control for sensorimotor aspects of the task, is a sensitive measure of executive function and working memory.

9.2 **Clinical and Functional Evaluations**

9.2.1 **Clinical Dementia Rating Scale – Sum of Boxes (CDR-SB)**

The CDR (Hughes, Berg et al. 1982; Morris 1993) 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.

At Screening the CDR global score will be used for eligibility purposes. For all other administrations, the 6 domain scores will be summed to get the Sum of Boxes (SB) score. Training on the use of the CDR will be conducted to standardize its administration across sites. The CDR online training tool resides on the Washington University, St. Louis website, with oversight provided by Dr. John C. Morris (Morris, Ernesto et al. 1997). The person administering the CDR should not also administer the ADAS-Cog12.

9.2.2 **ADCS-MCI Activities of Daily Living (ADCS-ADL-MCI)**

The Alzheimer's Disease Cooperative Study - Activities of Daily Living Scale (ADCS-ADL) is an

activities of daily living questionnaire aimed at detecting functional decline in people with Mild Cognitive Impairment (MCI) (Grundman, Petersen et al. 2004). It was adapted from an inventory developed by the ADCS to assess functional performance in subjects with Alzheimer's disease (Galasko, Bennett et al. 1997). In a structured interview format, informants are queried as to whether participants attempted each item in the inventory during the prior 4 weeks and their level of performance. The ADCS-ADL-MCI scale discriminates well between normal controls and patients with mild AD or MCI. It has good test-retest reliability. The questions focus predominantly on instrumental activities of daily living scales (e.g. shopping, preparing meals, using household appliances, keeping appointments, reading).

9.2.3 **Neuropsychiatric Inventory (NPI)**

The NPI is a well-validated, reliable, multi-item instrument to assess psychopathology in AD based on interview with the study partner (Cummings 1997). The NPI evaluates both the frequency and severity of 10 neuropsychiatric disturbances. Frequency assessments range from 1 (occasionally, less than once per week) to 4 (very frequently, once or more per day or continuously) as well as severity (1=mild, 2=moderate, 3=severe). The overall score and the score for each subscale are the product of severity and frequency.

9.2.4 **Modified Hachinski**

This brief questionnaire incorporates questions about medical history, cognitive symptoms and features of stroke, reported by a study partner as well as the neurological examination and neuroimaging studies (Rosen, Terry et al. 1980).

9.3 **Research Satisfaction Survey**

A Research Satisfaction Survey will be administered in order to evaluate the participant's satisfaction with the current study. The survey will also reveal specific aspects of the study that participants may dislike, thus improvements can be made in the design of future studies. Past studies in multiple fields have also shown that consumer input and feedback is an important element in increasing retention (e.g., in psychotherapy) (Duncan, Miller et al. 2010) (Miller, Duncan et al. 2005).

9.4 **Treatment Blinding Questionnaire**

The Treatment Blinding Questionnaire assesses the perception of blind being maintained until the end of the study. The questionnaire is to be completed by the study participant, study partner, principal investigator and ADAS rater at the end of the blinded phase of the study.

10.0 **STUDY METHODS**

10.1 **Safety Assessments**

At each study visit, all participants will undergo a nasal examination and any occurrence of adverse events will be reviewed and documented; concomitant medications will be recorded as well. Physical and neurological examination and routine laboratory testing will occur at Screening, Month 6, Month 12 and Month 18 visits. An MRI will be conducted at Screening and repeated at Month 12 for safety assessment. Study staff will telephone each study participant who undergoes the optional lumbar puncture, or a person designated to speak for him/her,

approximately 24 hours after the procedure to confirm the participant's well being and query about any new adverse events. All adverse events will be reported to the DSMB. Safety reports will be prepared and submitted to the DSMB for periodic review.

10.2 **Physical and Neurological Examination**

A brief physical examination will be performed by a medically qualified professional every six months. A review of the major body systems will be performed for example: skin, head/ears/eyes/nose/throat (HEENT), cardiovascular, pulmonary, abdomen, musculoskeletal, neurological, and gastrointestinal. Assessments of height (Screening visit only), weight, and vital signs (systolic and diastolic blood pressure, pulse, temperature, and respiration) are included. Neurological examination will include an assessment of cranial nerves, strength, coordination, reflexes, sensation, tremor and gait at every study visit.

A nasal examination will be performed every three months at each visit. The examination will assess irritation or other abnormalities of the nares.

10.3 **Electrocardiogram (ECG)**

A standard 12-lead resting ECG will be performed at Screening visit. The ECG report will be reviewed, signed, and dated by the local site Principal Investigator (or a medically qualified individual delegated by the PI). Those with clinically significant ECG findings will be referred for follow-up as deemed appropriate by the investigator and may be excluded from the study.

10.4 **Clinical Laboratory Evaluations**

All routine laboratory samples will be analyzed by a central laboratory, which will provide a procedures manual and supplies. Lab reports will be reviewed, signed and dated by the local site Principal Investigator (or a medically-qualified individual delegated by the PI). If a value is outside of the laboratory's normal range, the clinician will indicate if it is clinically significant or not. If clinically significant, lab tests may need to be repeated and follow up with the participant's PCP should occur.

Clinical laboratory assessments include:

- Chemistry Panel
- TSH
- CBC | Differential
- Vitamin B12 (Screening visit only)
- HCY | MMA (Note: HCY & MMA reflex tests are only run if the B12 level is $\leq$200 pg/mL)
- HgA1c
- U/A with microscopic (Screening visit only)

11.0 **BIOMARKER STUDIES**

11.1 **CSF**

It is expected that many participants will receive optional lumbar punctures pre and post-treatment. All samples should be collected in the morning before breakfast and after an overnight fast, if possible. Participants who have contraindications to lumbar puncture including

prior lumbosacral spine surgery, severe degenerative joint disease or deformity of the spine, platelets <100,000 or a history of a bleeding disorder cannot undergo a lumbar puncture in this trial, nor can participants who are taking anticoagulants, warfarin (Coumadin) and dabigatran (Pradaxa). Based on clinician judgment and depending on the clinical indication, it may be suitable to discontinue participants from their anti-platelet agent (e.g., aspirin, Plavix, NSAIDs) for 5-7 days prior to lumbar puncture and until at least 24 hours after lumbar puncture if the participant voluntarily agrees to the lumbar puncture component of this study. It is not required that participants be discontinued from their anti-platelet agent in order to screen and enroll in the study.

At baseline, the optional lumbar puncture must be conducted prior to initiation of study drug. At Month 12, the optional lumbar puncture should be performed after (or more than 72 hours before) the MRI scan while the participant is still actively taking study drug. Additionally, the time of the last INI dosage and the time of CSF collection will be recorded. LP under fluoroscopy is allowed, if needed or required per local regulations. A post lumbar puncture telephone follow-up call will be completed approximately 24 hours after participant has undergone the procedure to assess safety.

A minimal total volume of CSF (20 ml) will be collected from participants undergoing the optional lumbar puncture for this study. To clear any blood from minor trauma associated with needle insertion, the first 1-2 mL of CSF are discarded (or more if needed). Collected CSF is aliquoted into sterile collection tubes. Approximately 2ml of CSF or volume per local laboratory requirements will be sent at ambient temperature to the local laboratory for protein, glucose and cell count. The remaining CSF will be shipped to the central biomarker laboratory. CSF must be immediately frozen upright on dry ice for at least 20 minutes before being packaged and shipped overnight frozen on dry ice to the central biomarker laboratory.

CSF samples will be used to measure levels of Abeta42, Abeta40, total tau, phospho-tau181, insulin, F2-Isoprostanes, cytokines, and BDNF. Assays will be performed by the central biomarker laboratory. CSF samples will also be frozen and stored for future analysis of putative biomarkers.

11.2 **Blood Collection**

Non-fasting blood samples will be collected at screening. Fasting blood samples (Baseline, Month 6, Month 12, and Month 18) should be collected in the morning before breakfast and after an overnight fast, if possible.

