Official Title: Study of Nasal Insulin to Fight Forgetfulness - Device Study
NCT03857321
IRB Approval Date: 12/07/2020

Device Study for Intranasal Delivery of Insulin

SNIFF Device

IND #: 119232

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PROTOCOL VERSION
Final Protocol v5.0 07-December-2020

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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)**
BDNF	**BRAIN-DERIVED NEUROTROPHIC FACTOR**
BID | 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**
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**

TITLE	Device Study for Intranasal Delivery of Insulin
PROJECT DIRECTOR	Suzanne Craft, Ph.D.
STUDY SPONSOR	Wake Forest University Health Sciences
STUDY PHASE	Phase II
INDICATION	Preclinical AD, Amnestic mild cognitive impairment (aMCI) or mild Alzheimer's disease (AD)
AIM OF STUDY	To determine the ability of an intranasal delivery device (Kurve ViaNase device) to increase levels of insulin in cerebrospinal fluid (CSF)
PRIMARY OBJECTIVE	To test the hypothesis that CSF insulin levels will increase 30 minutes after receiving a 20 International Units dose of insulin delivered with either the ViaNase device, compared to levels achieved 30 minutes after placebo.
SECONDARY OBJECTIVES	1. To test the hypothesis that memory performance measured with a list learning test (Auditory Verbal Learning Test) will be enhanced after insulin administration relative to placebo 2. To test the hypothesis that the CSF Aβ42/40 and the CSF Aβ/tau ratios will increase after insulin administration relative to placebo
PRIMARY OUTCOME MEASURE	CSF insulin
SECONDARY OUTCOME MEASURES	Memory test, CSF insulin, Aβ40, Aβ42, total tau, and phospho-tau 181
STUDY DESIGN	Single-site, double-blind, placebo-controlled study
SAMPLE SIZE	• n=30
SUMMARY OF KEY ELIGIBILITY CRITERIA	• Cognitively normal or • Diagnosis of aMCI (Petersen criteria) • Age: 55 to 85 yrs (inclusive)
DRUG DOSAGE & FORMULATION	20 International Units Humulin® R U-100 or matching placebo (saline)
DURATION OF PARTICIPATION	The approximate timeline for this 21 month study is projected as: 1) approximately 1 months for study startup activities including IND submission and IRB approval, and training; 2) approximately 18 months for data collection; 3) 2 months for assay and data analysis
PLACEBO	A matching placebo (sterile saline) will be used
ROUTE OF ADMINISTRATION	Intranasal
PROCEDURES	Physical and neurological exam, nasal examination, lumbar puncture, Vitals, Clinical Labs, CSF and blood analysis & banking 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 provide information to assist in examining a novel therapeutic approach using intranasally administered insulin (INI) that has shown promise in short-term clinical trials. In a recent longer term trial in which INI was delivered with two devices (Kurve ViaNase device and Impel Precision Olfactory Delivery (POD) device, a different pattern of results was observed between the two devices, suggesting that differences in delivery systems can impact the therapeutic effects of insulin. The proposed proof of concept study will examine whether the device that was associated with clinical improvement is able to increase CSF insulin levels 30 minutes after administration, a timepoint which has been show to represent the greatest increase in CSF insulin in a previous study (Born et al. Nature Neuroscience, 2002 Jun;5(6):514-6). If successful, information gained from the study will inform the design of future Phase III trials of intranasal insulin.

1.1 Primary Aim

To test the hypothesis that CSF insulin levels will increase 30 minutes after receiving a 20 International Units dose of insulin delivered with the ViaNase or device, compared to levels achieved 30 minutes after placebo

1.1.1 Secondary Aim 1

1. To test the hypothesis that memory performance measured with a list learning test (Auditory Verbal Learning Test) will be enhanced after insulin administration relative to placebo

1.1.2 Secondary Aim 2

1. To test the hypothesis that the CSF Aβ42/40 and the CSF Aβ42/tau ratios will increase after insulin administration relative to placebo

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 International Units/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 intranasal delivery device investigated in this study has been used in ongoing and previous studies at Wake Forest:

