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Protocol for a double-blind placebo-controlled randomised controlled trial assessing the impact of oral semaglutide in amyloid positivity (ISAP) in community dwelling UK adults

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ABSTRACT

Introduction Glucagon-like peptide-1 receptor agonists (GLP-1 RAs), currently marketed for type 2 diabetes and obesity, may offer novel mechanisms to delay or prevent neurotoxicity associated with Alzheimer's disease (AD). The impact of semaglutide in amyloid positivity (ISAP) trial is investigating whether the GLP-1 RA semaglutide reduces accumulation in the brain of cortical tau protein and neuroinflammation in individuals with preclinical/prodromal AD.

Methods and analysis ISAP is an investigator-led, randomised, double-blind, superiority trial of oral semaglutide compared with placebo. Up to 88 individuals aged ≥55 years with brain amyloid positivity as assessed by positron emission tomography (PET) or cerebrospinal fluid, and no or mild cognitive impairment, will be randomised. People with the low-affinity binding variant of the rs6971 allele of the Translocator Protein 18 kDa (TSPO) gene, which can interfere with interpreting TSPO PET scans (a measure of neuroinflammation), will be excluded.

At baseline, participants undergo tau, TSPO PET and MRI scanning, and provide data on physical activity and cognition. Eligible individuals are randomised in a 1:1 ratio to once-daily oral semaglutide or placebo, starting at 3 mg and up-titrating to 14 mg over 8 weeks. They will attend safety visits and provide blood samples to measure AD biomarkers at weeks 4, 8, 26 and 39. All cognitive assessments are repeated at week 26. The last study visit will be at week 52, when all baseline measurements will be repeated. The primary end point is the 1-year change in tau PET signal.

Ethics and dissemination The study was approved by the West Midlands—Edgbaston Research Ethics Committee (22/WM/0013). The results of the study will be disseminated through scientific presentations and peer-reviewed publications.

Trial registration number ISRCTN71283871.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ Interventional study in a predementia population with pathological evidence of potential Alzheimer's disease (AD).
⇒ Randomisation in a trial setting minimises potential between-group difference at baseline.
⇒ Measures of brain tau will test the primary hypothesis that glucagon-like peptide-1 receptor agonists interact with core AD pathophysiology.
⇒ The study is not powered to establish the efficacy of semaglutide in preclinical AD.

INTRODUCTION

Alzheimer's disease (AD), characterised by synaptic dysfunction and neurodegeneration, is thought to be triggered by the accumulation in the brain of amyloid plaques and neurofibrillary tangles, aggregates of hyperphosphorylated τ proteins.[1] This process likely begins decades before symptoms of AD become evident, with supra-threshold levels of cortical amyloid triggering the AD pathophysiological cascade.[1] 'Amyloid positivity' on positron emission tomography (PET) scans or in cerebrospinal fluid (CSF) assays in cognitively healthy individuals is diagnostic of the preclinical stages of AD.[2] While treatments designed to reduce accumulations of these abnormal levels of protein have recently been shown to associate with modest clinical benefit in patients with AD (mild cognitive impairment (MCI) or dementia), they are nonetheless associated with significant adverse effects, and delivering them in routine clinical practice requires a sizeable investment and reorganisation of services. Therefore, secondary prevention strategies in people with preclinical disease is unlikely to rest primarily on amyloid clearance approaches. An alternative strategy for both treatment and secondary AD prevention is

the repurposing of existing compounds which has shown efficacy elsewhere (eg, COVID-19).[3 4] In relation to AD, this strategy is underdeveloped.

Repurposing compounds reduces risk, cost and time to providing a new treatment.[4] Drug development risk is reduced through a well-established safety profile and thus bypasses the need for early preclinical safety testing and iterative chemical compound optimisation. Additionally, significant knowledge of clinical safety can be gained from existing literature, pharmacovigilance, and clinical experience. Further advantages of this strategy include real-world evidence of effectiveness, and biosamples from previous trials. For AD, repurposing is particularly relevant as pathways thought to propagate both AD and non-AD neurodegeneration (neuroinflammation, central nervous system insulin resistance (IR) and cerebrovascular pathology) can already be targeted by approved compounds and thus represent viable treatment targets.

Several compounds have been identified as high priority targets for repurposing in AD. Among these, a class of medications developed for the treatment of type 2 diabetes (T2D) known as glucagon-like peptide-1 receptor agonists (GLP-1 RAs) offers promise through strong epidemiological and preclinical data suggesting neuroprotective effects. GLP-1 RAs are incretins which enhance glucose-dependent insulin secretion, slow gastric emptying and reduce both postprandial glucagon secretion and food intake.[5] The effect is reduced postprandial blood glucose without the risk of hypoglycaemia. They are currently marketed for glycaemic control in T2D, and for weight loss in individuals with obesity or overweight individuals with comorbidities).[6] Importantly for dementia research, these classes of compounds cross the blood-brain barrier (BBB) and have been shown to be neuroprotective in animal models of neurodegeneration[7 8]; additionally, they can be given safely in non-diabetic populations due to the low risk of hypoglycaemia.

The evidence for potential GLP-1 RA efficacy in AD comes from pharmacoepidemiological studies that have demonstrated an association of their use in T2D and reduced incidence of dementia.[9 10] A nested case-control study based on dementia diagnosis within a cohort of 176 people with T2D showed that GLP-1 RAs were associated with a significantly reduced odds of dementia after adjusting for demographic confounders, acute and chronic diabetes complications and use of other types of antidiabetic agents (OR 0.58, 95% CI 0.42 to 0.81, relative to placebo).[10] In addition, increasing exposure to GLP-1 RAs over time resulted in further gradual decrease in the odds of developing dementia. A paper reporting the dementia-related outcomes in 15 820 people with T2D from three double-blind randomised placebo-controlled cardiovascular trials (Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome Results (LEADER), Peptide Innovation for Early Diabetes Treatment 6 (PIONEER 6) and Trial to Evaluate Cardiovascular and Other Long-term Outcomes with Semaglutide in Subjects with Type 2 Diabetes (SUSTAIN-6)) and a Danish healthcare register-based cohort (120 054 individuals) found similar results.[9] The authors reported a relative risk reduction for dementia with HRs for GLP-1 RAs of 0.47 (95% CI 0.25 to 0.86) and 0.89 (95% CI 0.86 to 0.93) relative to other diabetes treatments and placebo in the trials and cohort data, respectively. Increase in yearly exposure to GLP-1 RAs was associated with further benefit related to dementia that primarily affected individuals ≤70 years of age.

Mechanisms for the potential disease-modifying action of GLP-1 RAs regarding dementia is probably multifactorial. A pilot trial in patients with AD showed liraglutide, compared with placebo, decreased the rate of decline of brain glucose metabolism[11] and increased the capacity of the BBB to transfer glucose.[12] While the pilot trial did not find an effect on amyloid accumulation in a secondary analysis, there is now evidence that tau protein changes are more tightly related to IR.[13] A preclinical study demonstrated that loss of tau function (tau deletion in knockout mice) was associated with impaired hippocampal and hypothalamic responses to insulin.[14] This builds on evidence demonstrating that tau hyperphosphorylation leads to neuronal IR and intracellular insulin accumulation.[15] These results add to the growing appreciation that tau's physiological role is significantly broader than neuronal structure support and includes regulation of insulin signalling, in addition to DNA protection from oxidative stress[16] and control of neuronal excitability.[17] Alternative potential mechanisms underlying GLP-1 RAs' effects in dementia are through inflammatory and cerebrovascular factors; neuroinflammation is a key driver of AD pathology[18] with GLP-1 RAs having been shown to regulate both systemic[19] and neuroinflammation.[20] For example, in a post hoc analysis of three trials of semaglutide, the agent was shown to reduce C reactive protein by 39%–48% after 68 weeks of treatment. In addition, cardiovascular risk factors mediate the risk of AD and dementia in general.[21] GLP-1 RAs have consistently been shown to reduce the risk of major cardiovascular and cerebrovascular events, which may in turn translate into lower risk of dementia.[22 23] Specifically, in the EXSCEL trial, exenatide reduced the occurrence of death caused by a composite of vascular events (cardiovascular causes, non-fatal myocardial infarction or non-fatal stroke),[22] while SUSTAIN 6 and PIONEER 6 trials found that semaglutide reduced the incidence of major cardiovascular events in patients with T2D.[23] Thus, there remains a need for research into the mechanism through which GLP-1 RAs may be beneficial in dementia. The knowledge gained could guide the design of trials aimed at demonstrating GLP-1 RA efficacy, particularly where a specific subgroup (eg, individuals with high cardiovascular risk or high levels of systemic inflammation or genetic risk for AD) is shown to be most likely to benefit from them.

Methods and analysis

ISAP is a randomised, double-blind, placebo-controlled parallel-group, superiority trial of oral semaglutide given

over a period of 12 months that aims to clarify the mechanism through which GLP-1 RAs may impact AD pathophysiology. It is recruiting adults in preclinical stages of AD through having high levels of cortical amyloid as assessed by PET. The study start date was 1 March 2021 with a planned end date of 15 March 2026.