At Baseline and Month 12 visits, fasting blood samples should be collected before CSF collection, which would be processed for fasting plasma, insulin, glucose, Abeta42, and Abeta40. Additional blood for plasma, serum and peripheral blood mononuclear cells (PBMCs) will be collected. The samples will shipped to the central biomarker laboratory for processing and banking.

11.3 **Insulin & Glucose (HgA1c Test)**

HgA1c test will be performed and fasting insulin and glucose values will be used to calculate the homeostasis model assessment of insulin resistance (HOMA-IR) for participants (Matthews, Hosker et al. 1985). A central laboratory will analyze the HgA1c test.

11.4 **Magnetic Resonance Imaging (MRI)**

Screening and Month 12 MRI will use the same imaging protocol, which will include a localizer scan, followed by a high-resolution 3D T1 structural series (MPRAGE or IR-SPGR), a T2-weighted series (FLAIR), a diffusion weighted scan and a gradient recalled echo scan. The volumetric analysis procedure will include corrections for gradient nonlinearities and intensity non-uniformity (Sled, Zijdenbos et al. 1998; Arnold, Liow et al. 2001; Jovicich, Czanner et al. 2006).

For clinical assessment of MR images and meeting of MRI screening criteria, images will be read by the site radiologist and submitted without patient identifiable information to the study EDC database, where quality checks will be performed. Images will be checked for image quality and adherence to scanning protocols. 3D T1-weighted datasets passing quality checks will be corrected for spatial distortion and for intensity variation. Baseline and follow-up datasets for each participant will be spatially registered to one another using rigid-body registration followed by nonlinear registration and neuroanatomic parcellation to quantify whole-brain and subregional volumetric change on a participant-by participant basis.

MRI should be conducted prior to the LP. 1.5 Tesla (1.5T) or 3.0 Tesla (3T) scanners that have passed the study's qualification procedures will be used. Participants must be scanned by the same scanner throughout the study.

11.5 **Genetic Samples, Storage and Future Use**

DNA will be extracted from participant blood samples and will be analyzed for ApoE genotyping. ApoE genotyping will be used as a weighting factor for the minimization strategy during randomization. This will allow secondary analyses of data on the impact of the ApoE genotype on putative biomarkers of AD, clinical outcome measures, and adverse events. Participants will be asked to consent to optional DNA banking for future research studies. ApoE genotyping will be performed and the DNA will be banked at the ADCS Biomarker Core at UCSD using established protocols.

12.0 **STATISTICAL PLAN**

The intent-to-treat (ITT) data set will include all eligible individuals who are randomized. The per-protocol (PP) data set will include all eligible participants who complete 12-month assessments for the primary analysis and receive 80% of prescribed treatment doses. To address the Primary Aim, the longitudinal scores for the first 12 months on the primary endpoint (ADAS-Cog12) as well as Secondary Aims (memory composite, Trails, CDR-SB, ADCS-ADL-MCI), will be analyzed using random effects (RE) models to assess the differences in the rate of change across treatment groups (insulin-treated vs. placebo). Fixed effects for time, treatment and time-by-treatment interaction will be included. The following covariates will be included in efficacy analyses if they meet the criteria described below: baseline MMSE, baseline age, years of education, gender, APOE-ε4 carrier status, and diagnosis. Each covariate will be included in RE, ANCOVA, and GEE models only if the following two conditions are satisfied: 1) dissimilarity at baseline as measured by Fisher's exact test $P < .1$; 2) a bivariate association exists between the covariate and the response (without regard to treatment) as measured by Spearman's correlation coefficient $P< 0.15$.

To address Secondary Aims 2 and 3, CSF biomarkers (Aβ42, tau, Aβ42/tau ratio) and MRI

volumes (hippocampus, entorhinal cortex) will be analyzed with a similar strategy except that only baseline and month 12 values will be used. Other CSF biomarkers will be treated similarly in exploratory analyses. To address Secondary Aims 3 and , the predictive ability of APOE-ε4 allele carriage and CSF biomarkers will be evaluated using RE models. A model will be developed predicting longitudinal ADAS-Cog12 response. CSF Aβ42 levels and APOE-ε4 allele carriage will be included as fixed main effects along with treatment, time, and treatment-by-time interaction. A fixed effect for the interaction of Aβ42 and APOE-ε4 will also be included. Potential confounders evaluated as described above will also be included as main effects. RE models make use of all observed data, and the assumption is made that any missing data is missing at random. Responses will be modeled as multivariate normal. Time will be treated as continuous and coded as months from baseline visit, (i.e. months = (exam date minus baseline date)/30.5]. The correlation structure for the model will be first order autoregessive. As sensitivity analyses, both GEE (generalized estimating equations) and ANCOVA modeling will be performed. As an additional secondary (sensitivity) analysis, the above RE analysis will be performed treating time as categorical; differences between the active group and placebo group at 12 months will be assessed via contrasts in the least squares means; this analysis does not assume linearity over time. Associated p-values, standard errors of the differences, and 95 percent confidence intervals for each model will be presented for all analyses.

The application of the ITT principle in a change score analysis requires the imputation of any missing “post” observations. A multiple imputation strategy will be utilized for missing endpoints. Missing post-baseline data will be imputed via multiple imputations from the general location model (Little and Rubin 1987). Point estimates, standard errors, confidence intervals, and p-values will be computed using standard inference procedures for multiple imputations (Little and Rubin 1987). Safety data will be analyzed using exact contingency table methods by study arms. AEs will also *be subcategorized by relationship to study drug (unrelated/possibly related/probably related).* Details of the analytic models will be stated in a formal statistical analysis plan (SAP) that will be generated and finalized within six months of anticipated data lock.

12.1 **Power Analyses**

Power calculations to estimate the sample sizes required to detect a difference between the rate of change in two groups were based on the two-sample t-test using the formula in Statistical Methods in Medical Research (Armitage and Berry 1994). Sample sizes per arm were estimated using ADNI 12-month change scores for the ADAS-Cog12 for the combined MCI and AD population, targeting power=.80, Type 1 error level=0.05, SD=5.72. Table 5 shows the range of estimates needed to detect absolute change score differences between groups ranging from 1.6-2.6 for dropout rates between 0.15-0.25. These estimates were derived with 6 month intervals between testing, and thus more frequent administration of the ADAS-Cog12 in this trial should provide even greater power. Similarly, a 4-month pilot trial (Craft, Baker et al. 2012) dropout rate was <7%, and although a higher dropout rate is expected with a longer, multi-site trial, the excellent safety profile and incentive of the open-label extension at the end of the trial should maximize retention. The addition of a delayed recall score should increase sensitivity for aMCI participants. For MRI endpoints, sample sizes needed to detect a 25% slowing in mean rate of decline in the entorhinal cortex over a 12-month period ranged from 70 for AD to 280 for aMCI (Holland, Brewer et al. 2009). Given that our n=240-300, and the sample will be composed of both aMCI and AD, we should have adequate power.

Table 5 | Range of estimates needed to detect absolute change score differences between groups ranging from 1.6-2.6 for dropout rates between 0.15-0.25

	1.6	1.7	1.8	1.9	2.0	2.1	2.2	2.3	2.4	2.5	2.6
Dropout rate .15	431	382	341	306	276	250	228	209	192	177	163
Dropout rate .175	436	387	345	309	279	253	231	211	194	179	165
Dropout rate .20	441	391	349	313	282	256	233	214	196	181	167
Dropout rate .225	446	395	353	317	286	259	236	216	198	183	169
Dropout rate .25	451	400	357	320	289	262	239	218	201	185	171

13.0 **POTENTIAL RISKS**

13.1 **Safety of Intranasal Insulin**

Safety issues pertaining to INI administration for the treatment of diabetes have been extensively explored for over two decades (Pontiroli, Alberetto et al. 1982). For diabetes treatment, absorption enhancers must be used to increase the transport of insulin across the nasal membrane to the periphery due to the fact that peripheral bioavailablity of insulin without absorption enhancers is less than 1% (Illum 2002). A recent safety study of INI administration without absorption enhancers demonstrated no treatment induced changes in blood glucose levels, nasal airway patency, or transnasal pressure gradient (Kupila, Sipila et al. 2003). There are no known serious risks associated with INI without enhancers. A recent industry report raised the issue of rare but significant increases in lung cancer in smokers treated with inhaled insulin; six of 4740 patients taking inhaled insulin developed lung cancer compared with one of 4292 patients who received an active comparator (incidence per 100 patient years exposure, 0.13 vs 0.02). However, the inhaled insulin protocol used for diabetes treatment in this report included absorption enhancers to maximize delivery to lungs, whereas the nose-to-brain delivery device to be used in this study greatly minimizes lung delivery.