1) An investigational device (ViaNase™) developed by Kurve Technology (Bothell, WA) will be used in this study; the current device is identical to the model used two studies approved by the Wake Forest IRB, SNIFF Long, IRB number 00023230 and Dr. Carol Bushnell's study of Intranasal Insulin and Post Stroke Cognition, IRB00029022.Typical spray bottle administration results in large droplets that penetrate only within the first 20% of the lower nasal cavity, and due to gravity and insufficient airflow, ~90% of the droplets wind up in the stomach. The ViaNase™ device delivers a substance throughout the nasal cavity (to the olfactory region and paranasal sinuses), thereby maximizing access to nose-to-brain channels. This controlled particle dispersion (CPD) occurs because droplet size is adjusted according to the weight of the substance through an individually optimized droplet generator resulting in maximal vertical distribution.

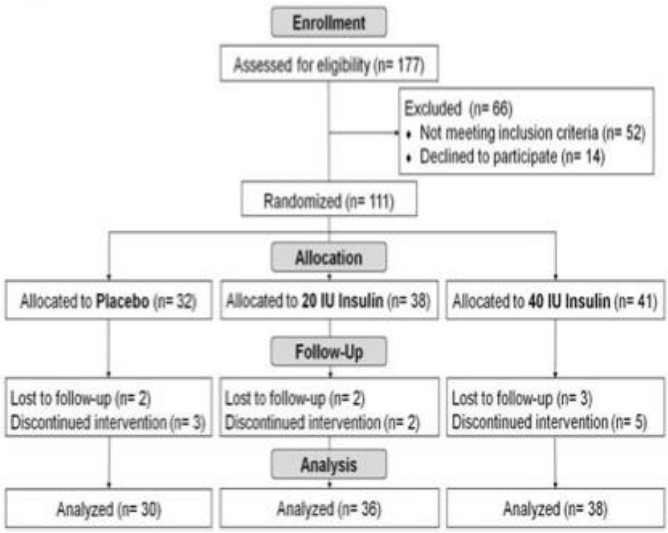
3.0 **PRELIMINARY STUDIES**

A preliminary study examined the impact of 4-month INI administration using the ViaNase device (10 or 20 International Units 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).

3.1 **Participants**

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

Figure 1 | Trial Enrollment Flow



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 International Units INI bid for a total daily dose of 20 International Units INI (n=36), 20 International Units INI bid for a total dose of 40 International Units (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.

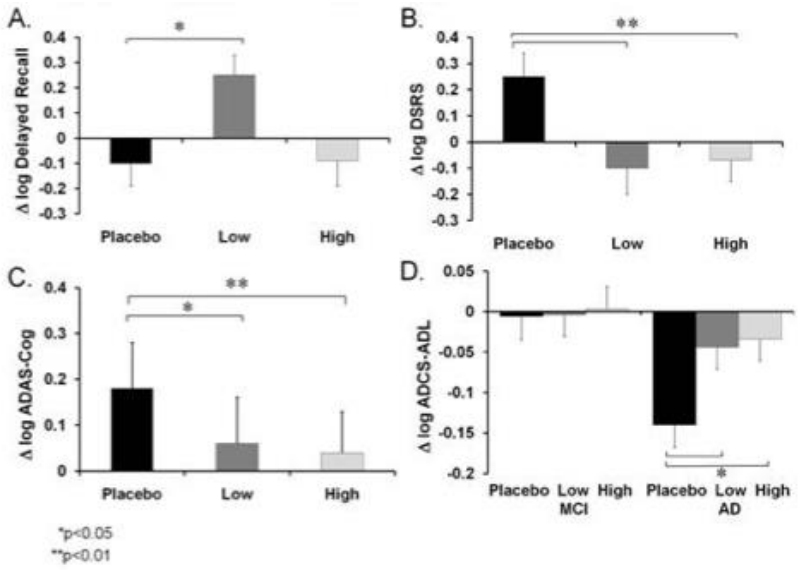
3.4 **Statistical Analyses**

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.

