A feasibility study of the combination of intranasal insulin with dulaglutide for cognition in older adults with metabolic syndrome at high dementia risk- Study rationale and design

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1. Introduction

Alzheimer's disease (AD) is characterized by the aggregation of amyloid plaques and neurofibrillary tangles as well as other brain pathologies instituting the clinical concept of AD and related disorders (ADRD) in the field[1]. The vast majority of randomized clinical trials (RCTs) for ADRD in the last decade has targeted amyloid β (Aβ), with good target engagement but limited evidence for cognitive improvements[2]. Less attention has been given to other mechanisms, including cerebrovascular disease, a major contributor to cognitive decline and dementia[3,4]. Furthermore, the focus of current RCTs is mostly on a single biological target, rather than multiple targets, despite ample evidence that various brain pathologies underlie ADRD.

It is well established that insulin receptors are densely localized in the hippocampus and in cortical areas, primarily in synapses, where insulin modulates synaptogenesis and synaptic remodeling, crucial for cognitive function[5]. Moreover, alterations on the insulin receptor signaling pathway are implicated in Aβ and phosphorylated tau (P-tau) generation[6]. Recently, insulin receptors have been shown to be located in microvessels rather than in the parenchyma, with lower concentrations in the parietal cortex of individuals with AD. Furthermore, higher vascular concentrations of insulin receptors were correlated with better cognitive functioning. This is consistent with cerebrovascular insulin resistance in the AD brain.[7]

Consistent with this neurobiological evidence, numerous clinical trials were performed to test the potential beneficial effect of intranasal insulin (INI) on cognition, some showing improvements in memory and executive functions in INI treated participants[8–11]. Interestingly, there were less white matter hyperintensities, presumably of vascular origin, in the INI arm of the safety, efficacy, and feasibility of INI for the Treatment of Mild Cognitive Impairment and Alzheimer Disease Dementia study (SNIFF)[12], supporting the cerebral vasculature as a main mechanism of action of this medication.

Another line of evidence pointing to the potential neuroprotective effects of diabetes medications relates to the glucagon-like peptide-1 receptor agonist (GLP-1 RA), a relatively new group of medications to treat type 2 diabetes (T2D). GLP-1 has emerged as an incretin hormone for its facilitation in insulin release and reduction of insulin resistance. However, GLP-1 possesses broader pharmacological effects. A growing body of literature, primarily based on in vitro and animal models, shows that GLP-1 RA has neuroprotective characteristics via numerous molecular mechanisms including enhancement of brain insulin signaling and neurogenesis, and reduction of oxidative stress, neuroinflammation, and neuroapoptosis[13]. Furthermore, in animal models, GLP-1 receptor activation leads to dilation of cortical arterioles and improved cerebral blood flow and brain perfusion[14]. Consistent with these results, the REWIND (Researching Cardiovascular Events with a Weekly Incretin in Diabetes) trial demonstrated that the GLP-1 RA dulaglutide, reduced a composite cardiovascular outcome[15] including ischemic strokes[16] in T2D patients (aged ≥50 years). Recently, a significant reduction in incident cognitive impairment in the dulaglutide arm compared to placebo has been reported[17].

Our group and others have shown that the combination of insulin with another diabetes medication may interact and improve cognition[18]. A combination therapy of two or more antidiabetic agents was more effective than monotherapy alone in controlling decline in physical and cognitive functioning among patients with T2D[19]. In vitro studies have shown that the combination of a T2D medications with insulin is associated with protection of synapses[20] and less aggregation of Aβ[21]. Using a mouse model of AD, treatment of combination of a GLP1-RA with INI, normalized expression of insulin signaling genes, compared to placebo, or each medication alone, and nominally, improved cognition[21].

The safety of INI[10] and dulaglutide[22] has been published and both have mostly a safe profile with minor and reversible adverse effects. However, the combination of both has not been tested. The evidence accrued on

the potential effects of the combined action of a T2D medication with insulin suggests neuroprotection, and provide a scientific rationale for the potential cognitive benefits of combination of INI with a T2D therapy in enhancing cognition by preventing, delaying, or possibly reversing neuropathologic processes that cause cognitive decline. Here we describe the rationale and design of a feasibility double blind, placebo-controlled pilot RCT of INI combined with dulaglutide in patients with both mild cognitive impairment (MCI), *i.e.*, at high risk for developing ADRD, and metabolic syndrome (MetS), *i.e.*, enriched with cerebrovascular disease.