Regarding the risk of hypoglycemia, at least five peer reviewed human studies (Kern, Born et al. 1999; Born, Lange et al. 2002; Kupila, Sipila et al. 2003; Benedict, Hallschmid et al. 2004; Stockhorst, de Fries et al. Submitted for publication) and four preliminary studies (Reger, Watson et al. 2006; Reger, Watson et al. 2008; Reger, Watson et al. 2008) (Craft, Baker et al. 2012) revealed no change in blood glucose levels following intranasal insulin administration with doses that included 40 IU 4 times daily for two months. There was one exception with the case of a single participant who experienced mild hypoglycemia (52 mg/dl) after skipping a meal and engaging in sustained vigorous exercise. In addition, a recent safety study (Kupila, Sipila et al. 2003) examined intranasal insulin administration of 60 IU once a day for three weeks in 20 healthy adults. This randomized, double-blind, placebo-controlled crossover trial measured blood glucose levels six times a day during the first two and the last two days of treatment. Pre- and post-treatment blood laboratory tests and nasal examinations were performed. The nasal studies included rhinoscopy to detect local irritation, a saccharin particle test to analyze mucocilary clearance, and rhinomanometry to evaluate nasal airway patency and transnasal pressure gradient. Results indicated no change in blood glucose values with insulin, and no change in the frequency of glucose values above 3.0 mmol/L. The only symptomatic hypoglycemic value occurred during placebo treatment. Insulin treatment had no effect on other laboratory values (C-peptide, total cholesterol, HDL, LDL, triglycerides, creatinine, glutamyl transferase), blood pressure, or body weight. In addition, nasal examinations revealed no adverse effects or functional disturbances following intranasal insulin administration. No serious adverse effects of treatment were observed in the preliminary studies (Reger, Watson et al. 2006; Reger, Watson et al. 2008; Reger, Watson et al. 2008; Craft, Baker et al. 2012). However,

there are fewer safety data for INI use by patients with AD and it is possible that long-term administration may induce CNS hyperinsulinemia or insulin resistance that may have deleterious effects. Such effects would presumably manifest as accelerated cognitive or functional decline. Thus an intensive safety-monitoring plan will be in place for this study that should detect such patterns and a conservative duration of treatment that balances safety and scientific considerations.

13.2 **Risks associated with use of the POD device**

There are risks that may occur from use of the investigational intranasal drug administration device, although these are not expected to be serious. It is important that participants carefully follow the instructions they are given about how to administer the study drug and how to maintain the intranasal drug administration device and its components. If they don't carefully follow the instructions it is possible that they may not receive the correct dose. They could also experience some discomfort to their eyes or face if they do not hold the device to their nose as directed. In addition, the contents of the POD device's canister are under pressure and should not be punctured or stored near heat or open flame. Finally, the POD tip on POD device is small and could be a choking hazard for children. The participant and their Study Partner will be trained on proper device operation and asked to maintain the device and its components properly.

13.3 **Optional Lumbar Puncture**

Lumbar puncture may be associated with pain during the performance of the procedure. This is usually temporary and confined to the lower back. Headache may occur in about 5% of elderly people who undergo lumbar puncture. Less commonly, in about 1-4% of participants, a persistent low-pressure headache may develop, probably due to leakage of CSF. Lower rates of post-LP headache have been noted in elderly patients, and when atraumatic (Sprotte) needles are used. If a post-LP headache persists it may need additional treatment, e.g. with fluids and analgesics. Uncommonly a blood patch (injection of some of the participant's blood to patch the CSF leak) may be needed. Potential but rare risks of lumbar puncture include infection, damage to nerves in the back, and bleeding into the CSF space. The risk of these is much less than 1%.

13.4 **MRI**

The risks of MRI primarily arise from the possible introduction of ferromagnetic objects into a high magnetic field, which can create a dangerous projectile or lead to dysfunction or heating of an implanted medical device. All participants will be rigorously screened by MRI personnel to be certain that they do not have any medical contraindications for MRI, which include metallic foreign bodies in the brain or eye or cardiac pacemaker. This safety screening is part of routine clinical practice at MRI centers and is performed before any participant is permitted to enter the scanning room. There is a slight risk of anxiety due to claustrophobia. Any participant who experiences anxiety when placed into the MR scanner will be removed from the scanner, offered reassurance by the MR tech doing the scan, and offered the option of continuing or terminating the scan. If the participant decides that the anxiety associated with MRI is uncomfortable for them and they wish to terminate the scan, then the examination will be ended at that time. There will be no attempt to coerce participants to complete exams that they are uncomfortable with. Participants uncomfortable with MRI scans should preferably not be included in this study. Use of anxiolytic agents for completion of MRI scans is discouraged, but allowed at the discretion of the site clinician.

13.5 **Blood Draw**

The risks of blood draw include pain from the needle, bruising or infection at the site of venipuncture, or fainting as a response to blood draw. Approximately 150 mls of blood will be drawn for routine and biomarker laboratory assessments over the course of this trial.

14.0 **PERSONNEL REQUIREMENTS**

The following staff-member roles will be required to conduct the protocol at each site. Details will be provided in the procedures manual.

- **Site Protocol Principal Investigator**: The Site Protocol Principal Investigator (PI) is responsible for the overall conduct of the study at the site. The PI will perform or supervise clinical evaluation of all participants and ensure protocol adherence. The PI will supervise project personnel and ensure that clinical raters maintain a high level of skill and accuracy in conducting assessments. The PI may also perform certain ratings him or herself.
- **Study Clinician:** This person will be responsible for conducting and supervising the medical evaluation (nasal examination, physical and neurological examinations), reviewing adverse events, interpreting laboratory results, and supervising clinical care provided to the participant during the protocol. The study clinician will also perform the optional lumbar punctures. The study clinician may perform the CDR-SB. The study clinician may be a physician, or if consistent with local practice and regulations, a nurse practitioner or physician's assistant. The site Principal Investigator may also serve as the study clinician.
- **Study Coordinator:** This person will be responsible for managing the day-to-day conduct of the trial. Duties may include tracking recruitment, ensuring accurate administration of all instruments at the site, maintaining case report forms, processing of laboratory samples, maintaining a log of treatment adherence, serving as liaison with the clinical monitor, coordinating clinic visits, and administering cognitive assessments.
- **Interviewer/Psychometrician:** This person will be responsible for administering the cognitive and functional assessments. The same individual should not administer both the ADAScog and the CDR-SB.
- **CDR Rater:** This person will render the CDR-SB rating based on clinical assessment of participant and study participant. Since the CDR-SB is a secondary outcome measure, it is important for the CDR rater to remain blinded to ADAS-cog data.
- **ADAS-Cog12 Rater**: This person should be blinded to the CDR-SB data.
- **Regulatory Affairs:** This person will be responsible for managing all regulatory related documents for the duration of the trial, including submitting all required regulatory and essential documents to the Coordinating Center Regulatory Affairs.
- **Billing Remittance and Statement**: This person will be responsible for reviewing and verifying payments from the Coordinating Center are in alignment with procedures completed, along with accepting and processing payments from the Coordinating Center.
- **MRI Contact**: This person will be responsible for conducting phantom and human volunteer scans using the appropriate scanning sequence, for site qualification purposes and as needed to assess for drift. As well as conducting participant MRI scans per protocol.