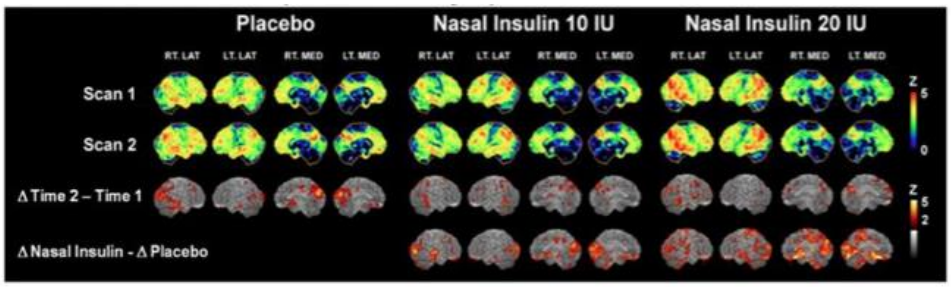


Table 2 | Z scores and stereotactic coordinates for areas of reduced progression for low and high does insulin groups compared to placebo

		Stereo Coordinates		
	Z	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 PRELIMINARY STUDY 2

A follow-up study has recently concluded and preliminary results have been analyzed**.**

Objectives: This study tested the effects of 40 International Units of intranasal insulin administered daily for 12 months, compared with placebo, on cognition, daily function and safety in adults with MCI or mild AD. Longer-term effects were examined in a six-month open-label extension offered to all participants. Safety and feasibility issues relating to the use of intranasal delivery devices were also evaluated. The trial is nearing completion; all participants have concluded the blinded phase.

Methods: Twenty-six sites enrolled 289 participants with MCI or mild AD in this randomized, double-blind, Phase II/III trial (NCT01767909). Adults 55 to 85 years of age with diagnoses of amnestic MCI or AD (National Institute on Aging-Alzheimer's Association criteria) with Mini-Mental State Exam (MMSE) scores >19, Clinical Dementia Ratings (CDR) of 0.5 or 1, and delayed Logical Memory scores within a specified education-adjusted range were eligible. Participants with diabetes requiring medication were excluded, as were participants who had used insulin within one year of the screening

visit. Participants were randomized on a 1:1 basis using a covariate-adaptive algorithm that weighted MMSE, apolipoprotein E-ε4 (APOE-ε4) allele carriage, study site, sex, and age based on previous work indicating these factors may impact treatment response. Participants received 40 International Units of insulin or insulin diluent placebo (Humulin R U-100 or insulin diluent, Eli Lilly, Indianapolis, USA) daily for 12 months. At the end of the 12-month blinded phase, all participants were offered open-label insulin treatment for 6 months. The primary outcome (Alzheimer's Disease Assessment Scale for Cognition-12/ADAS-Cog12) was administered at baseline and then at 3 month intervals. Secondary functional outcomes (Alzheimer's Disease Cooperative Study-Activities of Daily Living Scale for MCI; CDR Sum of Boxes) were assessed at 6 month intervals, as was a memory composite (Free and Cued Selective Reminding Test and Story Recall). Cerebrospinal fluid biomarkers (Aβ42 and Aβ42/tau ratio) and magnetic resonance imaging hippocampal and entorhinal cortex volumes were measured at baseline and after 12 months.

Device issues. Intranasal delivery device monitoring revealed no safety issues. However, for the first 49 participants, the delivery device had frequent malfunctions (i.e. failure to turn on) that impacted dosing reliability. At that time, a newly available device was introduced (Precision Olfactory Device/POD, Impel NeuroPharma, Seattle, USA) which was used by the remaining 240 participants with good reliability.

Results: Demographic characteristics of enrolled participants are presented in Table 1. Retention was excellent, with only 25 participants discontinuing treatment during the blinded phase, and 15 also discontinuing study visits during the blinded phase. Quarterly DSMB reviews did not detect any safety issues and approved unmodified continuation of the trial.

Table 1. Baseline Participant Characteristics	
N (F/M)	289 (134 / 155)
Age (years)	70.95 ± 7.1
Diagnosis (MCI/AD)	105 / 184
MMSE	24.8 ± 2.7
Logical Memory	2.1 ± 2.7
APOE (ε4+/ε4-)	193 / 96

Data from the subgroup of participants who used the POD were analyzed separately from the ViaNase group. The primary analysis of the POD group showed was negative; no difference in ADAS-Cog12 scores were observed after 12 months of INI treatment compared with placebo (Fig.1). In contrast, analysis of the ViaNase group showed beneficial effects of insulin compared with placebo; the effect was significant at 6 months (p<0.01), and persisted at 12 months (p=0.09; Fig. 2).