The primary goals of the study are to examine the ease of use of the intranasal device and the subcutaneous injection of dulaglutide, the adherence to the combined treatment, and the safety profile of the combination of INI with dulaglutide. Secondary goals of the study are to compare the effect of the combination of INI and dulaglutide on cognition compared with each of the medications alone. To explore target engagement, the study will have neurobiological secondary outcomes, namely, cerebral blood flow, brain metabolism, expression of insulin signaling genes from brain-derived exosomes, and blood biomarkers of AD and neurodegeneration. This study might provide the basis for a multi-center large-scale RCT of the cognitive benefits of the combination of INI with dulaglutide in individuals enriched for cerebrovascular disease at high dementia risk.

2. **Methods**

2.1 **Participants**

Recruitment: The study will take place at the Joseph Sagol Neuroscience Center, Sheba Medical Center, Israel. All participants will sign informed consent. The study has been approved by the Sheba Helsinki Committee and is registered in ClinicalTrials.gov (xxx). Participants will be recruited through the Maccabi Health Services in Israel. Israel has socialized medicine so every citizen pertains to one of four health maintenance organizations (HMOs). Maccabi is the second largest HMO representing a cross-section of the population. Maccabi will provide our study with detailed information on diagnoses, bloods, and medications used since 1998 for each participant. We have been successfully using this exquisite source of recruitment in our other clinical trials[23,24] and observational studies[25,26] primarily through the Maccabi website, Facebook groups, and companies (*e.g.*, Infinity) who provide information to people interested in medical topics. The Maccabi data will provide the proposed RCT with the opportunity to examine the role of potential contributors to the effects of combination of INI and dulaglutide on brain and cognition in this pilot RCT, to identify groups that benefit most, refining the design for a future large-scale multi-center RCT.

The study will recruit 80 participants, 20 in each of the following four groups: 1) INI and dulaglutide injection, 2) intranasal placebo and dulaglutide injection, 3) INI and placebo injection, and 4) intranasal placebo and placebo injection. INI will be given twice daily (20IU each time for a total of 40IU daily) and 1.5 mg of dulaglutide will be injected subcutaneously weekly.

Eligibility criteria: Eligibility criteria are described in Table 1. In short, older adults ($\geq$60Y) with MCI (based on a MOCA <27 and a clinical dementia rating scale [CDR] score of 0.5) and MetS will be included. The rationale for the choice of the MCI definition deserves further explanation. Individuals with MetS have impairments in several cognitive domains, including executive functions and memory[27]. In the CDR, a few scenarios could lead to a CDR score=0.5. A score of 0.5 in the memory domain defines a CDR score of 0.5. However, a score of 0.5 in at least two other domains (orientation, judgement and problem solving, community affairs, home and hobbies, and personal care) also defines a CDR=0.5, even if the memory domain has a score of zero (*i.e.*, no memory problems). Therefore, we view the use of CDR as advantageous for this study, as it captures a range of impairments consistent with the cognitive profile of MetS individuals. Its combination with the requirement of a MOCA<27 ensures important cognitive impairment with measurable consequences but no dementia.

MetS will be defined based on modified ATP III 2021, requiring a) abdominal obesity (waist circumference >102cm for men and >88cm for women), b) glucose intolerance (fasting glucose>110 mg/dL) and at least one of the following—c) dyslipidemia (high triglycerides [>150 mg/dL] and low HDL [<40mg/dL for men and <50 mg/dL for women]), or d) elevated blood pressure (>130/>85 mmHg). Cognitive assessment will be

in Hebrew, so participants must be fluent in Hebrew. The study requires an active study partner. As detailed in **Table 1**, exclusion criteria include diabetes (of any type) and medications that may affect glucose metabolism (including a GLP-1RA), a diagnosis of dementia and its subtypes, conditions that may directly affect cognition, short life expectancy or a condition that precludes consistent participation in the study, and contraindications to insulin or dulaglutide.