15.0 **STUDY DRUG**

All participants will take two daily doses of INI (20 IU bid for a total of 40 IU daily) or placebo, administered approximately 30-60 minutes after breakfast and dinner. Participants should be instructed to skip the morning dose of study drug on study visit days to avoid the acute effects of insulin.

15.1 **Humulin® R U-100 Insulin**

Humulin® R U-100 (NDC: 0002-8215, Eli Lilly & Company) is a polypeptide hormone structurally identical to human insulin synthesized through rDNA technology in a special non-disease-producing laboratory strain of *Escherichia coli* bacteria. Humulin R U-100 has the empirical formula $C_{257}H_{383}N_{65}O_{77}S_6$ and a molecular weight of 5808 Da. Humulin R U-100 is a sterile, clear, aqueous, and colorless solution that contains human insulin (rDNA origin) 100 units/mL, glycerin 16 mg/mL and metacresol 2.5 mg/mL, endogenous zinc (approximately 0.015 mg/100 units) and water for injection. The pH is 7.0 to 7.8. Sodium hydroxide and/or hydrochloric acid may be added during manufacture to adjust the pH.

More information about the Humulin® R U-100 insulin, including risks, contraindication and adverse reactions can be found in the the US package insert (USPI).

15.2 **Placebo (Sterile Diluent)**

The matching placebo/sterile diluent (NDC: 0002-0800-01, Eli Lilly & Company) will be comprised of: Glycerin, 16 mg; Metacresol, 1.6 mg; Phenol, 0.65 mg; Sodium Phosphate Dibasic, 3.8 mg dissolved in 1 ml distilled water.

15.3 **Randomization**

We will use a semi-crossover design (Jarjoura 2003). Initially, eligible participants will be randomized on a 1:1 schedule to receive placebo or 20 IU bid of INI using the following randomization strategy: ApoE-e4 carrier status (yes or no), gender, MMSE score (high, low). Study site and age will be weighted in a covariate-adaptive randomization strategy to achieve optimal balance between treatment arms on important factors that may influence treatment outcome. After 12 months of double-blinded treatment with placebo or 20 IU bid of insulin, all participants will receive 20 IU INI bid in a 6-month, open-label extension.

15.4 **Blinding**

For the initial 12-month period, neither participants, study partners, site personnel, most Coordinating Center staff, nor PI will know whether the participant is receiving active study drug or placebo. Exceptions may be personnel involved in preparing the DSMB reports, members of the DSMB and personnel involved in managing certain aspects of the study drug supply chain.

15.5 **Study Drug Administration**

Participants will use a needleless syringe and a vial adapter to load study drug into the POD tip. Package labels and study diary will instruct participants and their study partners to administer each dose 30-60 minutes after breakfast and dinner. If a dose is missed, it should not be replaced.

15.6 **Intranasal Administration**

The POD device specifically targets olfactory delivery to maximize drug transport to the CNS by releasing a measured insulin dose (0.1 mL) into a POD tip that rests inside the participant's nostril. The insulin or placebo is delivered by actuating a propellant, delivering 10 IU to the upper nasal cavity each time the device is activated for a total daily dose of 40 IU. A pilot study (Core Human Factors, Inc., 2012) evaluating the usability and tolerability of the POD device demonstrated that participants preferred the POD device over a conventional nasal pump.

Participation of the study partner in the administration of the study drug is recommended for all participants regardless of which device is used. If site clinicians determine that assistance is needed, study partners will participate in placing the study drug into the device and administering the insulin or placebo twice daily (after breakfast and dinner). Participants and study partners will be trained in use of the delivery device. If the study partner is not available to assist with the administration of the study drug twice a day, another person (such as a family member or friend or other) may be appropriate to assist the participant. The person (or persons) who assists with the study drug administration must be properly trained prior to assisting.

15.7 **Compliance**

Compliance will be assessed by on-site vial counts preferably during each study visit. Participants will be instructed to return their study diary and all used and unused study drug vials at each visit. Site personnel will count returned vials and evaluate compliance preferably while the participant and study partner are still on-site. Participants found to be more than 20% noncompliant will receive specific instruction from site personnel, including re-instruction on study procedures and additional telephone contact between visits.

15.8 **Dose Adjustments**

Dose adjustments by the Investigator are generally not permitted, however if a participant experiences an AE that is clinically significant, the Investigator has the option of interrupting therapy. The Investigator should contact the Project Director to obtain approval and instructions for a re-challenge.

15.9 **Breaking the Blind**

In the case of an adverse event requiring treatment, sites will be directed to treat each participant as if he/she were receiving active study drug. Decisions regarding breaking the blind must be made in conjunction with the Project Director or Medical Director. If the blind has been broken in any instance, the PI must document the following information on the participant's case report form: the date, the site personnel exposed to the treatment assignment, and the reason the blind was broken.

15.10 **Storage**

Participant and study partners will be instructed to keep the study drug at a controlled temperature in the refrigerator.

15.11 **Drug Accountability**

Participants must return all used, unused and partially used study drug vials to the investigator. All returned study drug vials will be accounted for by the clinical monitor during monitor visits and disposed of according to local site procedures.

16.0 **ADVERSE EVENTS**

16.1 **Definition**

An adverse event (AE) is defined as per the Code of Federal Regulation Title 21 Part 312.

http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=312.32

Adverse events which occur after informed consent is signed include but are not limited to: (1) worsening or change in nature, severity, or frequency of conditions or symptoms present at the start of the study; (2) participant deterioration due to primary illness; (3) intercurrent illness; and (4) drug interaction. An abnormal laboratory value will only be reported as an AE if the investigator considers it to be an AE, or if it leads to the participant being withdrawn from the study.

The investigator should attempt to establish a diagnosis of the event based on signs, symptoms, and or other clinical information. In such cases, the diagnosis should be documented as the AE and not the individual signs or symptoms. Symptoms and conditions present at the beginning of the study will be characterized, so that AEs can be defined as any new symptom, or any increase in frequency or severity of an existing symptom.

Following questioning and evaluation, all AEs, whether determined to be related or unrelated to the study drug by a medically qualified site PI or clinician (MD, DO, NP or PA), must be documented in the participant's medical records, in accordance with the investigator's normal clinical practice, and on the AE *e*-CRF. Each AE is evaluated for duration, severity, seriousness, and causal relationship to the study drug.

16.2 **Following Up on AEs**

The investigator is obliged to follow participants with AEs until the events have subsided, the conditions are considered medically stable, or the participants are no longer available for follow up. Participants who discontinue due to adverse experiences will be treated and followed according to established medical practice. All pertinent information will be entered into the *e*-CRF. All adverse events will be reported to the DSMB. Adverse events will be rated as mild, moderate or severe. This will also pertain to abnormal laboratory values deemed clinically significant by the site clinician.

17.0 **SERIOUS ADVERSE EVENTS (SAE)**

17.1 **Definition**

A serious adverse event is defined as per the Code of Federal Regulation Title 21 Part 312

http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=312.32

17.2 **Reporting SAEs**

Any serious and adverse event due to any cause, which occurs during the course of the investigation (i.e. anytime after informed consent, regardless of study drug exposure) or within 30 days of receiving the last dose or last study visit, whichever is longer, must be reported to the Project Director and the Coordinating Center within 24 hours of learning of the event. All serious adverse events will be reported to the DSMB.

18.0 **ETHICS & REGULATORY CONSIDERATIONS**

18.1 **Ethical Standard**

Study investigators are charged with conducting this study in full conformity with:

1. Good Clinical Practice (GCP) guidelines, as defined by the International Conference on Harmonisation (ICH) Guideline, Topic E6
2. The United States Code of Federal Regulations, Title 21, Part 50 (21CFR50) – Protection of Human Subjects
3. 21CFR56 – Institutional Review Boards (IRBs)
4. HIPAA
5. State and Federal regulations and all other applicable local regulatory requirements and laws.

Study personnel involved in conducting this study will be qualified by education, training and experience to perform their respective task(s) in accordance with GCP.