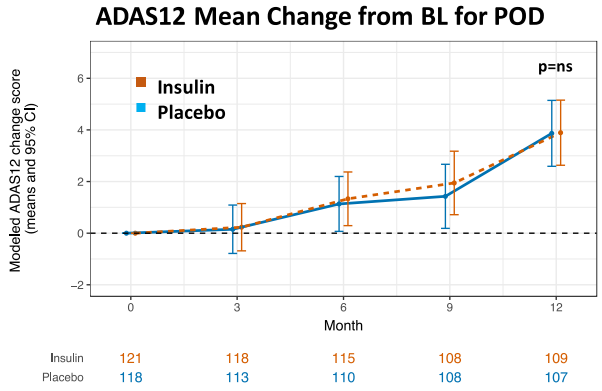


Fig. 1

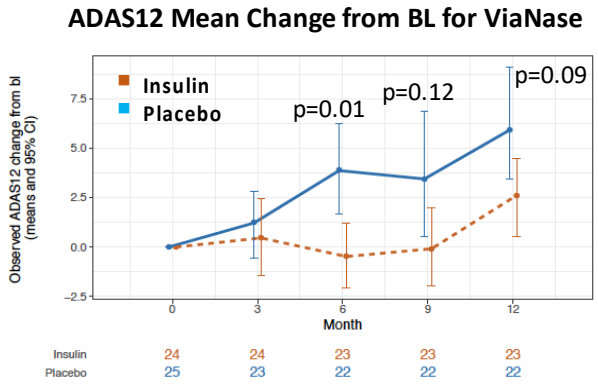


Fig. 2

Implication: These results suggest that the two delivery devices may be providing different doses of insulin to the CNS. The ViaNase device results are consistent with multiple previous studies. The POD had not been used previously in clinical trials with insulin, and thus was relatively untested in terms of delivery efficacy, although modeling in organoid nasal cavities was conducted to verify that the target amount of insulin was dispersed to the target location in the nasal cavity. These divergent results can potentially be clarified by conducting a proof of concept study, in which insulin or placebo will be administered and then insulin in CSF will be measured 30 minutes after administration. Results

of this proof of concept study will help confirm the delivery capabilities of the ViaNase device, information that is essential to the design of a future Phase III trial.

4.1 Rationale for Dosage Selection

The dosage selected (20 International Units INI) has been used in two prior studies with positive results as described above.

4.2 Rationale for Primary and Secondary Outcome Measures

Previous work demonstrates that following intranasal insulin administration, insulin levels are increased in the CSF, reflecting entry into the CNS, and with the greatest increases observed 30 minutes after administration (Born et al. Nature Neuroscience, 2002 Jun;5(6):514-6). The observation that CSF levels are increased following insulin administration with the ViaNase device will validate that insulin was delivered to the CNS and thus that the positive results for the ADAS-Cog12 for the POD cohort in the recent study are possibly due to its ability to successfully deliver insulin to the CNS.

Regarding secondary measures, in previous studies intranasal insulin acutely improved memory performance and affected CSF levels of AD biomarkers (Reger et al. 2008; Craft et al. 2012). Thus examining effects on these measures will provide supportive information about access to and efficacy in the CNS.

4.3 Rationale for Design of Trial

This study is designed as an acute administration, proof of concept study to determine whether administration of intranasal insulin results in increased delivery to the CNS as evidenced by increased CSF insulin levels relative to placebo.

4.4 Rationale for Biofluids

The CSF biomarkers insulin, Aβ42, Aβ40 and total tau will be measured. 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 may be measured by qualified investigators in the future given adequate rationale and feasibility.

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.

5.0 STUDY OVERVIEW

The study will consist of a single site, randomized, double-blind trial comparing the acute effects of INI (20 International Units) or placebo delivered with the ViaNase device on CSF insulin levels, AD biomarkers and memory. At study entry, participants will be randomized to receive either an acute dose of insulin or of placebo first, and the other substance on a second visit. Participants who are cognitively normal or who have aMCI (n=30) will be enrolled. The primary outcome measure will consist of CSF insulin levels. Secondary measures will include a memory test and CSF biomarker levels.