Primary objective

The primary objective is to evaluate the impact of oral semaglutide compared with placebo on tau accumulation in amyloid-positive dementia-free ageing adults.

Primary end point

The primary end point for the study is cortical tau accumulation over 1 year as assessed by tau PET.

Secondary analyses

We will examine the effects of semaglutide compared with placebo on brain neuroinflammation as determined by the Translocator Protein 18 kDa (TSPO) PET and plasma assays of biomarkers relevant to dementia, cognition, neurodegeneration as determined by MRI, AD plasma biomarkers and physical activity variation as determined by wrist-worn actigraphy.

Exploratory analyses

Exploratory analyses of plasma samples using multidimensional protein assays will include established AD biomarkers, such as amyloid β 42 over 40 ratio (Aβ42/Aβ40), phosphorylated tau forms (181 and 217), neurofilament light as a neurodegeneration marker and glial fibrillar acidic protein (GFAP) as an astrocytic activation marker.[24] We will also investigate the plasma of individuals who at screening have amyloid-negative PET scans and are excluded from randomisation to (1) inform screening procedures for future predementia AD trials and (2) define how individuals who are not eligible for the trial differ from those included to help inform the generalisability of the study results. Plasma will be retained for further proteomic exploratory analyses. See table 1 for a summary of outcome measures.

Study subjects

Amyloid-positive community dwelling UK-based volunteers (determined through PET amyloid scanning or CSF) of both sexes aged ≥55 years with no or MCI (evidenced through a score of 0.5 or below on the Clinical Dementia Rating (CDR) scale). See online supplemental appendix 1 for a full list of trial inclusion and exclusion criteria.

We will recruit using existing recruitment resources (eg, electronic research registers or research volunteer lists) or electronic healthcare records in primary or secondary care (subject to a valid consent to be approached for research). The stratification algorithm we use combines diagnosis of MCI, carriage of the apolipoprotein E

Table 1 Primary, secondary and exploratory outcomes

Outcomes	Method	Measurements
Primary		
Annualised cortical tau change	PET tau	Baseline, week 52
Secondary and exploratory outcome measures		
Cortical neuroinflammatory signal	TSPO PET	Baseline, week 52
Plasma biomarkers of neuroinflammation	Plasma GFAP	Screening, baseline, weeks 4, 8, 26, 39 and 52
Plasma AD biomarkers	Plasma p-tau181 and Aβ42/40	Screening, baseline, weeks 4, 8, 26, 39 and 52
Cognition (in-clinic)	ACE-III, Cambridge Cognition CANTAB battery	Baseline, weeks 26 and 52
Cognition (remote)	Cognitron	Baseline, weeks 26 and 52
Adverse events	Presence of adverse events	Baseline, weeks 4, 8, 26, 39, 52 and follow-up call
Neurodegeneration (imaging)	Hippocampal MRI volume	Baseline and 52 weeks
Neurodegeneration (plasma)	Plasma NFL	Screening, baseline, weeks 4, 8, 26, 39 and 52
Depression and anxiety levels	CES-D and HAI scales	Baseline, week 52
Distress at AD risk disclosure	Genetic testing for AD scale	Weeks 26 and 52
Quality of life	EQ-5D-5L scale	Baseline, week 52
Level and pattern of physical activity, and circadian rhythms	Wrist-worn actigraphy	Baseline, week 52

ACE-III, Addenbrooke's Cognitive Assessment; AD, Alzheimer's disease; CES-D, Center for Epidemiological Studies Depression; GFAP, glial fibrillar acidic protein; HAI, Health Anxiety Inventory; NFL, neurofilament light; PET, positron emission tomography; TSPO, Translocator Protein 18 kDa.

(*APOE*) ε4 allele, family history of dementia in a first-degree relative, advancing age (treated as continuous variable), sex and where available cognitive function and AD biomarkers in plasma.

Patient and public involvement

Members of the public were involved in the design stages of the study during a patient and public involvement (PPI) group meeting on 12 March 2020. The PPI input informed the study design and prioritising amyloid PET over lumbar puncture as a screening method. A member of the public also sits on the ISAP trial steering committee.

STUDY PROCEDURES

Screening visit

At the screening visit, subjects provide written informed consent with a medically trained study investigator (consent form available as a online supplemental file) and undergo a medical history and physical examination, interview for the presence of dementia (CDR scale) and blood samples. These samples inform eligibility, determine TSPO allele carriership (low-affinity binding variant of the rs6971 allele of the TSPO gene is an exclusion criterion) and provide samples for research biomarker analyses. An informant indicated by the participant is approached to collect information relevant to the CDR scale. Individuals that meet study criteria then undergo amyloid PET testing to determine their amyloid status.

For each PET scan, participants receive an intravenous injection of the appropriate radiotracer (florbetaben or if unavailable, florbetapir or flutemetamol with a maximum of 300 MBq activity per scan) with data acquisition in the region of 20–30 min (depending on tracers) following an uptake period appropriate for the particular radioligand and its kinetics.Amyloid scans are read clinically and full quantification is also performed.

Amyloid status will also be possible to establish alternatively through CSF sampling (subject to ethical amendment currently under consideration) depending on study logistics and participant preference. A minimum of 500 µL sample will be analysed using the Lumipulse G600II automated assay (Fujirebio) for Aβ42 and Aβ40. Aβ42/Aβ40 ratio will be used due to previously demonstrated high concordance with amyloid PET relative to Aβ42 alone and defined a positivity cut-off of 0.065.[25]

Baseline visit

Participants eligible for the study based on screening procedures are invited to a baseline study visit when amyloid status (positive or negative) is disclosed to them in accordance with the ISAP study Amyloid Disclosure standard operating procedure. Baseline measures of cognition are recorded using computerised testing (CANTAB battery focusing on attention, episodic memory, processing speed, working memory and executive function) and the Addenbrooke's Cognitive Examination III, 2017 (ACE-III). Participants complete questionnaires probing health-related quality of life (EQ-5D-5L), health anxiety (Health Anxiety Inventory), affective symptoms (Center for Epidemiological Studies Depression Scale) and disclosure of dementia risk. Participants are then allocated at random to semaglutide or matching placebo in a 1:1 ratio. Treatment assignments are performed using a computerised procedure with minimisation (adaptive stratified sampling) based on T2D (yes/no), MCI (yes/no) and trial site to maintain balance between treatment groups. Participant replacement is not permitted.

Before initiating study treatment, participants are required to undergo tau PET, TSPO PET and MRI scanning as well as completing their remote actigraphy and cognition assessments.

Tau positron emission tomography

A target dose of 185 MBq using one of three tau PET tracers depending on availability ([18F]PI-2620; [18F]AV1451; [18F]MK-6240) is administered intravenously. Image acquisition takes place 30 min after appropriate uptake time depending on the ligand. PET images are co-registered to their T1-weighted MRI, and fully quantified.

Translocator Protein 18 kDa positron emission tomography

A target dose of 185 MBq 18F-DPA714 is injected intravenously followed by up to 60 min duration of image acquisition. The images are co-registered to a T1-weighted MRI, and then transformed into Montreal Neurological Institute space and fully quantified.

Magnetic resonance imaging

The MRI acquisition protocol consists of the following sequences: T1-weighted imaging, three-dimensional T2-fluid-attenuated inversion recovery (FLAIR), axial T2-weighted, diffusion weighted and T2*-weighted imaging. The analysis of the MRI protocol will comprise measures of (i) macrostructural change derived from volumetric T1-weighted imaging including global and regional atrophy rates; (ii) microstructural pathology and loss of connectivity through change in grey and white matter diffusivity (diffusion-weighted imaging derived from fractional anisotropy, axial and radial diffusivity); (iii) vascular burden and microhaemorrhages derived from FLAIR, T2-weighted and quantitative susceptibility mapping; (iv) total and regional white matter hyperintensities volumes as well as Fazekas visual scoring of cerebrovascular burden.

Actigraphy

Wrist-worn actigraphs (AX3 device, Axivity, purchased 'off-the-shelf' and used within its indication), will be distributed to participants who will be requested to wear them for 7 days after the baseline visit. The 7-day assessment period can commence up to 3 days after the baseline visit to allow for any delay in shipment.

Remote cognitive assessment

A neuropsychological battery using the Cognitron cognitive testing platform[26] will be employed to gather data remotely. The battery will consist of executive function (Verbal Reasoning), attention (Simple Reaction Time, Choice Reaction Time, Digit Vigilance), working Memory (Paired Associate Learning, Self-Ordered Search, Digit Span) and episodic memory (Delayed Word Recognition) tasks. Eligible participants will be required to complete sessions of testing using a PC or a tablet on three consecutive days in the week after the baseline visit.