18.2 **Institutional Review Board (IRB)**

Each participating institution must provide for the review and approval of this protocol and the associated informed consent documents and recruitment material by an appropriate IRB registered with the Office for Human Research Protections (OHRP). Any amendments to the protocol or consent materials must also be approved before they are placed into use. In the United States, only institutions holding a current US Federalwide Assurance issued by OHRP may participate.

The investigator must obtain approval from the IRB for all subsequent protocol amendments and, when warranted, changes to the informed consent document. Protocol and informed consent form amendments can be made only with the prior approval of the Coordinating Center. The investigator may not implement any protocol deviation without prior notification to the Coordinating Center and prior review and documented approval of the IRB, except where necessary to eliminate an immediate hazard to study participants, or when change(s) involve only logistical or administrative aspects of the trial (ICH 4.5.4). The investigator shall notify the IRB of deviations from the protocol or serious adverse events occurring at the site, in accordance with local procedures.

18.3 **Informed Consent & HIPAA Authorization**

In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirement(s), and should adhere to GCP and to the ethical principles that have their origin in the Declaration of Helsinki. Prior to the beginning of the trial, the investigator should have the IRB's written approval of the written informed consent form (ICF) and any other written information to be provided to participants. Participants, their relatives, guardians, or authorized representatives and study partners will be given ample opportunity to inquire about the details of the study. Prior to a subject's participation in the trial, the written informed consent form and HIPAA Authorization should be signed and personally dated by the subject and/or the subject's legally authorized representative, the study partner and by the person who conducted the informed consent discussion. Participants should be provided a copy of the signed ICF.

The informed consent will not only cover consent for the trial itself, but for the genetic research, biomarker studies, biological sample storage and imaging scans as well. The consent for storage will include consent to access stored data, biological samples, and imaging data for secondary analyses. Consent forms will specify that DNA and biomarker samples are for research purposes only; the tests on the DNA and biomarker samples are not diagnostic in nature and participants will never receive results. MRI scan findings of clinical significance, determined by the site radiologist, can be shared with participants per site clinician discretion.

Consent forms will be developed by the Coordinating Center in collaboration with the Project Director (PD). The sample consent form includes all of the required elements of informed consent required by the FDA and GCP. Once developed and approved by the PD, the sample is sent to sites participating in the protocol where it is tailored to include site-specific information as well as to meet local IRB consent form regulations. Each site's PI, under the guidance of his/her IRB, is responsible for ensuring that all applicable state laws are met with regards to judgment of competency and the consent form process. Each study site must submit its letter of IRB-approval and the approved consent form to Regulatory Affairs at the Coordinating Center along with other required regulatory records and essential documents in order to be approved to receive study drug/supplies and enroll participants into this study. During the first monitoring visit following participant enrollment, the clinical monitors will review the consent forms and verify that the proper signatures have been obtained.

18.4 **Participant Confidentiality | HIPAA**

Participant confidentiality is strictly held in trust by the participating investigators, research staff, and the sponsoring institution and their agents. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. 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 sponsoring institution. Authorized representatives of the Coordinating Center 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. Any data, specimens, forms, reports, and other records that leave the site will be identified only by a subject identification number to maintain confidentiality. All records will be kept in a locked file cabinet. All computer entry and networking programs will be done using subject IDs only. Information will not be released without written permission of

the subject, except as necessary for monitoring by the Coordinating Center, their representatives,, IRB, FDA, NIA, and the OHRP.

Information about subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed HIPAA Authorization informing the subject of the following:

- What protected health information (PHI) will be collected from participants in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research participant to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. Each site PI, under the guidance of his/her IRB, is responsible for ensuring that all applicable HIPAA regulations and State laws are met.

19.0 GENETIC RESEARCH & STORAGE OF GENETIC MATERIAL

The DNA is banked in locked freezers in the ADCS Biomarker Core at UCSD. Sample tubes are bar-coded and linked to participant ID number only and banked without personal identifiers. The presence of the sample is recorded into a computerized inventory database that is encrypted and password-protected.

Only DNA from consenting participants will be banked and used to facilitate future research on aging and dementia, particularly in the discovery of genetic polymorphisms that may influence risk of developing AD. Collection of DNA will permit qualified investigators to probe candidate genetic polymorphisms as predictors of outcome in future studies. The samples will be stored by the ADCS as long as funding is available from the NIH. If funding should lapse completely, the UCSD ADRC will provide responsible custodianship of the ADCS biospecimen bank.

An online request form is available through the ADCS website for investigators wishing to access DNA samples stored by the ADCS. Specific procedures for requesting samples for future genetic studies have been prepared by the ADCS DNA subcommittee in accordance with the recommendations of the NBAC Human Biological Materials Report. These DNA guidelines were developed in accordance with the American Society for Human Genetics' position paper on the NBAC report and the Ad Hoc Committee on Stored Tissue of the College of American Pathologists.

19.1 Storage of Biospecimen Samples

All biospecimens being banked for future AD biomarker research will be shipped to and stored by the ADCS Biomarker Core at UCSD.

20.0 RISKS AND BENEFITS ASSOCIATED WITH THIS STUDY

20.1 Potential Benefits of the Proposed Research to Human Subjects

There is an urgent need to identify promising treatments for patients with AD and its prodrome aMCI. In a previous trial (Craft, Baker et al. 2012), intranasal insulin safely improved delayed

memory and function in these patients. The trial is designed to answer the important question of whether intranasal insulin can safely provide clinically meaningful benefit to adults with AD and aMCI.

There are significant potential scientific and clinical benefits for the participant population. Currently, little is known about the relationship between insulin abnormalities, A regulation, and cognitive impairment in AD. As impaired insulin metabolism may contribute to the symptoms and pathophysiology of AD, this study has the potential to identify significant mechanisms related to cognitive decline and suggest novel therapeutic strategies for patients with AD. In contrast, the relatively minor risks posed by the intranasal administration, cognitive testing, MRI and LP are outweighed by the value of the scientific investigations outlined in this study.

20.2 **Inclusion of Women and Minorities**

There are currently no studies that definitively support or negate the existence of significant differences in response to intranasal insulin in subgroups defined by gender or ethnic background. A specific goal percentage for women and minority enrollment is not set for this study. However, we will monitor minority enrollment throughout the study and make special effort to encourage minority enrollment. Minority enrollment will be facilitated through minority outreach effort coordinated by the Recruitment and Retention group overseen by the Coordinating Center. Each site will be encouraged to employ specific efforts (e.g. media and community outreach activities) to attract appropriate minority participants to the trial. Spanish translation is available for a variety of instruments deployed in this trial. The success of minority recruitment is based on the specific efforts of each site (e.g. media and community outreach efforts) as well as the criteria for entry. This trial has been designed to maximize minority participation in both ways. No participant will be excluded due to his or her sex, race, or ethnic group.

No studies have been completed that examine racial or ethnic differences in response to treatment with intranasal insulin. One study has been published examining gender differences in which 32 healthy participants were administered 160 IU regular human insulin or placebo intranasally; acute effects on cognitive performance were examined as outcomes. Their findings indicated that women showed the most cognitive benefit on hippocampal-dependent measures, and no sex differences on hippocampal-independent tasks (Benedict, Kern et al. 2008). Although this one study provides only modest support for sex differences, the current study will conduct secondary analyses designed to detect significant differences in intervention effect between sexes.

20.3 **Inclusion of Children as Participants in Research Involving Human Subjects**

Children will not be included.

21.0 **STUDY MONITORING & DATA COLLECTION**

Clinical monitors are responsible for contacting and visiting the investigator for the purpose of inspecting the facilities and, upon request, inspecting various records of the trial (e.g., case report forms and other pertinent data).

Clinical monitors are responsible for inspecting the online case report forms at regular intervals throughout the study to verify adherence to the protocol, completeness and accuracy of the

data, and adherence to local regulations on the conduct of clinical research. The investigator agrees to cooperate with the monitor to ensure that any problems detected in the course of these monitoring visits are resolved.