5.1 Study Population

A total of 30 adults who are cognitively normal or diagnosed with aMCI will be enrolled in this trial. We expect to enroll approximately 50% of participants from each group. 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 conducted by the investigators and staff of the Clinical Core of the Wake Forest Alzheimer's Disease Research Center (ADRC).

5.2 Diagnosis Criteria

Diagnoses will be assigned by consensus of investigators from the Clinical Core of the Wake Forest ADRC using criteria specified by the NIA and Alzheimer's Association MCI workgroup (Petersen, Doody et al. 2001, Albert, Dekosky et al. 2011).

Criteria for cognitively normal adults:

a) No evidence of significant cognitive impairment on objective testing
b) Clinical Dementia Rating Scores of 0

Diagnosis of aMCI requires:

a) Evidence of a decline in episodic memory (memory scores below age and education-based norms)
b) General preservation of independence in functional abilities
c) Absence of dementia

5.3 Inclusion Criteria

The following inclusion criteria* will be used:

1. Age 55 to 85 (inclusive)
2. Fluent in English
3. Cognitively normal or diagnosis of aMCI by criteria described above
4. MMSE ≥ 24 at screening. Approval for exceptions can be requested to the Project Director for minority or low education participants
5. CDR 0-0.5 (inclusive) at screening
6. Stable medical condition for 3 months prior to screening visit
7. Stable medications for 4 weeks prior to the screening and study visits
8. Clinical laboratory values must be within normal limits or, if abnormal, must be judged to be clinically insignificant by the study physician

*Exceptions to these criteria may be considered on a case-by-case basis at the discretion of the Project Director and study physician, and must be approved in advance by the IRB.

5.4 Exclusion Criteria

The following exclusion criteria* will be used:

1. A diagnosis of dementia
2. History of a clinically significant stroke
3. 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
4. Sensory impairment that would preclude the participant from participating in or cooperating with the protocol
5. Diabetes (type I or type II) requiring pharmacologic treatment (including both insulin dependent and non-insulin dependent diabetes mellitus)
6. Current or past use of insulin or any other anti-diabetic medication within 5 years of Screening visit.
7. Evidence of any significant clinical disorder or laboratory finding including clinically significant or unstable hematologic, hepatic, cardiovascular, pulmonary, gastrointestinal, endocrine,

metabolic, renal or other systemic disease or laboratory abnormality

8. Active neoplastic disease, history of cancer five years prior to screening (history of skin melanoma or stable prostate cancer are not exclusionary)
9. History of seizure within past five years
10. 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)
11. 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
12. Use of anticoagulants warfarin (Coumadin) and dabigatran (Pradaxa) (due to LP requirement)
13. Residence in a skilled nursing facility at screening
14. Use of an investigational agent within two months of screening visit
15. 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 and Study Physician, and must be approved in advance by the IRB.

5.5 Recruitment and Retention Strategies

Recruitment will occur primarily from the Clinical Core of the Wake Forest ADRC. Some participants may also be identified from community recruitment efforts.

6.0 STUDY TIMELINE

The approximate timeline for this twenty-one-month study is projected as follows: 1) approximately one months for study startup activities; 2) study visits and data collection will occur over an eighteen-month period; 3) CSF and blood analyses, data analyses and study dissemination will occur over the final 2 months.

7.0 DESCRIPTION OF STUDY VISITS

The “Schedule of Study Procedures and Assessments” in Table 4 provides an overview of study visit activities. The primary and secondary outcome measure will be measured at both study visits. Genotyping will occur at Screening for participants who have not previously received APOE genotypes through the ADRC.

7.1 Screening (Visit 1)

The purpose of this visit is to determine study eligibility. Potential participants 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 Story Recall to determine study eligibility. Screening may be waived for participants who have received evaluations from the ADRC within the past 12 months.

In addition, information regarding demographics, concurrent medications, medical history and adverse events will be gathered from the participant. Vital signs, height and weight will be measured. A brief physical and neurological examination (which include a nasal examination) 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. These procedures may be waived for participants who have received evaluations from the ADRC within the past 12-months.

Blood will be drawn 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.

7.2 **Baseline (Visit 2)**

Results from all screening procedures must be reviewed and all inclusion/exclusion criteria must be met prior to proceeding to baseline. For participants whose screening visit was waived because they had received ADRC evaluations within the past 12-months will sign an informed consent form and HIPAA Authorization prior to administration of any study-related procedures.