Table 1 Eligibility Criteria

Inclusion	Exclusion
• 60+ years of age	• Diabetes
• MetS	• Diagnosis of dementia
• Diagnosis of MCI	• Conditions that may affect cognition (*e.g.*, CVA, TBI, PD, schizophrenia, substance abuse)
• MOCA<27	• Short life expectancy
• Clinical dementia rating=0.5	• Medical condition that impedes consistent participation in the study (such as active cancer)
• Fluency in Hebrew	• Medications that may affect glucose metabolism such as corticosteroids
• Availability of study partner	• contraindications for either insulin or dulaglutide, taking GLP-1 RA

2.2 Randomization and blinding of intervention

Following baseline assessment, eligible participants will be randomized with equal probability to each of the 4 study groups. All participants and research staff will be blinded to group assignment, except the researcher who generates the randomization list and who will be blinded to cognitive and clinical assessments. An independent company (Formulex) will mark the dulaglutide and placebo syringes and send them to the Sheba pharmacy who will insert the insulin and placebo to the intranasal device (Kurve device) and combine them into treatment packs according to the randomization schedule. This company will not be exposed to participants' data.

Each patient will be supplied with a treatment pack which will contain the Kurve device (described below) for daily use (twice a day intranasal administration of either insulin or placebo) and 12 syringes of dulaglutide/placebo (once weekly, subcutaneous injection of dulaglutide or placebo, supplied for 3 months). The placebo used in this study is identical to the active formula but does not contain the active ingredient. The participants, and researchers involved in data collection and outcomes assessment will be blind to the group's allocation.

2.3 Procedures

Table 2 presents study procedures for each participant. Potential participants will be invited to the Sagol Neuroscience Center at Sheba and sign informed consent. Then the study physician will perform a clinical evaluation, collect medical information, draw blood for metabolic and lipid markers and anthropometrics for assessment of MetS. CDR and MOCA for MCI classification will be administered by a neuropsychologist. A diagnostic consensus conference comprised of a neurologist, geriatric psychiatrist, and a neuropsychologist will combine medical and cognitive assessments to adjudicate eligibility. Eligible participants will be invited to a baseline assessment that will be split into two days. The first day will include a full cognitive and functional assessments, blood draws (for AD biomarkers, metabolic markers [fasting glucose, HbA1c, lipid profile, insulin], and brain-derived exosomes for assessment of insulin signaling-related proteins), brain MRI, as well as training on the administration of the drugs. The second day will include the FDG-PET and additional training in drug administration if necessary. Adverse event monitoring and reporting on glucose values will occur daily through the phone app described below, and face to face

every three months, when medications will also be dispensed. The study physician will review daily the AEs. The same assessments performed at baseline will be performed at the 6-month follow up. Final cognitive assessment will be performed at 12 months.

Potential adverse events include dizziness, headaches, nausea, diarrhea, vomiting, abdominal pain, constipation, decreased appetite, fatigue, hypoglycemic events (severe and non-severe). Participants will be provided with a glucometer and will be instructed to measure their glucose levels in any suspected hypoglycemia event (neuroglycopenic or adrenergic symptoms). All suspected events and glucose levels during these events will be recorded in the study app. Severe Hypoglycemia will be defined as an event requiring assistance of another person due to altered consciousness to actively administer carbohydrate, glucagon, or other resuscitative actions. In addition, the following AE will be adjudicated: acute pancreatitis, any cancer (specifically pancreatic cancer, medullary and c cell hyperplasia, thyroid cancer), clinically significant supraventricular arrhythmias and other conduction disorders, hypersensitivity and allergic reactions.

Table 2 Study procedures and assessment schedule

Study Activity	Screen	Baseline/ beginning of intervention	Month 3	Month 6	Month 9	Month 12
Informed consent, medical history, cognitive assessment (for MCI classification), clinical evaluation	X					
Medication review	X	X				
Physical exam	X					X
Vitals, concomitant medication, metabolic markers in blood (HbA1c and glucose, lipid profile, insulin)	X	X	X	X	X	X
Weight and waist circumference	X	X		X		X
Cognitive testing		X				X
MRI-ASL, FDG-PET, bloods for ADRD biomarkers, brain-derived exosomes for brain IRSP, physical capacity assessment, functional assessment (CDR, ADL, IADL)		X *		X		
Training of drugs administration		X				
Adverse event monitoring, coordinator video calls		X	X	X	X	X
Drug dispensing		X	X	X	X	X