In accordance with ICH GCP, representatives of the Coordinating Center may select this study for audit. Inspection of site facilities (e.g., pharmacy, laboratories) and review of study-related records will occur to evaluate the trial conduct and compliance with the protocol, GCP, and applicable regulatory requirements.

Results of locally administered tests such as ECG tracings and local clinical laboratory reports must be retained as source documents.

No study document shall be destroyed without prior written agreement between the Coordinating Center and the investigator. Should the investigator wish to assign study records to another party or move them to another location, he/she may do so only with the prior notification and written consent of the ATRI/RA.

21.1 **Communication Plan & Site Monitoring**

Communication between the PI, the Coordinating Center, and the sites will occur through several mechanisms. Sites will communicate performance data to the clinical operations team, Regulatory Affairs and the Clinical Monitoring groups on an ongoing basis. These statistics will be reported during bi-weekly conference calls attended by the PD and the study team. Sites will also receive formal monitoring visits approximately twice yearly in which all ongoing trial operations and source documents are reviewed. Visit finding information is provided to the PI, following each monitoring visit.

22.0 **DATA AND SAFETY MONITORING PLAN AND BOARD**

The Medical and Safety Core will be responsible for medical management of the trial including standardized coding of AEs and central medical monitoring. Safety reports will be created for the DSMB on a quarterly basis. Operations Core personnel will generate any aggregate summary tables, as needed, to the PI and the clinical sites to fulfill their regulatory/reporting obligations to the FDA, their respective site IRBs, as well as to the NIA, and other oversight and regulatory agencies. The Data Safety and Monitoring Board will monitor this trial. No investigator involved in the trial is a member of the DSMB. The initial task of the DSMB will be to review the protocol to identify any necessary modifications. If modifications are necessary, revisions will be reviewed by the DSMB prior to its recommendation on initiation of the project. The DSMB, based on its review of the protocol, will identify the data parameters and format of the information to be regularly reported. The DSMB will be informed of the occurrence of any serious adverse events and immediately notified of fatal or life threatening events. The DSMB may at any time request additional information from the study PI. The DSMB will initially be provided with data blinded to treatment status, but they may request unblinded data if there is a safety concern. The DSMB and NIA representative will meet in person or by conference call on a quarterly basis. Based on the review of safety data, the DSMB will make recommendations regarding the conduct of the study. These may include amending safety monitoring procedures, modifying the protocol or consent, terminating the study or continuing the study as designed. The Board will also be informed in a real-time basis of all immediately reportable AE (FDA defined serious AE). All reports are stripped of identifying information. These processes ensure substantial oversight and case review to alert the investigators, in a timely manner, to any safety

issues that may arise.

23.0 PUBLICATION POLICY

The results of this study will be published. To coordinate dissemination of data from this study, a publication committee will be formed. The committee will consist of the Protocol Committee, interested Principal Investigators and appropriate Coordinating Center personnel. The committee will solicit input and assistance from other Investigators as appropriate and adhere to all Coordinating Center Publications Policies.

24.0 CLINICAL TRIAL REGISTRATION & SHARING OF FINAL RESEARCH DATA

This trial has been registered on www.ClinicalTrials.gov (NCT01767909).

Data from this research will be shared with other researchers pursuant to the 02/26/2003 “NIH Final Statement on Sharing Research Data”. The research grant contains a data sharing policy consistent with the goals of the NIH but which also respects the rights of commercial partners. NIH believes that data sharing is important for further translation of research results into knowledge, products, and procedures to improve human health. The NIH endorses the sharing of final research data to serve these and other important scientific goals. To protect subjects’ rights and confidentiality, identifiers will be removed from the data before they are shared.

25.0 TABLE 4: SCHEDULE OF PROCEDURES AND ASSESSMENTS

Visit #	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Visit Name/Month	**Screen**	**Baseline**	**3**	**6**	**9**	**12**	**15***	**18***
Informed Consent	X							
Demographics	X							
Medical History	X							
Concomitant Meds	X	X	X	X	X	X	X	X
Nasal Exam	X	X	X	X	X	X	X	X
Physical and Neurological Exam	X			X		X		X
Vital Signs	X	X	X	X	X	X	X	X
Height	X							
Weight	X	X	X	X	X	X	X	X
ECG	X							
Modified Hachinski	X							
Logical Memory II Subscale (Wechsler Memory Scale)	X							
ADAS-cog12		X	X	X	X	X	X	X
MMSE	X			X		X		X
Memory Composite (Story Recall, FCSRT)		X		X		X		X
Trail-making Test (Part A & B)		X		X		X		X
ADCS-ADL-MCI		X		X		X		X
NPI		X		X		X		X
CDR	X			X		X		X
Treatment Blinding Questionnaire						X		
Research Satisfaction Survey		X	X	X		X		X
Adverse Events	X	X	X	X	X	X	X	X
Urinalysis	X							
Blood Draw	X	X		X		X		X
- Clinical Labs	X			X		X		X
- ApoE Genotyping | DNA Banking[1]	X							
- Biomarkers | Serum | PBMCs | Sample Banking		X		X		X		X
Optional LP		X				X		
- CSF Biomarkers | Banking[2]		X				X		
- post-procedure safety telephone check		X				X		
MRI	X[3]					X		
INI Device Training		X						
Distribute replacement POD device components			X	X	X	X	X	
Study Diary Return			X	X	X	X	X	X
Drug Compliance			X	X	X	X	X	X
Return of devices and components								X

*Open Label visits
[1]DNA banking is optional
[2]CSF banking is optional
[3]Screening MRI to be conducted after confirmation from clinician that the participant has met inclusion/exclusion criteria

26.0 **LITERATURE CITED**

Aisen, P. S., S. Andrieu, et al. (2011). "Report of the task force on designing clinical trials in early (predementia) AD." Neurology **76**(3): 280-286.

Albert, M. S., S. T. Dekosky, et al. (2011). "The diagnosis of mild cognitive impairment due to Alzheimer's disease: Recommendations from the National Institute on Aging and Alzheimer's Association workgroup." Alzheimers Dement.

Armitage, P. and G. Berry (1994). Statistical methods in medical research. Oxford, Boston, Blackwell Scientific Publications.

Arnold, J. B., J. S. Liow, et al. (2001). "Qualitative and quantitative evaluation of six algorithms for correcting intensity nonuniformity effects." Neuroimage **13**(5): 931-943.

Baker, H. and R. F. Spencer (1986). "Transneuronal transport of peroxidase-conjugated wheat germ agglutinin (WGA-HRP) from the olfactory epithelium to the brain of the adult rat." Exp Brain Res **63**(3): 461-473.

Baker, L. D., D. J. Cross, et al. (2011). "Insulin resistance and Alzheimer-like reductions in regional cerebral glucose metabolism for cognitively normal adults with prediabetes or early type 2 diabetes." Arch Neurol **68**(1): 51-57.

Balin, B. J., R. D. Broadwell, et al. (1986). "Avenues for entry of peripherally administered protein to the central nervous system in mouse, rat, and squirrel monkey." J Comp Neurol **251**(2): 260-280.

Benedict, C., M. Hallschmid, et al. (2004). "Intranasal insulin improves memory in humans." Psychoneuroendocrinology **29**(10): 1326-1334.

Benedict, C., W. Kern, et al. (2008). "Differential sensitivity of men and women to anorexigenic and memory-improving effects of intranasal insulin." J Clin Endocrinol Metab **93**(4): 1339-1344.

Bodian, D. and H. A. Howe (1941). "Experimental studies on intraneuronal spread of poliomyelitis virus." Bull Johns Hopkins Hosp **68**: 248-267.

Born, J., T. Lange, et al. (2002). "Sniffing neuropeptides: a transnasal approach to the human brain." Nat Neurosci **5**(6): 514-516.

Broadwell, R. D. and B. J. Balin (1985). "Endocytic and exocytic pathways of the neuronal secretory process and trans-synaptic transfer of wheat germ agglutinin-horseradish peroxidase in vivo." J Comp Neurol **242**(4): 632-650.