According to the randomization schedule, the participant will receive a dose of 20 International Units insulin or placebo administered with the ViaNase device. They will then begin preparations for the lumbar puncture. After preparation, the immediate recall section of the AVLT will be administered. Blood for plasma biomarkers will then be collected immediately before and after participants undergo the lumbar puncture. Fasting plasma insulin, glucose, Aβ40 and Aβ42 will be measured. Additional plasma and serum will be collected and banked.

Lumbar puncture will then be performed in the morning after a minimum 8-hour overnight fast. CSF samples will be used to measure levels of insulin, Aβ42, Aβ40, total tau, and phospho-tau181. Additionally, CSF sample will be banked for future exploratory analysis. CSF will be collected and immediately frozen upright on dry ice. Site staff will call the participant within 24 hours from the lumbar puncture procedure to inquire about the participant's well-being and possible adverse events. Following the lumbar puncture, the delayed recall section of the AVLT will be administered. Participants will then receive a snack and instructions about post-lumbar puncture care.

7.3 **Visit 3**

Visit 3 will be scheduled within 2 to 6 weeks following Visit 1. Vital signs and weight will be obtained, a nasal examination will be conducted, adverse events and concurrent medications will be recorded. Procedures will follow the schedule outlined for Visit 1, except that the alternate compound (either saline or 20 International Units insulin) will be administered.

8.0 **STUDY-SPECIFIC PROCEDURES**

8.1 **Memory Evaluation Instruments Administered to the Participant**

Objective tests of cognitive function will include MMSE, Story Recall**,** and the Rey Auditory Verbal Learning Test.

8.2 **Clinical and Functional Evaluations**

8.2.1 **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).

9.0 STUDY METHODS

9.1 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. In addition, 24 hours after the Lumbar Puncture, each study participant, or a person designated to speak for them will be contacted by phone to confirm the participant's well being and queried about any new adverse events. All adverse events will be reported to the Wake Forest DSMB. Safety reports will be prepared by study team and submitted to the DSMB for periodic review.

9.2 Physical and Neurological Examination

A brief physical examination will be performed by a medically qualified professional at the screening visit. 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 also be performed. The examination will assess irritation or other abnormalities of the nares.

9.3 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 investigator. 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.

9.4 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 Study Physician. 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.

10.0 BIOMARKER STUDIES

10.1 CSF

All CSF samples will be collected in the morning before breakfast and after an overnight fast. Participants who are taking anticoagulants, warfarin (Coumadin) and dabigatran (Pradaxa) should not be screened for this trial, as these are prohibited medications. 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. It is not required that participants be discontinued from their anti-platelet agent in order to screen and enroll in the study.

A minimal total volume of CSF (25 ml) will be required 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 microtubes. Approximately 2ml of CSF or volume per local laboratory requirements will be sent at ambient temperature to the CRU laboratory for protein, glucose and cell count. The remaining CSF will be immediately frozen upright on dry ice for at least 20 minutes then stored at -70 until analysis.

CSF samples will be used to measure levels of insulin, Aβ42, Aβ40, total tau, and phospho-tau181. Assays will be performed by the Wake Forest ADRC Biomarker Service. CSF samples will also be frozen and stored for future analysis of putative biomarkers.

10.2 **Blood Collection at Lumbar Puncture Visits**

All samples will be collected in the morning before breakfast and after a minimum 8-hour overnight fast. Blood samples will be collected before CSF collection, which would be processed for fasting plasma insulin, glucose, Aβ42, and Aβ40. Additional blood for plasma and serum will be processed and banked.

10.3 **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 by Dr. Don Bowden under the auspices of the ADRC Biomarker service using established protocols.

11.0 **STATISTICAL PLAN**

Statistical analyses will be conducted by the ADRC Biostatistics Core. To address the Primary Aim, CSF insulin values will be subjected to a repeated measures analysis of variance with treatment (saline vs. placebo) as the repeated measure, with age and baseline MMSE as covariates. Secondary analyses will examine the effect of diagnostic status (cognitively normal vs. MCI) and APOE genotype. Secondary analyses will also examine treatment-related differences for other CSF and plasma biomarkers, and for memory scores.