*MRI and FDR-PET will be performed in separate days

2.4 Treatment related methods

2.4.1 Intranasal insulin administration

The choice of INI, rather than injected insulin, stems from the enormous risks accompanying insulin injection, especially to non-T2D older adults. The study will use the ViaNase; Kurve Technology intranasal device (See **Figure 1**). This device has been used in other studies of persons with AD[12,28,29] and has shown insulin penetration into the brain via CSF studies[12,28]. Through sniffing, the medication crosses the blood-brain barrier (BBB) at the top of the nasal cavity[28,29](see **Figure 2**).
Participants will be instructed to press a switch that will turn on the device, engaging a pump that releases a nebulized stream of insulin through a nose piece into a nostril for 20 seconds (the device includes an electronic timer), after which the device switches off. The process is then repeated in the other nostril. We have decided on administration of 20IU of INI twice per day for one year as the literature suggests this as the optimal dosage[9]. The Kurve device was successfully used in a recently published RCT with 80% of the participants using the device >65% of the time[30]

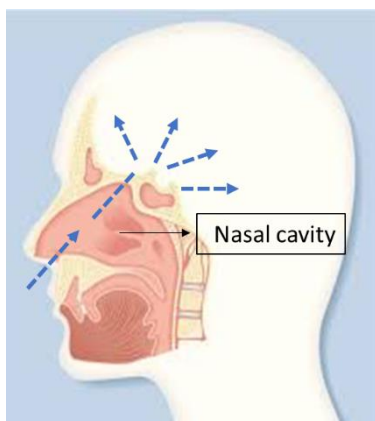*Figure 1* ViaNase; Kurve Technology intranasal device

Figure 2 The medication crosses the BBB at the top of the nasal cavity through sniffing

2.4.2 Dulaglutide administration

Individuals will be instructed to start with subcutaneous injection of a dose of 0.75 mg once weekly. If they do not experience any adverse event or has mild gastrointestinal AEs (nausea, diarrhea, vomiting, abdominal pain, decreased appetite, indigestion, fatigue[31,32]), they will be instructed to raise the dose to 1.5 mg per week (final dose) in the following week and stay at this dose for one year. Severity of AEs will be scaled on a 5-point Likert scale from 1 (no symptoms) to 5 (very severe symptoms) [33].

People with moderate or severe AEs will raise the dose from 0.75 to 1.5 after two weeks, and only if the AEs subside. If such adverse events persist over 3 weeks, the participants will not continue in the study. At any time if people suffer from severe symptoms, the medication will be terminated. Re-challenge will be proposed in certain cases at the investigator's discretion.

Participants will be instructed to inject dulaglutide on the same day of the week. It should be injected into pinched skin of abdomen or thigh. Participants and informants will be taught by the study physician how to inject the drug.

2.4.3 Video calls to ensure appropriate administration of drugs

In the first two weeks, the coordinator will accompany by video the administration of INI on a daily basis (and weekly for dulaglutide). During the third and fourth weeks, the coordinator will accompany the participants via video call every other day. From the fifth week of the study and on, the coordinator will verify once weekly that administration continues being performed appropriately. From the second video call and on, we will collect AEs, and ask to receive a report of glucose values recorded. In addition to ensuring optimal correctness of administration, this strategy will also assure optimal adherence and an opportunity to closely collect adverse events, if they occur.

2.4.4 RedCap application to ensure adherence

Study data will be collected and managed using the REDCap (Research Electronic Data Capture) electronic data capture tools hosted at the Sheba Medical Center. REDCap is a secure, web-based software platform designed to support data management for research studies. The app will be developed by our group as done for another RCT (clinicaltrials.gov #xxx), and will include two main large "buttons", one for reporting of adverse events, and the other for reporting of use of medication. It will be downloaded to both the participant and caregiver phones. Participants will be asked to report daily on their intervention administration as instructed. For dulaglutide or its respective placebo- they will be asked to report 1) if they injected the medication (yes/ no), 2) at what time, 3) what is the dose injected (0.75/1.5 mg), 4) what is the injection location (abdomen/thigh), and 5) if there were any difficulties with injection. They will be also asked to report about adverse events, if they experienced any (nausea, diarrhea, vomiting, abdominal pain, decreased appetite, indigestion or fatigue and other), and then they will score the severity of the adverse event on a 5-point Likert scale from 1 (no symptoms) to 5 (very severe symptoms). For INI and its

respective placebo- they will be asked to report 1) if they inhaled the medication (yes/no), 2) at what time, 3) if they use the device as instructed (20 seconds into each nostril- yes/no), 4) if there were any difficulties with using the device, 5) if they experience any adverse events (dizziness, headache or other). If adverse events are reported, participants will be asked to score their severity on the 5-point Likert scale. In addition to the drug administration and AE questions, the app will include space for reporting glucose levels. The daily app report is anticipated to last 1-3 minutes.