Once scanning and remote assessment procedures are complete, randomised participants initiate treatment with 3 mg oral semaglutide/placebo once daily and follow a 8-week dose escalation regimen until reaching the treatment dose of 14 mg oral semaglutide/placebo once daily. Participants to take the drug with half a glass of water and (i) to not split, crush or chew the tablet, (ii) to take it in the morning before any oral intake, (iii) not to eat, drink or take any other medication for 30 min after administration. Participants should remain on the 14 mg dose level until the end of treatment visit, but treatment interruptions are allowed, for example, if there are issues with poor tolerability or treatment emergent adverse events (AEs). Unscheduled visits are arranged as required in instances where a change in dose is necessitated to dispense a lower dose of the investigational medicinal product (IMP). If participants are unable to tolerate the IMP despite dose reductions or interruptions, the IMP is discontinued permanently. In this instance, participants should continue to follow the trial schedule without being withdrawn from the trial. Unblinding is possible in a medical emergency through the software employed for randomisation; unblinding will result in withdrawal from the study.

Follow-up visits

Participants will attend visits at weeks 4, 8, 26 and 39 to monitor safety (emergence of AEs, changes to questionnaires relevant to health anxiety, depression and distress) and obtain blood for research purposes.

Final visit (week 52)

The study will close out with a final visit at which all assessments from the baseline visit will be repeated: in-clinic cognitive testing (ACE-III, CANTAB) and EQ-5D-5L will be performed and scanning (PET Tau, PET TSPO and MRI) will be arranged. Participants will complete a final period of remote cognitive testing and actigraphy monitoring in the week before the final visit.

Follow-up (week 57)

The study team will follow participants up with a telephone call to determine if any AEs have occurred in the period since discontinuing the medication.

Total radiation exposure

The maximum total radiation protocol dose (TRPD) from this study is 28.4 mSv. For comparison, the average annual natural background radiation dose in the UK is 2.7 mSv. The TRPD incurred in this study can be compared with approximately 12 years of natural background radiation exposure. The risk from exposure to ionising radiation is the induction of fatal cancers and, assuming a risk for the UK population of both sexes for ages 18–64 years of 5% per Sievert (Documents of the National Radiological Protection Board Vol 4, No 4, 1993), the additional lifetime risk of inducing a fatal cancer in a healthy individual is approximately 1 in 704 from a dose of 28.4 mSv. This should be compared with the natural incidence rate for cancer in the UK, which is one in three.

Sample size and power considerations

The proportion of participants with T2D will be limited to 30% to minimise the possible impact of any diabetes-specific effects of semaglutide. Table 2 shows 12 power estimates for 3 possible tau PET change effect sizes (based on the difference in mean 1-year change in tau accumulation between the semaglutide and placebo treatment

Table 2 Sample size estimations

Total number scanned for amyloid=316 Number randomised=88 Number of completers=75 (=88×0.86)	Power estimate* assuming tau PET mean annual change (SD) of 0.05 (0.04)† when untreated		Power estimate* assuming tau PET mean annual change (SD) of 2.01 (2.97)‡ when untreated	
One-year change from baseline in mean tau accumulation with semaglutide	Alpha 0.05	Alpha 0.10	Alpha 0.05	Alpha 0.10
20% lower compared with placebo. Effect differences of 0.01 and 0.402, respectively	19.7%	29.9%	9.2%	16.0%
30% lower compared with placebo. Effect differences of 0.015 and 0.603, respectively	38.1%	50.8%	14.6%	23.4%
40% lower compared with placebo. Effect differences of 0.02 and 0.804, respectively	59.8%	71.6%	22.3%	33.0%

316 individuals need to be screened to randomise 88 participants, with 75 participants completing the study, assuming a 14% dropout rate.
*Power calculation is based on a two-sample t-test assuming equal variance.
†Hanseeuw, BJ *et al*.[32] 2019 PMID: 31157827.
‡Whittington A, Gunn R.[33] 2021 PMID: 33517326.

groups) and two alpha (type 1 error) values, assuming 3.6 individuals need to be scanned to identify 1 amyloid-positive individual, 10% of the total participants recruited will be excluded for reasons unrelated to use of TSPO ligand, 10% have a genetic variant that precludes use of TSPO ligand and 14% potentially lost to follow-up. The study will therefore inform the size of the effect of semaglutide on tau PET accumulation rates for future confirmatory trials.

Statistical analysis

A description of the planned statistical analysis can be found in the Statistical Analysis Plan (online supplemental appendix 1).

ETHICS AND DISSEMINATION

ISAP was approved by the West Midlands—Edgbaston Research Ethics Committee (22/WM/0013). Any amendments are communicated by the trial team to the relevant parties. Dissemination of the study results will take place through peer-reviewed publications and scientific meeting presentations. Access to data for exploratory analyses and approval of publications, including authorship, will be subject to approval by the Trial Steering Committee.

KEY ISSUES IN TRIAL DESIGN

Participant stratification

The goal of the ISAP trial is to examine the impact of GLP-1 receptor agonism in predementia AD compared with placebo. Evidencing abnormal cortical amyloid using either PET or CSF is currently the most widely accepted method for establishing that an individual is on the AD pathophysiology continuum irrespective of the presence of clinical signs and symptoms.[27 28] On this basis, amyloid positivity is a standard criterion for inclusion in AD-modifying treatments trials.[29] Amyloid positivity has been demonstrated to predict conversion to AD dementia, both in those with a degree of cognitive impairment[30] as well as asymptomatic individuals.[31] Amyloid positivity also increases the rate at which pathology more tightly correlated with neurodegeneration, such as tau, accumulates.[32 33] The established position of amyloid positivity as AD continuum biomarker and its impact on tau accumulation guided us in our choice of inclusion criterion. The decision to focus on PET-based testing was driven by a patient and public consultation in the study design stages which showed a preference for PET over lumbar punctures for potential participant

A major issue faced by trials relying on amyloid positivity is how to minimise the number of people excluded because of negative amyloid status when tested. A strategy of relying solely on age is high risk as a minority of cognitively unimpaired individuals are amyloid positive.[34] *APOE* ε4 allele carriership is the most reliable predictor of abnormal amyloid load, increasing the likelihood by twofold to threefold regardless of the age group.[34] Having MCI increases the risk substantially, whereby 27%–71% are amyloid positive and again this risk is mediated by *APOE* ε4 carriership.[34] Efforts to further improve the prediction in cognitively unimpaired individuals have led to predictive models that include a variety of dementia risk-related data.[35 36] For the purposes of the ISAP trial, we opted for a pragmatic risk-stratification method based on the type of data available through electronic research registers in the UK (PROTECT cohort, https://www.protectstudy.org.uk/[37] and Dementias Platform UK Great Minds[38]): diagnosis of MCI, APOE4 carriership, family history of dementia in a first-degree relative, age and sex. We estimated that through this stratification method, we would need to scan 3.6 individuals to identify 1 amyloid-positive case.

Efficacy biomarkers

Evidencing the effects of novel therapeutics in clinically silent or minimally symptomatic individuals is a major challenge for AD research. Various biomarkers are under investigation as surrogates of treatment response in prodromal AD.[39] Of these, tau PET has the best evidence—it co-localises with neurodegeneration,[40] predicts cognitive status[41] and mirrors the clinical phenotype.[42] In contrast, the site and extent of amyloid deposition does not correlate with neurodegeneration.[41 43] In a head-to-head comparison versus MRI and amyloid PET, tau PET was shown to be the strongest predictor of cognitive decline and neurodegeneration in both cognitively impaired and healthy individuals.[44] For this reason, it is currently the biomarker of choice for tracking treatment effects across the AD spectrum.[45]

While well validated, tau PET is limited in its utility for large-scale clinical trials because of its cost, invasiveness and reliance on infrastructure.[45] Future research efforts therefore are directed towards identifying more scalable biomarkers. Plasma biomarkers of AD are in prime position to act as surrogates for developing AD pathology through their recently demonstrated strong associations.[46] In fact, the plasma p-tau181 assay has been shown to become abnormal even before tau PET becomes abnormal which may make it even more suitable for early therapeutic signal detection,[47] and similar results exist for Aβ42/Aβ40, p-tau217 and GFAP.[25 48] Digitally derived measures may offer an alternative or complementary method for tracking effects in prodromal AD through the high density of data obtainable through passive and active cognitive function monitoring.[49] To explore this important facet of prodromal AD trial methodology, in ISAP we implemented a high-density schedule of blood and digital biomarker sampling so that we can compare longitudinal variations in these biomarkers with the gold standard of tau PET.

Amyloid disclosure to potential participants

Disclosure of increased risk for AD to cognitively normal individuals indicated by a positive amyloid result is

inherent in preclinical AD trials. Whether this risk disclosure exposes individuals to psychological sequelae has been explored in previous research such as the anti-amyloid treatment in asymptomatic AD (A4) study.[50] In that trial, cognitively normal individuals who tested amyloid positive were given a monoclonal antibody, solanezumab, to assess its impact on the rate of memory impairment progression. The A4 team found that individuals who learned that they have elevated amyloid did not experience an increase in depressive, anxiety or suicidality symptoms.[51] Others have similarly reported low risk from psychological harm of amyloid disclosure.[52] The process of conveying amyloid positivity however remains a key ethical consideration of preclinical AD trials and formal processes have been developed.[53] We have followed these recommendations in the ISAP amyloid disclosure standard operating procedure; these will likely continue to evolve in future studies as the exact prognostic significance of amyloid PET and other AD biomarkers (eg, tau PET) in predementia adults becomes apparent.