Cavanna, A. E. and M. R. Trimble (2006). "The precuneus: a review of its functional anatomy and behavioural correlates." Brain **129**(Pt 3): 564-583.

Chiu, S. L., C. M. Chen, et al. (2008). "Insulin receptor signaling regulates synapse number, dendritic plasticity, and circuit function in vivo." Neuron **58**(5): 708-719.

Core Human Factors, Inc. for Impel Neuropharma (2012). "Usability and Tolerability Testing of the Pressurized Olfactory Delivery (POD) Device: 1-40.

Craft, S., S. Asthana, et al. (1999). "Enhancement of memory in Alzheimer disease with insulin and somatostatin, but not glucose." Arch Gen Psychiatry **56**(12): 1135-1140.

Craft, S., S. Asthana, et al. (1999). "Insulin metabolism in Alzheimer's disease differs according to apolipoprotein E genotype and gender." Neuroendocrinology **70**(2): 146-152.

Craft, S., L. D. Baker, et al. (2012). "Intranasal insulin therapy for Alzheimer disease and amnestic mild cognitive impairment: a pilot clinical trial." Arch Neurol **69**(1): 29-38.

Craft, S., L. D. Baker, et al. (2012). "Intranasal Insulin Therapy for AD and MCI: A Pilot Clinical Trial." Arch Neurol **69**: 29-38.

Craft, S., J. Newcomer, et al. (1996). "Memory improvement following induced hyperinsulinemia in Alzheimer's disease." Neurobiol Aging **17**(1): 123-130.

Craft, S., E. Peskind, et al. (1998). "Cerebrospinal fluid and plasma insulin levels in Alzheimer's disease: relationship to severity of dementia and apolipoprotein E genotype." Neurology **50**(1): 164-168.

Craft, S. and G. S. Watson (2004). "Insulin and neurodegenerative disease: shared and specific mechanisms." Lancet Neurol **3**(3): 169-178.

Cummings, J. L. (1997). "The Neuropsychiatric Inventory: assessing psychopathology in dementia patients." Neurology **48**(5 Suppl 6): S10-16.

De Felice, F. G., M. N. Vieira, et al. (2009). "Protection of synapses against Alzheimer's-linked toxins: insulin signaling prevents the pathogenic binding of Abeta oligomers." Proc Natl Acad Sci U S A **106**(6): 1971-1976.

Duncan, B. L., S. D. Miller, et al. (2010). The heart and soul of change: Delivering what works in therapy, American Psychological Association.

Faber, H. K. (1938). "The early lesions of poliomyelitis after intranasal inoculation." J Pediat **13**(1): 10-37.

Fairbrother, R. W. and E. W. Hurst (1930). "The pathogenesis of, and propagation of the virus in, experimental poliomyelitis." J Path Bact **33**: 17-45.

Ferris S.H., Aisen P.S., et al. (2006). " Alzheimer's Disease Cooperative Study Group. ADCS Prevention Instrument Project: overview and initial results." Alzheimer Dis Assoc Disord **20**(4)(suppl3): S109-S123.

Folstein, M. F., S. E. Folstein, et al. (1975). "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician." J Psychiatr Res **12**(3): 189-198.

Francis, G. J., J. A. Martinez, et al. (2008). "Intranasal insulin prevents cognitive decline, cerebral atrophy and white matter changes in murine type I diabetic encephalopathy." Brain **131**(Pt 12): 3311-3334.

Frey, W. H. (2002). "Intranasal delivery: Bypassing the blood-brain barrier to deliver therapeutic agents to the brain and spinal cord." Drug Delivery Technology **2**(5): 46-49.

Frolich, L., D. Blum-Degen, et al. (1998). "Brain insulin and insulin receptors in aging and sporadic Alzheimer's disease." J Neural Transm **105**(4-5): 423-438.

Galasko, D., D. Bennett, et al. (1997). "An inventory to assess activities of daily living for clinical trials in Alzheimer's disease. The Alzheimer's Disease Cooperative Study." Alzheimer Dis Assoc Disord **11 Suppl 2**: S33-39.

Gasparini, L., G. K. Gouras, et al. (2001). "Stimulation of beta-amyloid precursor protein trafficking by insulin reduces intraneuronal beta-amyloid and requires mitogen-activated protein kinase signaling." J Neurosci **21**(8): 2561-2570.

Gil-Bea, F. J., M. Solas, et al. (2010). "Insulin levels are decreased in the cerebrospinal fluid of women with prodomal Alzheimer's disease." J Alzheimers Dis **22**(2): 405-413.

Grillo, C. A., G. G. Piroli, et al. (2009). "Insulin-stimulated translocation of GLUT4 to the plasma membrane in rat hippocampus is PI3-kinase dependent." Brain Res **1296**: 35-45.

Grober, E., H. Buschke, et al. (1988). "Screening for dementia by memory testing." Neurology **38**(6): 900-903.

Grober E., Hall C.B., et al. (2008). "Memory impairment, executive dysfunction, and intellectual decline in preclinical Alzheimer's disease." J Int Neuropsych Soc **14**(2): 266-278.

Grossman, M., J. Farmer, et al. (2005). "Cerebrospinal fluid profile in frontotemporal dementia and Alzheimer's disease." Ann Neurol **57**(5): 721-729.

Grundman, M., R. C. Petersen, et al. (2004). "Mild cognitive impairment can be distinguished from Alzheimer disease and normal aging for clinical trials." Arch Neurol **61**(1): 59-66.

Hallschmid, M., C. Benedict, et al. (2008). "Obese men respond to cognitive but not to catabolic brain insulin signaling." Int J Obes (Lond) **32**(2): 275-282.

Holland, D., J. B. Brewer, et al. (2009). "Subregional neuroanatomical change as a biomarker for Alzheimer's disease." Proc Natl Acad Sci U S A **106**(49): 20954-20959.

Hong, M. and V. M. Lee (1997). "Insulin and insulin-like growth factor-1 regulate tau phosphorylation in cultured human neurons." J Biol Chem **272**(31): 19547-19553.

Hughes, C. P., L. Berg, et al. (1982). "A new clinical scale for the staging of dementia." Br J Psychiatry **140**: 566-572.

Illum, L. (2002). "Nasal drug delivery: new developments and strategies." Drug Discov Today **7**(23): 1184-1189.

Impel NeuroPharma. "A drug-device compatibility and functionality veritification of the Impel NeuroPharma clinical Precision Olfactory Delivery device and the Novolog® insulin formulation." Impel Document CD-1304-003, 2015.

Jack, C. R., Jr., M. Slomkowski, et al. (2003). "MRI as a biomarker of disease progression in a therapeutic trial of milameline for AD." Neurology **60**(2): 253-260.

Jarjoura, D. (2003). "Crossing controls to treatment in repeated-measures trials." Control Clin Trials **24**(3): 306-323.

Jovicich, J., S. Czanner, et al. (2006). "Reliability in multi-site structural MRI studies: effects of gradient non-linearity correction on phantom and human data." Neuroimage **30**(2): 436-443.

Kern, W., J. Born, et al. (1999). "Central nervous system effects of intranasally administered insulin during euglycemia in men." Diabetes **48**(3): 557-563.

Kristensson, K. and Y. Olsson (1971). "Uptake of exogenous proteins in mouse olfactory cells." Acta Neuropathol **19**(2): 145-154.

Kupila, A., J. Sipila, et al. (2003). "Intranasally administered insulin intended for prevention of type 1 diabetes--a safety study in healthy adults." Diabetes Metab Res Rev **19**(5): 415-420.

Larsen, D. L., C. C. Attkisson, et al. (1979). "Assessment of client/patient satisfaction: development of a general scale." Eval Program Plann **2**(3): 197-207.

Lee, C. C., Y. M. Kuo, et al. (2009). "Insulin rescues amyloid beta-induced impairment of hippocampal long-term potentiation." Neurobiol Aging **30**(3): 377-387.