3. <u>Outcome measures</u>

<u>Primary cognitive outcome</u>: The primary cognitive outcome is a balanced composite sum of z-scores of four executive function tests (Trails B, Digit-Symbol, digit span, and Category Fluency), and four episodic memory tests (immediate and delayed recall of the word list from the ADAS-Cog, and immediate and delayed recall of Logical Memory Story I from the Wechsler Memory Test). Z-scores are reversed if necessary so that a positive value refers to good cognition. All tests have published norms for Hebrew in T2D older adults[34].

<u>Secondary cognitive outcomes</u>: Secondary cognitive outcomes will be the domain-specific composites, for executive functions and episodic memory. Such composites are commonly used in both pharmacological and non-pharmacological trials. The executive function composite will be an important outcome in light of the substantial involvement of this domain in vascular cognitive impairment[27].

<u>Secondary functional outcomes</u>: Three measures will be the clinical dementia rating scale sum of boxes (CDR-SB), which summarizes impairment in 6 domains (memory, orientation, judgment/problem solving, community affairs, home/hobbies, and personal care)[35] based on subject and informant interviews, the Katz index of independence in activities of daily living (ADL) and Brody instrumental activities of daily living (IADL)[36] questionnaires. We will also assess depression with the Beck Depression Inventory, and physical capacity using commonly used tests including grip strength, 6-meter walk, timed up and go, balance testing, 30-sec chair stand and the Fried frailty scale.

3.1 <u>Neurobiological outcomes, neuroimaging aquisition</u>

Magnetic resonance imaging (MRI) will be done on a Phillips 3T Ingenia (Ehrlangen, Germany) using a 32 Ch head coil. The MRI protocol will include high-resolution (1mm3) 3-dimensional fast spoiled gradient echo T1-weighted, high-resolution (1mm3) 3-dimensional T2-FLAIR, pcArterial Spin Labeling (ASL), and multi-shell diffusion imaging with the b-values b=0, 1000, and 2500s/mm^2.
FDG-PET will be performed after a 4 hour fast while closely monitoring blood glucose levels and with blood glucose levels lower than 180 mg/dl. PET scans will be performed on a Philips Vereos PET/CT scanner in 3D acquisition mode. A low-dose CT scan will be performed for attenuation correction prior to all scans. Image acquisition will begin after 30 minutes of eyes-open quiet rest post-injection of 5.0±0.5 mCi of [F18]FDG and include six 5-min frames. The PET data will then be reconstructed and corrected for attenuation, scanner normalization and scatter radiation.

3.2 <u>Neuroimaging outcomes</u>
Primary neuroimaging outcome
<u>Cerebral blood flow (CBF)</u>: CBF will be assessed via arterial spin labeling (ASL). Analysis will focus on mean CBF in dorsolateral prefrontal cortex, medial and lateral temporal lobe, and medial and lateral parietal cortex, defined by region of interest (ROI) being studied from the automated anatomical labeling (AAL) atlas. In addition, we will perform exploratory voxel-wise analyses (additional information on procedures and image processing is provided in the Electronic Supplement)[37,38]

<u>[F18]FDG-PET</u>: Reconstructed PET data will be realigned, summed and coregistered with reslicing to participants' T1-weighted MRIs. Voxel-wise standardized uptake value ratio (SUVR) images will be created in native MRI space with the pons as reference region. [F18]FDG values will be extracted from ADRD vulnerable regions of interest (dorsolateral prefrontal cortex, medial and lateral temporal lobe, and medial

and lateral parietal cortex). In addition, PET images will be warped into MNI space and smoothed for voxel wise analysis.

Secondary neuroimaging outcomes

<u>White matter hyperintensities (WMH)</u>**:** Total WMH volume will be quantified from 3D T2-FLAIR using the Lesion Segmentation Tool.

<u>Gray matter (GM) volume</u>**:** Regional cortical GM, hippocampal volumes, and intracranial volume will be extracted from T1-weighted scans using CAT12

<u>Tissue microstructure</u>: Diffusion weighted imaging will measure microstructural alterations indicative of tissue injury and neurodegeneration. Neurite orientation dispersion and density imaging modeling will be applied as previously implemented by our group[39].