CONCLUSION

GLP-1 RAs are a promising drug class for both secondary prevention and treatment of dementia. Repurposing licensed compounds reduces the risk, cost and time to develop a new treatment and remains largely untested in AD. Studies that evaluate the potential mechanism of action of repurposed compounds in AD are a critical part of the development process as they can provide proof-of-concept, highlight novel treatment approaches and inform the appropriate power of future confirmatory trials. Through a randomised placebo-controlled trial design, ISAP aims to deliver on these opportunities for oral semaglutide while also providing a valuable biomarker dataset linking gold standard PET measures of cortical tau and neuroinflammation to promising plasma and digital biomarkers relevant to preclinical AD.

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Contributors IK led on the study design and conduct of the study as Chief Investigator and site lead; AIA contributed to study design and trial oversight as lead of the Oxford Diabetes Trials Unit; RRH contributed to study design; PE contributed to study design and conduct of the study as site lead; BT contributed to study design, led the statistical analysis plan generation and is the main statistician of the study; AH and PH contributed on the provision of remote cognitive testing technology; JEM, BRU, LC, JB, CMu contributed as site leads; HZ contributed to the fluid biomarker analysis plans; CMa contributed as participant identification centre site lead; FC contributed to the provision of in-clinic cognitive testing.

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scientific advisory boards and/or as a consultant for AbbVie, Acumen, Alector, Alzinova, ALZPath, Annexon, Apellis, Artery Therapeutics, AZTherapies, Cognito Therapeutics, CogRx, Denali, Eisai, Nervgen, Novo Nordisk, Optoceutics, Passage Bio, Pinteon Therapeutics, Prothena, Red Abbey Labs, reMYND, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, Wave, has given lectures with honoraria in symposia sponsored by Cellectricon, Fujirebio, 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 Programme (outside submitted work). PE was a consultant to Pfizer, Novo Nordisk, Roche, AstraZeneca, Piramal Life Science, GE Healthcare. He has received speaker fees from Novo Nordisk, Pfizer, Nordea, Piramal Life Science. He has received educational and research grants from GE Healthcare, Novo Nordisk, Piramal Life Science/Life Molecular Imaging, Avid Radiopharmaceuticals and Eli Lilly. He was a member of the Scientific Advisory Board for Novo Nordisk and Cytodyn. CM consults for Biogen, Roche, Eli Lilly, Eisai, IONIS, Alnylam, Prevail, WAVE. She has been awarded an investigator grant from Biogen for ultrafast MRI programme.

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REFERENCES

1 Wilson DM III, Cookson MR, Van Den Bosch L, *et al*. Hallmarks of neurodegenerative diseases. *Cell* 2023;186:693–714.
2 Jack CR, Bennett DA, Blennow K, *et al*. A/T/N: an unbiased descriptive classification scheme for alzheimer disease biomarkers. *Neurology* 2016;87:539–47.
3 Horby P, Lim WS, *et al*, Group RC. Dexamethasone in hospitalized patients with COVID-19. *N Engl J Med* 2021;384:693–704.
4 Ballard C, Aarsland D, Cummings J, *et al*. Drug repositioning and repurposing for alzheimer disease. *Nat Rev Neurol* 2020;16:661–73.
5 Nauck MA, Quast DR, Wefers J, *et al*. GLP-1 receptor agonists in the treatment of type 2 diabetes - state-of-the-art. *Mol Metab* 2021;46:101102.
6 Medicines E, Compendium, Available: https://www.medicines.org.uk/emc/product/9750/smpc#gref
7 Hunter K, Hölscher C. Drugs developed to treat diabetes, liraglutide and lixisenatide, cross the blood brain barrier and enhance neurogenesis. *BMC Neurosci* 2012;13:33.
8 Nguyen T, Wen S, Gong M, *et al*. Dapagliflozin activates neurons in the central nervous system and regulates cardiovascular activity by inhibiting SGLT-2 in mice. *Diabetes Metab Syndr Obes* 2020;13:2781–99.
9 Nørgaard CH, Friedrich S, Hansen CT, *et al*. Treatment with glucagon-like Peptide-1 receptor agonists and incidence of dementia: data from pooled double-blind randomized controlled trials and nationwide disease and prescription registers. *Alzheimers Dement (N Y)* 2022;8:e12268.
10 Wium-Andersen IK, Osler M, Jørgensen MB, *et al*. Antidiabetic medication and risk of dementia in patients with type 2 diabetes: a nested case-control study. *Eur J Endocrinol* 2019;181:499–507.
11 Gejl M, Gjedde A, Egefjord L, *et al*. In Alzheimer's disease, 6-month treatment with GLP-1 analog prevents decline of brain glucose metabolism: randomized, placebo-controlled, double-blind clinical trial. *Front Aging Neurosci* 2016;8:108.
12 Gejl M, Brock B, Egefjord L, *et al*. Blood-brain glucose transfer in alzheimer's disease: effect of GLP-1 analog treatment. *Sci Rep* 2017;7:17490.
13 Mullins RJ, Diehl TC, Chia CW, *et al*. Insulin resistance as a link between amyloid-beta and tau pathologies in alzheimer's disease. *Front Aging Neurosci* 2017;9:118.
14 Marciniak E, Leboucher A, Caron E, *et al*. Tau deletion promotes brain insulin resistance. *J Exp Med* 2017;214:2257–69.
15 Rodriguez-Rodriguez P, Sandebring-Matton A, Merino-Serrais P, *et al*. Tau hyperphosphorylation induces oligomeric insulin accumulation and insulin resistance in neurons. *Brain* 2017;140:3269–85.
16 Sultan A, Nesslany F, Violet M, *et al*. Nuclear tau, a key player in neuronal DNA protection. *J Biol Chem* 2011;286:4566–75.
17 Ittner LM, Ke YD, Delerue F, *et al*. Dendritic function of tau mediates amyloid-beta toxicity in alzheimer's disease mouse models. *Cell* 2010;142:387–97.
18 Leng F, Edison P. Neuroinflammation and microglial activation in alzheimer disease: where do we go from here *Nat Rev Neurol* 2021;17:157–72.
19 Verma S, Bhatta M, Davies M, *et al*. Effects of once-weekly semaglutide 2.4 mg on c-reactive protein in adults with overweight or obesity (STEP 1, 2, and 3): exploratory analyses of three randomised, double-blind, placebo-controlled, phase 3 trials. *EClinicalMedicine* 2023;55:101737.
20 Yoon G, Kim YK, Song J. Glucagon-like Peptide-1 suppresses neuroinflammation and improves neural structure. *Pharmacol Res* 2020;152:104615.
21 Livingston G, Huntley J, Sommerlad A, *et al*. Dementia prevention, intervention, and care: 2020 report of the lancet commission. *Lancet* 2020;396::413–46:.
22 Holman RR, Bethel MA, Mentz RJ, *et al*. Effects of once-weekly exenatide on cardiovascular outcomes in type 2 diabetes. *N Engl J Med* 2017;377:1228–39.
23 Rossing P, Bain SC, Bosch-Traberg H, *et al*. Effect of semaglutide on major adverse cardiovascular events by baseline kidney parameters in participants with type 2 diabetes and at high risk of cardiovascular disease: SUSTAIN 6 and PIONEER 6 post hoc pooled analysis. *Cardiovasc Diabetol* 2023;22:220.
24 Zetterberg H. Biofluid-based biomarkers for alzheimer's disease-related pathologies: an update and synthesis of the literature. *Alzheimers Dement* 2022;18:1687–93.
25 Keshavan A, Wellington H, Chen Z, *et al*. Concordance of CSF measures of alzheimer's pathology with amyloid PET status in a preclinical cohort: a comparison of lumipulse and established immunoassays. *Alzheimers Dement (Amst)* 2020;12:e12097.
26 Hampshire A, Hellyer P. Available: https://www.cognitron.co.uk
27 Sperling RA, Aisen PS, Beckett LA, *et al*. Toward defining the preclinical stages of alzheimer's disease: recommendations from the national institute on aging-alzheimer's association workgroups on diagnostic guidelines for alzheimer's disease. *Alzheimers Dement* 2011;7:280–92.
28 Jack CR, Bennett DA, Blennow K, *et al*. NIA-AA research framework: toward a biological definition of alzheimer's disease. *Alzheimers Dement* 2018;14:535–62.
29 Rabinovici GD. Controversy and progress in alzheimer's disease - FDA approval of aducanumab. *N Engl J Med* 2021;385:771–4.
30 Sörensen A, Blazhenets G, Schiller F, *et al*. Amyloid biomarkers as predictors of conversion from mild cognitive impairment to alzheimer's dementia: a comparison of methods. *Alzheimers Res Ther* 2020;12:155.
31 Donohue MC, Sperling RA, Petersen R, *et al*. Association between elevated brain amyloid and subsequent cognitive decline among cognitively normal persons. *JAMA* 2017;317:2305–16.
32 Hanseeuw BJ, Betensky RA, Jacobs HIL, *et al*. Association of amyloid and tau with cognition in preclinical alzheimer disease: a longitudinal study. *JAMA Neurol* 2019;76:915–24.
33 Whittington A, Gunn RN. Alzheimer's disease neuroimaging I. Tau(IQ): a canonical image based algorithm to quantify tau PET scans. *J Nucl Med* 2021;62:1292–300.
34 Jansen WJ, Ossenkoppele R, Knol DL, *et al*. Prevalence of cerebral amyloid pathology in persons without dementia: a meta-analysis. *JAMA* 2015;313:1924–38.
35 Petersen KK, Lipton RB, Grober E, *et al*. Predicting amyloid positivity in cognitively unimpaired older adults: a machine learning approach using A4 data. *Neurology* 2022;98:e2425–35.
36 Calvin CM, de Boer C, Raymont V, *et al*. 'Prediction of alzheimer's disease biomarker status defined by the 'ATN framework' among cognitively healthy individuals: results from the EPAD longitudinal cohort study'. *Alzheimers Res Ther* 2020;12:143.