11.1 **Power Analyses**

As this study is designed as a proof of concept study to determine whether the ViaNAse device is able to deliver insulin to the CNS as evidenced by increased CSF insulin levels following insulin administration relative to saline placebo, power calculations have not been conducted. However, the proposed sample size is larger than two previous studies (Born et al. 2002; Fishel et al. that showed that administering insulin increased CSF insulin levels.

12.0 **POTENTIAL RISKS**

12.1 **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 International Units 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 International Units once a day for three weeks in 21 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).

12.2 **Risks associated with use of the ViaNase® device**

Because the use of the device will be supervised directly by the study nurse the risks of adverse events is extremely low. Participants could experience some discomfort to their eyes or face if they do not hold the device to their nose as directed; however, again, the study nurse will carefully instruct and oversee device use so that any errors can be immediately corrected.

12.3 **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%.

12.4 **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 50mls of blood will be drawn for routine and biomarker laboratory assessments over the course of this study.

13.0 **PERSONNEL REQUIREMENTS**

The following staff-member roles will be required to conduct the protocol.

- **Principal Investigator**: The Principal Investigator (PI; Suzanne Craft, PhD) is responsible for the overall conduct of the study. 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.
- **Study Physician:** Benjamin Williams, MD, PhD will serve as Study Physician for the trial. He 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 study. He will also supervise, and on some occasions may perform the lumbar punctures.
- **Study Nurse/Coordinator:** Deborah Dahl, RN will serve as Study Nurse/Coordinator for the trial. She 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, and coordinating clinic visits. She will also oversee the use and maintenance of the devices, and coordinate with the Pharmacy regarding ordering and storage of saline and insulin.

- **Interviewer/Psychometrician:** This person will be responsible for administering the memory assessments.
- **CDR Rater:** This person will render the CDR-SB rating based on clinical assessment of participant and study participant.
- **Regulatory Affairs:** Sarah Bohlman will be responsible for managing all regulatory related documents for the duration of the trial.

14.0 STUDY DRUG

All participants will take receive one dose of INI (20 International Units) or placebo, administered approximately 30 minutes prior to the LP.

14.1 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).

14.2 Placebo (Sterile Saline)

The placebo will consist of sterile saline.

14.3 Randomization

Eligible participants will be randomized on a 1:1 schedule to receive either insulin or saline first.

14.4 Blinding

Neither participants or site personnel will know whether insulin or saline is being administered. Exceptions will be the study nurse who is directly involved in preparing the insulin or placebo, as well as preparing DSMB reports.

14.5 Study Drug Dispensing

Study drug will be inserted into the chamber of the ViaNase™ by the study nurse.

14.6 Intranasal Administration

Insulin or placebo will be administered with a ViaNase™ drug delivery device (Kurve Technology, Bothell, WA). ViaNase™ specifically targets olfactory delivery to maximize drug transport to the CNS. This device releases a metered insulin dose into a chamber covering the participant's nose. The insulin or placebo is then inhaled by breathing evenly over a specified period. This method allows administration of smaller particle sizes to increase deposition in the upper nasal cavity while minimizing transport to the lungs. A volume of about 0.7 mL of insulin or placebo will be administered each time.

14.7 Storage

Insulin and placebo will be maintained at a controlled temperature.

14.8 **Drug Accountability**
The study nurse coordinator will maintain a log of study drug usage.

15.0 **ADVERSE EVENTS**

15.1 **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.

15.2 **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 adverse events will be reported to the Wake Forest 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.

16.0 **SERIOUS ADVERSE EVENTS (SAE)**

16.1 **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

16.2 **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), will be reported to the PI and study physician within 24 hours of learning of the event. All serious adverse events will be reported to the Wake Forest IRB and DSMB within 7 days of study personnel learning of the event.

17.0 ETHICS & REGULATORY CONSIDERATIONS

17.1 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.

17.2 Institutional Review Board (IRB)

This protocol and the associated informed consent documents and recruitment material will be approved by the Wake Forest IRB which is 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. The investigator shall notify the IRB of deviations from the protocol or serious adverse events occurring at the site, in accordance with local procedures.

17.3 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 and biological sample storage. The consent for storage will include consent to access stored data and biological samples 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.

17.4 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. 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 IRB, DSMB, 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.