3.3 Secondary neurobiological outcome- Brain-derived exosome IRSP measures

Brain-derived exosomes are extracellular nanovesicles (EVs) that are collectively released by all cell lineages of the central nervous system and contain cargo from their original cells. They are emerging as key mediators of communication and waste management among neurons, glial cells and connective tissue during both physiological and pathological conditions in the brain. This measure will be analyzed by our collaborator Dimitrios Kapogiannis as detailed in[40]. Blood samples will be collected in the morning, after 12 h fasting by venipuncture into vacutainer K2EDTA tubes. Immunoprecipitation will be used to isolate enriched sub-populations of neuronal-origin EVs (nEVs) from blood and to measure biomarkers reflecting diverse aspects of AD pathogenesis, such as amyloid B (Aβ) and tau cascades, insulin resistance, and synaptic loss. Total tau, p181-tau, p231-tau, p(Tyr)-IRS-1, p-IGF1R, pIR, p70S6K(T389), pGSK-3B(Ser9), and pAkt(Ser473) using electrochemiluminescence assays will be measured by using colorimetric enzyme-linked immunosorbent assay (ELISA) to measure EV marker Alix.

3.4 Secondary ADRD-blood biomarkers

There is evidence suggesting that INI may confer benefits against Aβ aggregation, the main neurobiological substrate of AD[41]. Thus, we will collect EDTA plasma and assess a panel of AD-related biomarkers. In collaboration with Dr. Henrik Zetterberg at the University of Gothenburg, we will measure biomarkers that reflect AD pathology, including Aβ42, Aβ40, and tau proteins (P-tau181, P-tau231, P-tau 217 and T-tau), using Single molecule array (Simoa) immunoassays developed for these markers [42,43]. Neurodegeneration and astrocytic markers, *i.e.*, neurofilament light (NfL) and glial fibrillary acidic protein (GFAP), will also be measured by Simoa[44][45,46]. All measurements will be performed using one batch of reagents at the end of the study on HD-X Analyzers (Quanterix, Billerica, MA) by board-certified laboratory technicians who will be blinded to clinical data.

4. <u>**Analytic plan**</u>

Table 3: Go/No go criteria	
Ease of use of intranasal and injection administration	After the first month (when the research coordinator will closely follow appropriate administration of drugs), >80% of correct use based on video follow ups of the coordinator
Adherence	Over 80% adherence of each of the medication administrations
Intranasal insulin adverse events	Not significantly above what has been published in the literature

This is a feasibility study so the main analyses are descriptive: Ease of use will be quantified by the number of times the coordinator will have to correct the participant with the use of the intranasal device or the injection in the video calls. Adherence to each of the drugs measured as number of days INI was used twice, once, or zero times and number of weeks for dulaglutide per the RedCap app reporting. We will

Dulaglutide adverse events	Not significantly above what has been published in the literature
Non published Insulin/dulaglutide adverse events	Not significantly above their frequency in the placebo group
Cognition	Participants on combination of INI with dulaglutide will not decline more than the placebo group on the primary cognitive outcome.
Neurobiological markers	No changes suggesting target engagement in any of the neurobiological outcomes

also collect dropout reasons. For comparisons among the four groups, we will examine the total number of adverse events, the proportion of days treatment adherence is achieved using the Kruskal-Wallis test, the dropout rate and the individual adverse event rate using the Fisher's exact test. The adverse events include dizziness, headaches, nausea, diarrhea, vomiting, abdominal pain, decreased appetite, or fatigue and severe hypoglycemia.

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Although the goal of the study is feasibility for designing a large scale RCT, we will explore changes from baseline to 6 months in cognition, CBF, glucose uptake, ADRD blood biomarkers, and brain-derived exosomes, using 2-way ANCOVA or the Kruskal-Wallis test to compare the four groups. Cognitive changes from baseline to 12-months will be tested to explore the long-term effects of the combination of INI with dulaglutide on cognition. ANCOVAs will include covariates (*e.g.*, age, sex, education) if the groups differ at baseline.

Power and sample size:
Table 3 define a Go/No go strategy for each of these feasibility conditions with criteria which will be applied in the decision process of whether a large-scale clinical trial of the combination of INI with dulaglutide should be further pursued. Below we detail the statistical power for ease of use and adherence, and then for safety, and show that we are adequately well-powered.