37 PROTECT study, Available: www.protectstudy.org.uk
38 Koychev I, Young S, Holve H, *et al*. Dementias platform UK clinical studies and great minds register: protocol of a targeted brain health studies recontact database. *BMJ Open* 2020;10:e040766.
39 Koychev I, Lawson J, Chessell T, *et al*. Deep and frequent phenotyping study protocol: an observational study in prodromal alzheimer's disease. *BMJ Open* 2019;9:e024498.
40 Jack CR Jr, Dickson DW, Parisi JE, *et al*. Antemortem MRI findings correlate with hippocampal neuropathology in typical aging and dementia. *Neurology* 2002;58:750–7.
41 Giannakopoulos P, Herrmann FR, Bussière T, *et al*. Tangle and neuron numbers, but not amyloid load, predict cognitive status in alzheimer's disease. *Neurology* 2003;60:1495–500.
42 Ossenkoppele R, Schonhaut DR, Schöll M, *et al*. Tau PET patterns mirror clinical and neuroanatomical variability in alzheimer's disease. *Brain* 2016;139:1551–67.
43 Bennett DA, Schneider JA, Wilson RS, *et al*. Neurofibrillary tangles mediate the association of amyloid load with clinical alzheimer disease and level of cognitive function. *Arch Neurol* 2004;61:378–84.
44 Ossenkoppele R, Smith R, Mattsson-Carlgren N, *et al*. Accuracy of tau positron emission tomography as a prognostic marker in preclinical and prodromal alzheimer disease: a head-to-head comparison against amyloid positron emission tomography and magnetic resonance imaging. *JAMA Neurol* 2021;78:961–71.
45 Groot C, Villeneuve S, Smith R, *et al*. Tau PET imaging in neurodegenerative disorders. *J Nucl Med* 2022;63:20S–26S.
46 Janelidze S, Berron D, Smith R, *et al*. Associations of plasma phospho-tau217 levels with tau positron emission tomography in early alzheimer disease. *JAMA Neurol* 2021;78:149–56.
47 Moscoso A, Grothe MJ, Ashton NJ, *et al*. Time course of phosphorylated-tau181 in blood across the alzheimer's disease spectrum. *Brain* 2021;144:325–39.
48 Ashton NJ, Janelidze S, Mattsson-Carlgren N, *et al*. Differential roles of Abeta42/40, P-Tau231 and P-Tau217 for Alzheimer's trial selection and disease monitoring. *Nat Med* 2022;28:2555–62.
49 Chinner A, Blane J, Lancaster C, *et al*. Digital technologies for the assessment of cognition: a clinical review. *Evid Based Ment Health* 2018;21:67–71.
50 Sperling RA, Rentz DM, Johnson KA, *et al*. The A4 study: stopping AD before symptoms begin *Sci Transl Med* 2014;6:228fs13.
51 Grill JD, Raman R, Ernstrom K, *et al*. Short-term psychological outcomes of disclosing amyloid imaging results to research participants who do not have cognitive impairment. *JAMA Neurol* 2020;77:1504–13.
52 de Wilde A, van Buchem MM, Otten RHJ, *et al*. Disclosure of amyloid positron emission tomography results to individuals without dementia: a systematic review. *Alzheimers Res Ther* 2018;10:72.
53 Harkins K, Sankar P, Sperling R, *et al*. Development of a process to disclose amyloid imaging results to cognitively normal older adult research participants. *Alzheimers Res Ther* 2015;7:26.

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Participant identification number: | | | | — | | | — | | | |

CONSENT FORM
Impact of Semaglutide in Amyloid Positivity (ISAP) study

Please initial each box if you agree

1 I confirm that I have read and understand the information sheet dated _____ (version X.X) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2 I understand that my participation is voluntary and that I am free to withdraw at any point, without giving any reason, without my medical care or legal rights being affected. ☐

3 I understand that I will only be included in the study if I am found to be suitable during the screening assessments. ☐

4 I have been advised about the potential risks associated with taking part in this research and have taken these into consideration before consenting to participate. ☐

5 I have been advised as to what I need to do for this research (especially with regard to semaglutide intake) and I agree to follow the instructions given to me. ☐

6 I understand that relevant sections of my medical notes and data collected during the study may be looked at by members of the site study team, monitors and designated individuals from the University of Oxford, the funder (Novo Nordisk), regulatory authorities and the participating Universities/NHS Trust(s), where it is relevant to my taking part in this study. I give permission for these individuals to have access to my records. ☐

7 I agree to my General Practitioner being informed of my participation in the study and of any abnormal results arising during the study that may be of clinical relevance. I agree to my GP providing researchers with health information relevant to my participation in the study in the event that I stop attending follow-up visits. ☐

8 I understand that the MRI/PET scans for the study are research scans that are not useful for medical diagnosis, and that scans are not routinely looked at by a doctor. If a concern is raised about a possible abnormality on my scan, I will only be informed if a doctor thinks it is medically important such that the finding has clear implications for my current or future health. ☐

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9	I agree for my personal and health data (such as my name, date of birth, NHS number, address) and participant code to be shared with members of the Imperial College London team performing genetic testing on my blood sample as well as imaging. I understand that the purpose of this is so that they can safely identify me for the purposes of the scans. I understand that the form with my personal information will be retained at Imperial College London for the duration of the study but the sample used for genetic testing will be destroyed after analysing. I understand that the results will be recorded on an electronic patient record and sent to the study site team.	☐
10	I understand that the coded information collected about me will be used to support other research in the future, and may be transferred to, and stored at, a destination outside the UK and the European Economic Area for analysis. I understand this research may involve commercial organisations.	☐
11	I agree for my voice responses from the tablet memory tasks to be recorded and analysed for the purposes of the study.	☐
12	I agree to donate blood samples and consider these samples a gift to the University of Oxford and understand I will not gain any direct personal or financial benefit from them.	☐
13	I understand and agree that my samples will be used in research aimed at understanding the genetic influences on disease and that the results of these investigations are unlikely to have any implications for me personally.	☐
14	I agree for my blood samples to be used in future research, here or abroad, which has ethics approval. I understand this research may involve commercial organisations.	☐
15	I agree to take part in the above study.	☐

Optional:	I agree for my contact details to be kept in a secure database for the purpose of contacting me about future ethically approved studies. I understand that agreeing to be contacted does not oblige me to participate in any further studies.	Yes No	☐ ☐
Optional	If recruited through an existing research register, I agree for my results from the baseline ISAP assessments to be shared with the referring researchers.	Yes No N/A	☐ ☐ ☐

Name of Participant	dd / mmm / yyyy Date	Signature
Name of person taking consent	dd / mmm / yyyy Date	Signature

When completed: 1 for participant (copy); 1 for researcher site file (original)

Impact of Semaglutide in Amyloid Positivity (ISAP) Study

Statistical Analysis Plan

Version 1.0

Date: 20th October 2023

Aligned with protocol version: 7.0, 28th August 2023

Ethics Ref: 22/WM/0013

IRAS Project ID: 300550

EudraCT Number: 2021-003328-34

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Contents

ABBREVIATIONS:

Aβ40	Amyloid beta 40
Aβ42	Amyloid beta 42
ACE-3	Addenbrooke's Cognitive Assessment
AD	Alzheimer's disease
AE	Adverse event
ANCOVA	Analysis of Covariance
AR	Adverse Reaction
CANTAB	Cambridge Neuropsychological Test Automated Battery
CDR	Clinical Dementia Rating
CI	Confidence Interval
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CNS	Central Nervous System
CSF	Cerebrospinal fluid
DAS	Data Specification
DMC	Data Monitoring Committee
DTU	Diabetes Trial Unit
ECG	Electrocardiogram
EDC	Electronic Data Capture
eGFR	Estimated Glomerular Filtration Rate
EQ-5D-5L	EuroQol-5 Dimension-5 Level
EudraCT	European Union Drug Regulating Authorities Clinical Trial Database
GCP	Good Clinical Practice
GFAP	Glial fibrillary acidic protein
GLP-1 RA	Glucagon-like peptide-1 receptor agonist
GP	General Practitioner
HbA1c	Glycated haemoglobin
HR	Hazards ratio
ICH	International Conference on Harmonisation
IMP	Investigational Medicinal Product
IQR	Interquartile Range
ISAP	Impact of Semaglutide in Amyloid Positivity
ITT	Intention To Treat

MAR	Missing at Random assumption
MCI	Mild Cognitive Impairment
mITT	Modified Intention To Treat
MM	Mixed Model
MODY	Maturity-onset diabetes of the young
MRC BSU	MRC Biostatistics Unit
MRI	Magnetic resonance imaging
NFL	Neurofilament light
NHS	National Health Service
PET	Positron emission tomography
PPS	Per-Protocol Set
p-tau181	Phosphorylated tau181
REC	Research Ethics Committee
RS	Randomised Set
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction
SD	Standard Deviation
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reactions
SUVR	Standardised Uptake Value Ratio
T2DM	Type 2 diabetes mellitus
TS	Treated Set
TSC	Trial Steering Committee
TSPO	Translocator protein

E-Sign ID: 54e08cc4-0d2e-4185-9d84-2287f4ade13f

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1.0 Purpose and Scope of the Statistical Analysis Plan

The purpose of this document is to set out the study objectives and hypotheses, the proposed presentation, analytical approaches and procedures necessary for reporting results for the main trial paper(s) of the multi-centre randomised, double-blind, controlled trial on the Impact of Semaglutide in Amyloid Positivity (ISAP).