Liu, Y., Johnson, M, et al. (2008). "Creation of a Standardized Geometry of the Human Nasal Anatomy." J Appl Physiol **106**: 784-795.

Little, R. J. A. and D. B. Rubin (1987). Statistical analysis with missing data. New York, Wiley.

Maes, O. C., H. M. Schipper, et al. (2009). "Methodology for discovery of Alzheimer's disease blood-based biomarkers." J Gerontol A Biol Sci Med Sci **64**(6): 636-645.

Matthews, D. R., J. P. Hosker, et al. (1985). "Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man." Diabetologia **28**(7): 412-419.

McKhann, G. M., D. S. Knopman, et al. (2011). "The diagnosis of dementia due to Alzheimer's disease: Recommendations from the National Institute on Aging and the Alzheimer's Association workgroup." Alzheimers Dement.

Miller, S. D., B. L. Duncan, et al. (2005). "The partners for change outcome management system." J Clin Psychol **61**(2): 199-208.

Minoshima, S., K. A. Frey, et al. (1995). "Preserved pontine glucose metabolism in Alzheimer disease: a reference region for functional brain image (PET) analysis." J Comput Assist Tomogr **19**(4): 541-547.

Minoshima, S., R. A. Koeppe, et al. (1994). "Anatomic standardization: linear scaling and nonlinear warping of functional brain images." J Nucl Med **35**(9): 1528-1537.

Mohs, R. C., D. Knopman, et al. (1997). "Development of cognitive instruments for use in clinical trials of antidementia drugs: additions to the Alzheimer's Disease Assessment Scale that broaden its scope. The Alzheimer's Disease Cooperative Study." Alzheimer Dis Assoc Disord **11 Suppl 2**: S13-21.

Morris, J. C. (1993). "The Clinical Dementia Rating (CDR): current version and scoring rules." Neurology **43**(11): 2412-2414.
Morris, J. C., C. Ernesto, et al. (1997). "Clinical dementia rating training and reliability in multicenter studies: the Alzheimer's Disease Cooperative Study experience." Neurology **48**(6): 1508-1510.
Petersen, R. C., P. S. Aisen, et al. (2010). "Alzheimer's Disease Neuroimaging Initiative (ADNI): clinical characterization." Neurology **74**(3): 201-209.
Pontiroli, A. E., M. Alberetto, et al. (1982). "Insulin given intranasally induces hypoglycaemia in normal and diabetic subjects." Br Med J (Clin Res Ed) **284**(6312): 303-306.
Reger, M. A., G. S. Watson, et al. (2006). "Effects of intranasal insulin on cognition in memory-impaired older adults: modulation by APOE genotype." Neurobiol Aging **27**(3): 451-458.
Reger, M. A., G. S. Watson, et al. (2008). "Intranasal insulin administration dose-dependently modulates verbal memory and plasma amyloid-beta in memory-impaired older adults." Journal of Alzheimers Disease **13**(3): 323-331.
Reger, M. A., G. S. Watson, et al. (2008). "Intranasal insulin improves cognition and modulates beta-amyloid in early AD." Neurology **70**(6): 440-448.
Reitan, R. M. (1958). "Validity of the trail making test as an indicator of organic brain damage." Perceptual & Motor Skills **8**(3): 271-276.
Rivera, E. J., A. Goldin, et al. (2005). "Insulin and insulin-like growth factor expression and function deteriorate with progression of Alzheimer's disease: link to brain reductions in acetylcholine." J Alzheimers Dis **8**(3): 247-268.
Rosen, W. G., R. C. Mohs, et al. (1984). "A new rating scale for Alzheimer's disease." Am J Psychiatry **141**(11): 1356-1364.
Rosen, W. G., R. D. Terry, et al. (1980). "Pathological verification of ischemic score in differentiation of dementias." Ann Neurol **7**(5): 486-488.
Rubin, D. B. (1987). Multiple Imputation for Nonresponse in Surveys. Hoboken, New Jersey, John Wiley & Sons, Inc.
Sakane, T., M. Akizuki, et al. (1995). "Direct drug transport from the rat nasal cavity to the cerebrospinal fluid: the relation to the molecular weight of drugs." J Pharm Pharmacol **47**(5): 379-381.
Sano, M., R. Raman, et al. (2011). "Adding Delayed Recall to the Alzheimer Disease Assessment Scale is Useful in Studies of Mild Cognitive Impairment But Not Alzheimer Disease." Alzheimer Dis Assoc Disord **25**(2): 122-127.
Schneider, L. S., R. E. Kennedy, et al. (2010). "Requiring an amyloid-beta1-42 biomarker for prodromal Alzheimer's disease or mild cognitive impairment does not lead to more efficient clinical trials." Alzheimers Dement **6**(5): 367-377.
Selkoe, D. J. (2008). "Soluble oligomers of the amyloid beta-protein impair synaptic plasticity and behavior." Behav Brain Res **192**(1): 106-113.
Shipley, M. T. (1985). "Transport of molecules from nose to brain: transneuronal anterograde and retrograde labeling in the rat olfactory system by wheat germ agglutinin-horseradish peroxidase applied to the nasal epithelium." Brain Res Bull **15**(2): 129-142.
Sled, J. G., A. P. Zijdenbos, et al. (1998). "A nonparametric method for automatic correction of intensity nonuniformity in MRI data." IEEE Trans Med Imaging **17**(1): 87-97.
and its CNS effects in healthy humans: unconditioned and conditioned responses."
Stockhorst, U., D. de Fries, et al. (2004). "Insulin and the CNS: effects on food intake, memory, and endocrine parameters and the role of intranasal insulin administration in humans." Physiol Behav **83**(1): 47-54.
Stockhorst, U., D. de Fries et al. (2011). "Unconditioned and conditioned effects of intranasally administered insulin vs placebo in healthy men: a randomised controlled trial." Diabetologia 54(6): 1502-06.

Talairach, J. and P. Tournoux (1988). Co-planar stereotaxic atlas of the human brain: 3-dimensional proportional system: an aproach to cerebral imaging. New York, New York, Thieme Medical Publishers.

Thorne, R. G., C. R. Emory, et al. (1995). "Quantitative analysis of the olfactory pathway for drug delivery to the brain." Brain Res **692**(1-2): 278-282.

Thorne, R. G., G. J. Pronk, et al. (2004). "Delivery of insulin-like growth factor-I to the rat brain and spinal cord along olfactory and trigeminal pathways following intranasal administration." Neuroscience **127**(2): 481-496.

Townsend, M., T. Mehta, et al. (2007). "Soluble Abeta inhibits specific signal transduction cascades common to the insulin receptor pathway." J Biol Chem **282**(46): 33305-33312.

Turner, R. S. (2003). "Biomarkers of Alzheimer's disease and mild cognitive impairment: are we there yet?" Exp Neurol **183**(1): 7-10.

Vemuri, P., H. J. Wiste, et al. (2009). "MRI and CSF biomarkers in normal, MCI, and AD subjects: predicting future clinical change." Neurology **73**(4): 294-301.

Wechsler, D. (1987). WMS-R Wechsler Memory Scale - Revised Manual. New York, The Psychological Corporation, Harcourt Brace Jovanovich, Inc.

Weiss, P. and Y. Holland (1967). "Neuronal dynamics and axonal flow, ii. The olfactory nerve as model test object." Proc Natl Acad Sci U S A **57**(2): 258-264.

Worsley, K. J., A. C. Evans, et al. (1992). "A three-dimensional statistical analysis for CBF activation studies in human brain." J Cereb Blood Flow Metab **12**(6): 900-918.

Zhao, L., B. Teter, et al. (2004). "Insulin-degrading enzyme as a downstream target of insulin receptor signaling cascade: implications for Alzheimer's disease intervention." J Neurosci **24**(49): 11120-11126.

Zhao, W. Q. and M. Townsend (2009). "Insulin resistance and amyloidogenesis as common molecular foundation for type 2 diabetes and Alzheimer's disease." Biochim Biophys Acta **1792**(5): 482-496.