18.0 GENETIC RESEARCH & STORAGE OF GENETIC MATERIAL

The DNA is banked in locked freezers in the ADRC Biomarker Service. Sample tubes are bar-coded and linked to participant ID number only and banked without personal identifiers.

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 investigators to probe candidate genetic polymorphisms as predictors of outcome in future studies. The samples will be stored by the ADRC.

18.1 Storage of Biospecimen Samples

All biospecimens being banked for future AD biomarker research will be stored by the ADRC Biomarker Service.

19.0 RISKS AND BENEFITS ASSOCIATED WITH THIS STUDY

19.1 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. In a recent trial, these results were replicated with the subgroup of participants who used the ViaNase device, but not with the POD cohort. The proposed trial is designed to answer the important question of whether the ViaNase device is effective in delivering intranasal insulin into the CNS. This knowledge will enable the conduct of future Phase III trials of intranasal insulin.

There are no significant potential clinical benefits for the participants in this study. Rather, there is a clear scientific benefit for the field as a whole. The relatively minor risks posed by the acute intranasal administration, cognitive testing, and LP are outweighed by the value of the scientific investigations outlined in this study.

19.2 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 Core at the ADRC. No participant will be excluded due to his or her sex, race, or ethnic group.

19.3 Inclusion of Children as Participants in Research Involving Human Subjects

Children will not be included.

19.4 Data and Safety Monitoring Plan and Board

The principal investigator will be responsible for the overall monitoring of the data and safety of study participants, with assistance by members of the study staff, and the WFUHS Institutional Data and Safety Monitoring Board (I-DSMB), which will be responsible for monitoring the safety of research participants.

The WFUHS Institutional Data & Safety Monitoring Board (I-DSMB) is a Dean-appointed multi-disciplinary, standing committee that is available to provide independent oversight for human research studies conducted by WFUHS or by WFUHS-affiliated faculty investigators. I-DSMB recommendations are reported to the study PI, who is responsible for forwarding them to the IRB and to the study sponsor.

While most I-DSMB reviews are conducted via electronic interactions, face-to-face meetings would occur as needed (e.g., for unplanned interim analyses, based on safety concerns).
All individual study discussions (online or face-to-face) are preceded by inquiring if any I-DSMB members have either a perceived or actual conflict of interest that could bias their ability to objectively monitor and make judgments about the study. Actual conflicts would mandate recusal from all closed-session discussions and relinquishment of voting for the study, while perceived conflicts may be dealt with by having the member in question simply abstain from voting. Conflict of interest (COI) management of studies monitored by the I-DSMB will be determined on a case-by-case basis.

Information regarding serious adverse events (SAEs) will be presented to the DSMB every six months. The DSMB may recommend stopping the trial before its planned conclusion if convincing evidence is observed of a treatment difference in adverse events. Recommendations of the DSMB after each review will be presented to the study PI and IRB. Participants will be screened at the beginning of the study and will be monitored carefully at each study visit.

20.0 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 ADCS personnel. The committee will solicit input and assistance from other Investigators as appropriate and adhere to all ADCS Publications Policies.

21.0 SHARING OF FINAL RESEARCH DATA

Data from this research will be shared with other researchers pursuant to the 02/26/2003 “NIH Final Statement on Sharing Research Data”. 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.

22.0 TABLE 4: SCHEDULE OF PROCEDURES AND ASSESSMENTS

Visit #	**1**	**2**	**3**
Visit Name	**Screen**	**Study 1**	**Study 2**
Informed Consent	X		
Demographics	X		
Medical History	X		
Concomitant Meds	X	X	X
Nasal Exam	X	X	X
Physical and Neurological Exam	X		
Vital Signs	X	X	X
Height	X		
Weight	X		
ECG	X		
Story Recall	X		
MMSE	X		
CDR	X		
Adverse Events	X	X	X
Blood Draw	X		
- Clinical Labs	X		
- ApoE Genotyping | DNA Banking[1]	X		
- Biomarkers | Plasma|Serum | Sample Banking		X	X
LP		X	X
- CSF Biomarkers | Banking[2]		X	X
- post-procedure safety telephone check		X	X
Auditory Verbal Learning Test		X	X

[1]DNA banking is optional
[2]CSF banking is optional

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