As there can typically be multiple analytic approaches and strategies for addressing a hypothesis, there is the potential for different results to be realised from the use of alternative approaches, methods, outcome definitions and data that may be involved. Therefore the results reported in the main trial paper(s) will follow the strategy set out in this Statistical Analysis Plan (SAP); developed prior to the availability of follow-up data and finalised before database lock. Changes within any subsequent version of the SAP prior to analysis will be dated, with the basis/justification for these changes recorded.

The rationale, decision and strategy to be followed, as described in this SAP, will comply with the study protocol, Good Clinical Practice (GCP) guidelines, the statistical guidance/principles set out in the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines E9 – Statistical Principles for Clinical Trials[1], the CONSORT statement[2] for reporting trials and other statutory and regulatory requirements as appropriate.

Note that there may be possible additional analytic decisions that need to be taken after database lock (e.g. based on viewing the observed distribution of the follow-up data, prior to the trial arm being made available). Any deviations from the SAP will be described and justified and will be appended to the SAP for record purposes.

Note finally that any post hoc analyses of a more exploratory nature or not directly related to the main aims of the trial are not bound by this plan.

2.0 Introduction

2.1 Background and Rationale

Alzheimer's Disease (AD), characterised by synaptic dysfunction and neurodegeneration, is thought to be triggered by the sequential accumulation of amyloid plaques and neurofibrillary tangles which are aggregates of hyperphosphorylated tau proteins[3]. This process is understood to begin decades before first symptoms, with supra-threshold levels of cortical amyloid accumulation deemed triggering the condition; such 'amyloid positivity' as evidenced by PET scans or cerebrospinal fluid (CSF) assays in cognitively healthy individuals is now considered diagnostic of their being in the preclinical stages of AD[4]. Treatments designed to directly reduce accumulations of these abnormal proteins have so far not yielded beneficial results. Currently approved AD therapies are hence limited to symptomatic treatment which are of limited benefit. Developing an effective disease modifying therapy for AD therefore remains as one of the key unmet needs of modern medicine owing to the prevalence of this

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condition and the associated disability, societal costs and increased mortality. Dementia and AD have been the number one cause of death in the UK in 2021.[5]

Glucagon-like peptide-1 receptor agonists (GLP-1 RAs) may offer novel mechanisms to delay or even prevent neurotoxicity in individuals at-risk for AD. Studies in preclinical models have shown that administrating a GLP-1 RA is associated with a reduction in the effect of neurotoxic agents, decreases in the extent of AD protein and neuroinflammatory burden, and improved memory function. Pooled data from three double-blind randomised placebo-controlled studies in patients with type 2 diabetes mellitus (T2DM) demonstrated, in exploratory analyses, a reduction in the risk of developing dementia in patients treated with a GLP-1 RA, compared with placebo, over a 3.6-year follow-up period (Hazards Ratio (HR) 0.47, 95%CI 0.25-0.86). Additionally, analysis of the REWIND trial showed that dulaglutide, another GLP-1RA, reduced cognitive impairment in T2DM participants. Internal analysis by Novo Nordisk on the TRUVEN Medicare Supplemental and Coordination of Benefits Database found that more than 2 years of GLP-1 RA exposure resulted in an approximately 30% decrease in the risk of dementia, compared with no GLP-1 RA exposure (HR 0.69, 95%CI 0.57-0.85). However, the mechanisms for the potential disease modifying action of GLP-1 RAs with regard to dementia remains unclear.

In ISAP, we aim to explore possible mechanisms underlying the potential disease modifying effects of semaglutide, in a group of individuals with preclinical AD defined as being amyloid positive on PET and having no diagnosis of dementia.

2.2 Objectives of the Trial

2.2.1 *Primary objective*

The primary objective of the trial is to explore the disease modifying effects of oral semaglutide on tau accumulation rates (determined by PET) in preclinical AD over 52-week follow-up.

2.2.2 *Key Exploratory objectives*

To assess the effects of oral semaglutide on neuroinflammation (as determined by PET and blood assays), plasma blood biomarkers of AD pathology, cognitive changes, safety, neurodegeneration biomarkers (as determined by MRI and blood assays), health-related quality of life, diurnal activity variation (as determined by wrist-worn actigraphy) in preclinical AD over 52-week follow-up.

2.3 Trial Design

This is a multi-centre, randomised, double-blind, placebo-controlled, parallel-group, superiority trial of oral semaglutide in preclinical AD participants with follow-up over 52 weeks.

2.4 Eligibility

2.4.1 *Trial participants*

Amyloid-positive healthy volunteers of both sexes aged 55 years and over with no or minimal Mild Cognitive Impairment (MCI), as determined by a Clinical Dementia Rating (CDR) scale score ≤0.5, who speak English fluently.

2.4.2 *Inclusion criteria*

- Participant is willing and able to give informed consent for participation in the trial.
- Male or female, aged 55 years or above.
- Amyloid-positivity as evidenced by PET.
- Fluent English speaker as assessed by the Investigator.
- In the Investigator's opinion, is able and willing to comply with all trial requirements.
- Willing to allow their General Practitioner (GP), if appropriate, to be notified of participation in the trial.
- Clinical Dementia Rating (CDR) ≤0.5.
- An informant that is available to the research team for the purposes of the CDR scoring.

2.4.3 *Exclusion criteria*

- Diagnosis of dementia.
- Treatment with a GLP-1 RA: current or in the past 6 months
- Women who are pregnant, breastfeeding or of childbearing potential (see Appendix D of Protocol for definition).
- People with type 1 diabetes mellitus, secondary diabetes, or maturity-onset diabetes of the young (MODY).
- People with T2DM who have pre-proliferative or proliferative diabetic retinopathy, or diabetic maculopathy.
- People with T2DM if the cap of 30% of participants with T2DM randomised has been met.
- Poorly controlled T2DM defined as HbA1c ≥10% (≥86 mmol/mol).
- Evidence of severe renal impairment or an estimated glomerular filtration rate (eGFR) derived from serum creatinine (using the simple CKD-EPI formula) of <30 mL/min/1.73 m^2.
- Evidence of hepatic cirrhosis as assessed by medical history.
- A psychiatric condition which in the opinion of the investigator may affect the safety of the participant or the outcomes of the study.
- Any contraindication for MRI or PET scans, including but not limited to: MR-incompatible pacemakers, pregnancy, aneurysm clip, implanted neural stimulator, implanted cardiac pacemaker or auto-defibrillator, cochlear implant, ocular foreign body, recent carotid stent, CSF shunt, other implanted medical device, e.g., Swan Ganz catheter, insulin pump, as assessed by a standard pre-MRI questionnaire.
- Participant with a life expectancy of less than 6 months.
- Currently enrolled in another investigational device or drug study, or less than 30 days between randomisation and ending another investigational device or drug study or receiving other investigational treatment(s). Patients participating in a purely observational trial will not be excluded.
- Presence or history of malignant neoplasm (other than basal or squamous cell skin cancer, in-situ carcinomas of the cervix, or in situ prostate cancer) within 5 years prior to the day of screening.

- Lack of access to a suitable digital technology to allow remote cognitive testing (PC or tablet connected to the internet).
- Significant eye or hearing impairment that in the opinion of the investigator may affect study procedures.
- People with the low-affinity binding variant of the rs6971 allele of the TSPO gene.
- Known or suspected hypersensitivity to trial product or related products.
- Poor venous access or other contraindications that would make blood sampling difficult.
- Participant that in the view of the investigator will experience significant distress in the event of a positive amyloid status disclosure. Such individuals will not undergo amyloid screening.
- Diabetic individuals treated with sulphonylureas or insulin where dose adjustment as described in protocol is not possible for whatever reason.
- Individuals with significant radiation exposure in the past year for whom in the opinion of the investigator the additional trial-related exposure will result in an unacceptable risk.

2.5 Randomisation

At their Randomisation Visit (Visit 2), study participants who fulfil all the inclusion criteria (see 2.4.2) and violate none of the exclusion criteria (see 2.4.3) will be assigned a unique randomisation number that will allow subsequent identification of their randomised treatment group allocation.