Power calculation for go/no go of ease of use and adherence- This figure shows the confidence levels for various sample proportions (*i.e.*, required percent of ease of use or adherence rate for a "go") estimated with a sample size of 20 and a lower confidence limit that is 0.1 below the sample proportion, using the simple asymptotic with continuity correction method[25]. We chose a one-sided lower limit confidence interval calculation because we are only interested in whether the rate of the feasibility measure is at least above a certain level[26] (*e.g.*, above 80% for ease of use or adherence). As shown in **Figure 3**, the confidence levels range from 75% to 87% for a proportion of 0.65 to 0.90. So, for example, the confidence level is 80% if the proportion of subjects in the combination group who adhere to the protocol is 80% (with a lower limit of 70%). This is an adequate confidence level.
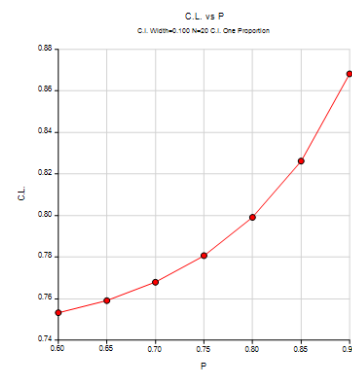
Power calculation for go/no go of safety: Because the proposed feasibility study is limited to 20 subjects per study arm, here we calculated the upper limit for the adverse event rate in the combination intervention group such that if we reject the null hypothesis, any adverse rate higher than the upper limit would establish harm for the combination group, namely, that proceeding to a larger study is not warranted. If the null hypothesis is not rejected, we will conclude that the combination intervention is likely safe justifying conducting a future larger study. For example, for an adverse event rate of 15% for the use of dulaglutide[27], if in the combination therapy arm we observe an adverse event rate >44%, the power is at the 80% level to claim that the adverse event rate is significantly higher for the combination therapy group. Any observed rate lower than the calculated rates for combination therapy in the **Table 4** (middle column) would signal safety and justify a larger-scale RCT.

Table 4: Power analyses for go/no go of safety		
Power	**Rate of adverse events for combination therapy (N=20)**	**Rate of adverse events for single therapy (N=20)**
80%	37%	10%
80%	44%	15%
80%	51%	20%
70%	33%	10%
70%	39%	15%
70%	46%	20%

5. **Discussion**

This is a double-blind placebo-controlled clinical trial investigating the feasibility, safety and challenges of INI combined with an injection of dulaglutide in older adults at high dementia risk due to MCI and at high risk for cerebrovascular disease due to MetS. Secondary goals are to test the efficacy of combination therapy on cognitive functions and on potential neurobiological outcomes, primarily cerebral blood flow and cerebral perfusion.

We have chosen to focus on MCI participants because they have a high risk of cognitive decline and dementia, but the neuropathologic processes that cause cognitive decline still have potential for prevention or delay. We also chose to focus on a population that has a high prevalence of cerebrovascular disease[47,48] since our and others' data indicate that the combined action of a T2D medication with insulin on cognition is likely mediated through cerebrovascular mechanisms[5]. Individuals with MetS have a conglomerate of cardiovascular risk factors (abdominal obesity, glucose intolerance, hypertension, and dyslipidemia), themselves associated with high dementia risk[18, 19], and have higher prevalence of white matter hyperintensities[20], small vessel disease[21], and hypometabolism[22, 23], enhancing the potential of this study to show target engagement, *i.e.*, to detect a signal of improvement in cerebrovascular measures by combination of INI with dulaglutide.

Numerous small INI RCTs[8,11,41], and two larger RCTs (the MemAID[8] and SNIFF[12] studies) tested the effect of INI on cognition. In the recent MemAID study, INI conferred beneficial effects on cerebral blood flow and cognition in older adults with T2D and in non-diabetic individuals with dysglycemia, compared to placebo[8]. However, in the large SNIFF trial, no cognitive advantage for INI was found when results were analyzed using data with all participants. Yet, in a post hoc analysis testing the effects of INI using the Kurve intranasal device, which will be used in our study, participants receiving INI had significantly better cognitive outcomes compared to those receiving placebo[12]. The discrepant results mandate additional research on the effects of INI on cognition and disentangling its underlying mechanisms of action.

To date, there is only one large RCT of a GLP-1 receptor agonist which examined cognition (albeit as an "add on" outcome), the REWIND[49] study. The study analyzed the effects of 1.5mg dulaglutide (mean administration=5.4 years) on incident cognitive impairment (a follow-up MoCA or DSST ≥1.5 SD below the baseline mean score) in 8828 initially cognitively normal adults (mean age=65) using intention-to-treat analysis (ITT)

After post-hoc adjustment for individual standardized baseline scores, the hazard of cognitive impairment was reduced by 14% in those assigned to dulaglutide (HR 0.86, 95% CI 0.79–0.95; p=0.0018). This result strongly supported our choice of dulaglutide and its potential in maintaining brain and cognitive health. Its effects on neurobiological outcomes have not been studied. Importantly, dulaglutide may protect the brain by several mechanisms associated with dementia risk: direct activation of the GLP-1 receptor, which is abundant in memory and learning regions[49]; and reduction of cardiovascular risk factors, cardiovascular disease, and stroke[16,31]. In addition to its potential neuroprotective and cognitive effects, dulaglutide has a good safety profile, making it the optimal choice, to date, for combining with INI.

There is growing evidence suggesting that the combination of insulin with another T2D medication, rather than each medication alone, may lead to neuroprotection. In the SALSA study, the combination of insulin with another T2D medication has been linked to slower cognitive decline than in a single drug or none[18]. Our group has shown in postmortem human brain[18] tissue that T2D medications are linked to improved insulin signaling and endothelial cell markers, and that these improvements were significantly stronger in vessels isolates than in tissue homogenate, pointing to an effect primarily through vascular mechanisms[5]. Indeed, in addition to the critical role of insulin signaling in neuronal health, even among persons without diabetes, it is implicated in cerebrovascular health and disease[50]. These results, together with results from the SNIFF study showing that INI diminished white matter hyperintensities, a characteristic of small vessel disease, and evidence from the REWIND (dulaglutide) clinical trial showing significantly lower risk for stroke in the dulaglutide arm[16,31], support our choice of cerebrovascular measures as neurobiological outcomes.

Since observational and postmortem human brain tissue studies by definition do not include controlled exposure to the T2D drugs, we have recently completed an animal model study using the AD-like animal

model Tg2576 mice. The study examined the effects of INI alone, exanatide (a GLP-1 receptor agonist) alone, the combination of INI with exanatide, and saline. Compared to no medications, the combination therapy significantly improved insulin signaling-related gene expression, and nominally improved memory[21], underscoring the need to investigate the potential cognitive benefits of combination therapy in a clinical trial context.

Additional support for the advantage of combination of insulin with another T2D medication over each medication alone comes from an in-vitro study[21] where downregulation of the insulin receptor on hippocampal neurons by Aβ-derived diffusible ligands was optimally reversed by the exposure of the receptor to the combination of insulin combined with rosiglitazone, an insulin-sensitizing drug for T2D. This study suggests that bolstering brain insulin signaling by combination therapy could have significant potential to slow or deter ADRD pathogenesis. The results of this study also provide a rationale to include, as secondary biological outcomes, AD-related biomarkers and insulin signaling-related proteins.

This body of evidence provided the foundation for the proposed combination therapy RCT. From a strategic point of view, our study relates to ADRD as multifaceted and complex, highly likely requiring multi-targeted treatments for neurobiological enhancements and ultimately significant cognitive benefits.

Our study has limitations. Participants are recruited only in Israel so the sample may not represent well other populations. However, Israel is characterized by waves of immigrants that reflect diverse ethnic backgrounds including approximately half Middle Eastern and North African (MENA). Moreover, the study does not have any exclusion criteria by race, ethnicity, or religion. Importantly, the proportion of older adults with MetS in Israel is very similar to its proportion in the Western world, 20%[51]. Our trial will also suffer from limited statistical power to detect significant findings related to the efficacy of combination therapy on cognition, largely due to small sample sizes and a short follow up period. However, we aim to provide new knowledge on the effect direction of cognitive outcomes, biomarkers, and brain outcomes. The main strength of the study is its novelty, the full design (four groups), the use of novel technology to ensure appropriate use and adherence to the drugs and monitor closely AEs, and the detailed, directly measured potential confounders which will be provided to the study team by the Maccabi Health Services.

If successful, this pilot study will provide the basis for a multi-center large-scale RCT for the cognitive benefits of the combination of INI with dulaglutide in individuals enriched for cerebrovascular disease and at high dementia risk. It is still unclear whether amyloid modulation leads to cognitive benefits, emphasizing the urgent need for novel therapeutic venues that engage other ADRD neurobiological targets. The accelerating prevalence of the MetS and ADRD in an aging population, further amplify the potential public health impact of this study.

Alzecure, Biogen, and Roche, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work).

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