Randomisation numbers will be allocated to eligible participants by the ISAP Electronic Data Capture (EDC) platform to assign semaglutide or matching placebo in an overall 1:1 allocation ratio. This assignment will be performed using a computerised procedure with minimisation (adaptive stratified sampling) to maintain balance between treatment groups based on 3 factors: T2DM (Yes/No), MCI (defined as CDR score 0.5) (Yes/No) and Site to maintain balance between treatment groups. No participant replacement will be allowed.

2.6 Sample Size

As a formal sample size estimation is infeasible in the absence of informative prior studies, the number of participants to be studied reflects the available funding. Table 1 shows twelve estimates of power for three possible tau PET change effect sizes and two alpha (Type 1 error) values. These calculations are based on the assumptions that there will be 10% of the total participants recruited (i.e. 39 of the estimated 390 participants recruited) excluded for reasons not related to use of TSPO ligand, followed by a further 10% of the remaining (i.e. 35 of 351) excluded due to having a genetic variant that precludes use of TSPO ligand. Additionally, we assume that 3.6 individuals will be needed to be scanned to identify one amyloid positive individual and that there will be 14% potential lost to follow-up.

Total number scanned for amyloid = 316 **Number randomised = 88** **Number of completers = 75 (=88*0.86)**	**Power levels assuming tau PET mean annual change (SD) of 0.05 (0.04)***	**Power levels assuming tau PET mean annual change (SD) of 2.01 (2.97)†**

One-year change from baseline in mean tau accumulation with semaglutide	Alpha 0.05	Alpha 0.10	Alpha 0.05	Alpha 0.10
20% lower compared with placebo	19.7%	29.9%	9.2%	16.0%
30% lower compared with placebo	38.1%	50.8%	14.6%	23.4%
40% lower compared with placebo	59.8%	71.6%	22.3%	33.0%

[*] *[6]Hanseeuw, BJ et al. 2019 PMID: 31157827;* [†] *[7]Whittington A, Gunn R. 2021 PMID: 33517326*

Table 1: Power calculations for a number of scenarios

2.7 Treatments

The trial is randomised with 2 arms with equal allocation of eligible participants in a 1:1 ratio to treatment or placebo.

Randomised participants will initiate treatment with 3 mg oral semaglutide/placebo once daily and follow a 4-week dose escalation regimen until reaching the treatment dose of 14 mg oral semaglutide/placebo once daily. Participants should remain on the 14 mg dose level until the end of treatment visits, but down titration and treatment restarts will be permitted where appropriate.

2.8 Blinding of Investigational Medicinal Product (IMP)

The placebo tablets will be identical in visual appearance to the IMP semaglutide tablet. Furthermore, the appearance of the semaglutide tablets will be the same irrespective of their dose.

2.9 Endpoints

2.9.1 *Primary outcome measure*

The primary endpoint is the annualized change in cortical PET tau standardised uptake value ratio (SUVR), derived from the difference in PET tau SUVR taken at the baseline and 52-week visits compared between treatment groups.

2.9.2 *Exploratory outcome measures*

- Annualised change in translocator protein (TSPO) PET SUVR over 52 weeks from baseline.
- Repeatedly measured plasma glial fibrillary acidic protein (GFAP) protein levels at screening, baseline, weeks 4, 8, 26, 39 and 52.
- Repeatedly measured plasma AD biomarkers (p-tau181, Aβ42/40 ratio) at screening, baseline, weeks 4, 8, 26, 39 and 52.
- Repeatedly measured pen and paper cognitive test (Addenbrooke's Cognitive Assessment (ACE-III)) scores at baseline, weeks 26 and 52.
- Repeatedly measured computerised in-clinic cognitive battery (CANTAB) at baseline, weeks 26 and 52.
- Repeatedly measured remote cognitive battery (Cognitron)[1] at baseline, weeks 26 and 52.
- Presence of Adverse Events (AEs), Serious Adverse Events (SAEs), Adverse Reactions (ARs), Serious Adverse Reactions (SARs) and Suspected Unexpected Serious Adverse Reactions (SUSARs) collected at baseline, weeks 4, 8, 26, 39, 52 and follow-up call.
- Annualized change in MRI-based neurodegeneration biomarkers (hippocampal volume) over 52 weeks from baseline.
- Repeatedly measured plasma neurofilament light (NFL) at screening, baseline, weeks 4, 8, 26, 39 and 52.
- Change in depression (CES-D) and anxiety (HAI) scores over 52 weeks from baseline
- Levels of distress at AD risk disclosure at weeks 26 and 52 (Impact of Genetic Testing for Alzheimer's disease scale)
- Change in quality of life as measured by EQ-5D-5L over 52 weeks from baseline.
- Change in level and pattern of activity and circadian rhythms as measured using wrist-worn actigraphy[1] (functional data) at baseline and 52 weeks.

Exact details on the list of data to be collected and the scheduling of procedures to be performed over the study period can be found in Appendix B of the ISAP Trial Protocol.

3.0 Analysis Populations

3.1 Target Population

The *target population*, to which inferences from the end of this trial are intended to generalise, is the population with pre-clinical AD participants aged 55 years and over.

3.2 Trial Population

The trial population, from which the study sample is drawn, is further defined to be participants aged 55 years or older in whom a positive screening based on clinical information for

[1] Actigraphy and remote cognitive testing will be completed in the week before randomization (baseline) and week before the final visit (52-week).

potentially higher risk of amyloid positivity and subsequent positive baseline amyloid beta PET imaging scan and meeting other defined trial eligibility criteria.

3.3 Trial Sample

The achieved trial sample comprises those participants who consent to participate and are actually randomised into this trial. These participants, whether treated or not, comprise the Randomised Set (RS). The Treated Set (TS) will include all subjects from the RS who were documented to have received at least one dose of study drug or placebo. This TS is the (modified) Intention To Treat (mITT) population.

The per-protocol set (PPS) includes all patients from the TS who provide evaluable data at baseline and on-treatment for the primary endpoint and are not affected by protocol violations (including non-compliance – evidence for less than 80% of all doses being taken) relevant to the statistical evaluation of the primary endpoint.

4.0 General Considerations

4.1 Timing of Analyses

The statistical analyses will be performed by the MRC Biostatistics Unit (MRC BSU), University of Cambridge and validated by an independent statistician. The main analyses will be on the securely, transferred, finalised and blinded data from the locked database, having been documented as meeting the cleaning and approval requirements of the Diabetes Trial Unit (DTU), University of Oxford Standard Operating Procedures (SOPs) and after the finalisation and approval of this SAP document.

No interim analyses are planned.

4.2 Stopping Rules

The trial may be prematurely discontinued by the Sponsor, Chief Investigator (CI) or Regulatory Authority on the basis of new safety information or for other reasons given by the Data Monitoring or Trial Steering Committee (DMC or TSC), the Regulatory Authority or Ethics Committee concerned. If the trial is prematurely discontinued, active study participants will be notified, and no further participant data collected. The Competent Authority and Research Ethics Committee (REC) will be informed within 15 days of the early termination of the trial.

The DMC will review the accumulating safety data every 6 months, or as deemed necessary. Interim safety analyses conducted by the DMC will be performed by the DTU Independent Statistician under the aegis of the DMC statistician and are detailed in a separate DMC SAP. There are no formal stopping rules. DMC guidance will be based on a per-protocol approach analysis, with an intention-to-treat approach used for sensitivity analyses. A statistical guideline of significance level for all-cause mortality at $p<0.01$ will be considered as evidence of recommending early stopping for safety.

4.3 Baseline Stratifiers, Baseline Outcomes and Subgroup Analyses

In the primary analyses, to assess whether there is a meaningful difference between the two arms in the annualized change in cortical PET tau SUVR, adjustments will be made for baseline variables used to minimise over in treatment allocation (i.e. T2DM status, MCI status and Site).

The corresponding baseline measure for a continuous outcome is often predictive of the outcome (and change in outcome) at follow-up, whereas standard errors of statistics derived from binary outcomes vary little with the prevalence to offer gains in precision from adjustment of baseline but expend degrees of freedom. Therefore, the corresponding baseline outcome will be an additional covariate when modelling continuous outcomes.

No subgroup analyses are planned.

4.4 Level of Significance

The primary hypothesis will be assessed using a two-tailed test at the 5%-level of significance. Specific *a priori* non-hierarchical secondary hypotheses will be evaluated using a two-sided 2.5%-level of significance. Other tests will be considered exploratory. However, 95% confidence intervals will be constructed around treatment effects. No adjustments will be made for multiple testing.

5.0 Descriptive Analysis

5.1 Recruitment and Follow-up Patterns

The flow of participants through the trial from enrolment to analysis will be in accordance with CONSORT guidelines[2] and will be similar to the flow diagram in Figure 1 which provides information about how the trial was conducted; reporting enrolment, allocation, follow-up and analysis of patients involved in the trial. This will include for each group, the number of participants randomised, the intention to treat (mITT) population, the numbers followed-up to be in the analysis of the primary outcome as well as the numbers and reasons missing data (e.g. withdrawn from treatment, lost to follow-up, died during the study) after randomisation.

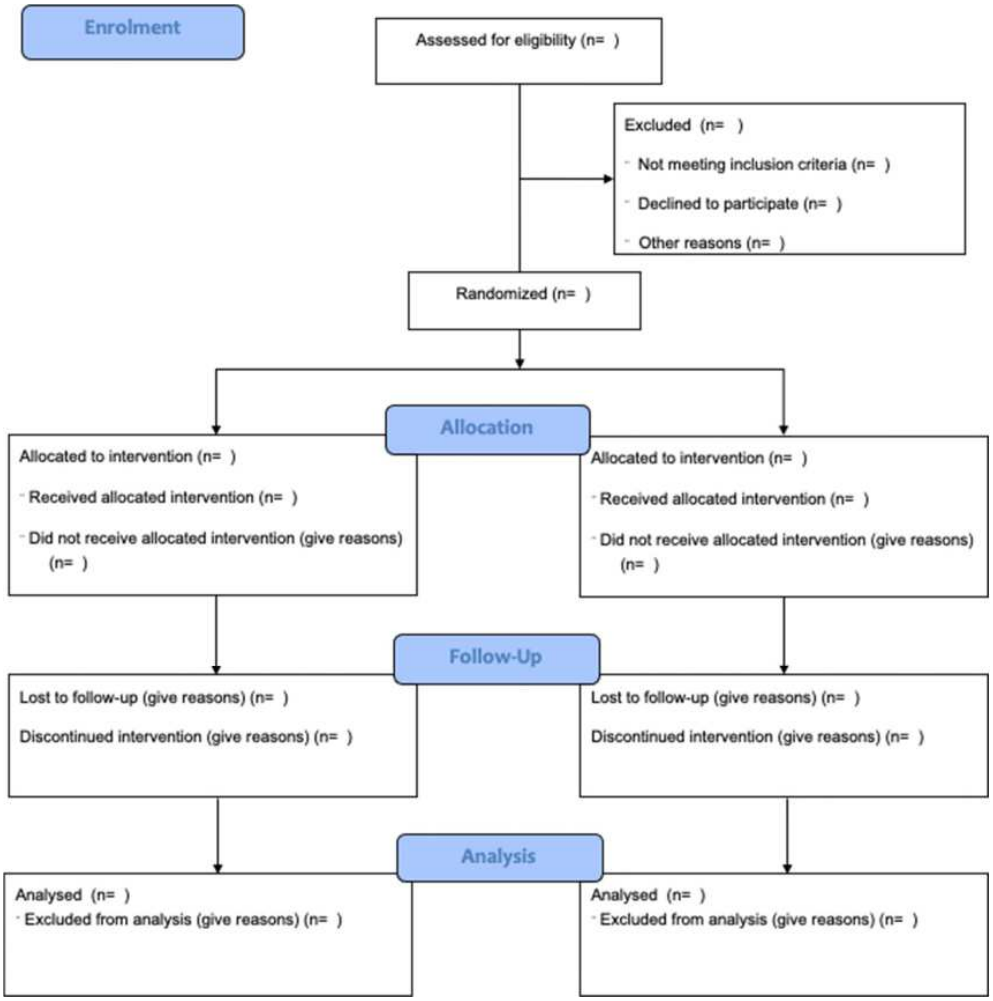

Figure 1: Consort 2010 flow diagram example

5.2 Baseline Characteristics

Baseline characteristics (including medical history and concomitant medications) of participants will be reported by treatment arm. No hypothesis testing will be carried out as any significant differences found are chance-generated (or failure of the implementation of the randomisation) and not for hypothesised reasons.

Continuous variables will be summarised using means and standard deviations (SDs) if (approximately) symmetrically distributed or after appropriately being transformed (e.g. logarithmic) or medians and interquartile ranges (IQRs) if skewed. Categorical variables will be presented as numbers and percentages.

5.3 Outcomes

Descriptive summaries of primary and key exploratory outcomes will be reported based on data from baseline and 52-week follow-up visits and presented in a similar manner as in the descriptive analyses of baseline characteristics.

Koychev I, *et al. BMJ Open* 2024; 14:e081401. doi: 10.1136/bmjopen-2023-081401

5.4 Aherence to Treatment and Lost to Follow-up and Pattern of Missingness

The number and proportion of participants, overall and by trial arm, who fail to adhere to treatment over the 52 weeks of the study will be assessed.

The numbers and proportions of participants who are lost to follow-up at each follow-up visit will be summarised in each trial arm. In addition, the pattern/extent of missing data will be quantified using possibly a graphical representation.

6.0 Comparative Analyses

6.1 Analysis of Primary Outcome

For the primary endpoint of annualised change in PET tau standardised uptake value ratio (SUVR), a modified intention to treat (mITT) superiority analysis will be the main analysis for comparing those randomised to the semaglutide treatment arm with those randomised to the placebo arm with respect to their mean annualised change in PET tau SUVR from baseline to 52 weeks in the TS population. An analysis of covariance (ANCOVA)/linear regression, where annualised change in PET tau SUVR is regressed on trial arm after adjusting for baseline PET tau SUVR and the variables used in minimisation (i.e. T2DM status, MCI status and Study Site), will be used to test the primary hypothesis of a treatment effect at the 5%-level of significance. The analysis will be repeated for the completers/complete cases (i.e. those who have both baseline and 52-week PET tau SUVRs).

In addition, a per protocol analysis of compliers will be performed in the PPS population.

Multiple imputation using chained equations[8], under a missing at random (MAR) assumption, will be used to impute missing outcome data. Sensitivity analysis[9] (e.g. tipping-point analysis) to potential informative dropout and low compliance will be considered. Model assumptions (e.g. assumptions of normality and constant variance) will be examined using residual and other diagnostic plots. Estimates of the treatment effect with corresponding standard error (SE) and 95% confidence interval will be reported.

6.2 Analysis of Secondary and Exploratory Outcomes

For continuous key exploratory outcomes measured at baseline and 52 weeks, similar approach to that for the primary analysis will be adopted. That is, an appropriate change measure will be derived and a modified ITT analysis of covariance/linear regression will be used to model the derived change on trial arm, its baseline value and the variables used in minimisation. Both completer and per protocol analyses will be performed. Multiple imputation using chained equations will be used to impute missing data.

For outcomes that are to be measured on more than 2 occasions, methods that take account of the longitudinal nature of the data will be used. Specifically, we will consider using mixed effects (ME) models, which are valid under the MAR assumption as they are likelihood-based. Estimates of the treatment effect with corresponding standard error (SE) and 95% confidence interval will be reported.

Koychev I, *et al. BMJ Open* 2024; 14:e081401. doi: 10.1136/bmjopen-2023-081401

6.3 Analysis of Safety Data

Safety data (e.g. adverse events (AEs) and serious adverse events (SAEs)) will be compared between the two trial arms either by comparing the mean rate of events or the proportion of participants experiencing an event in each arms over the follow-up period (including the follow-up call). The estimated risk ratios or absolute risk differences, whichever more appropriate, will be reported with confidence intervals.

7.0 Software

7.1 Data Management

The EDC system used for the trial will be OpenClinica version 4.0. It has been validated by the DTU in accordance with IT005 "Validation of Computerised Systems" as a Good Clinical Practice (GCP) compliant system and is currently operating in a validated state.

The full specification of the database is documented in the Data Specification (DAS), which will be updated to reflect any changes made throughout the study.

Once 20% of participants have completed the trial, a blinded dataset will be made available to the University of Cambridge MRC Biostatistics Unit (MRC BSU) via the DTU secure server to facilitate the construction and testing of analytic programs/analysis scripts. The full dataset will be transferred securely after database lock. Any data quality or management issues that arise after transfer will be resolved between the DTU and the MRC BSU.

7.2 Statistical Analysis

All statistical analyses will be carried out using the R statistical software environment[10] and/or STATA software, version 15[11] or higher. The analyses will be conducted by the MRC BSU. Analyses will be performed blinded to the disclosure of trial arms. An independent statistician who has access to the analysis plan and the full cleaned and quality assured dataset will repeat the primary analysis to ensure reproducibility. Any discrepancies (beyond those due to the effect of random number generation from statistical methods such as multiple imputation) between the findings from the independent statistician and the MRC BSU will be resolved by:

1. Assessing the consistency of their primary analysis plans with those set out in the SAP;
2. Comparing their primary analysis scripts; and
3. Referring to a third party if resolution cannot be achieved between the independent statistician and the MRC BSU.

8.0 References

1. International Conference on Harmonization (ICH). Statistical Principles for Clinical Trials E9.1998. Available at www.ich.org.

2. Schulz KF, Altman DG, Moher D; CONSORT Group. CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials. BMJ 2010; 340:c332.
3. Jack CR, Knopman DS, Jagust WJ, Petersen RC, Weiner MW, Aisen PS, Shaw LM, Vemuri P, Wiste HJ, Weigand SD, Lesnick TG. Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers. The lancet neurology. 2013;12(2):207-16.
4. Jack CR, Bennett DA, Blennow K, Carrillo MC, Feldman HH, Frisoni GB, Hampel H, Jagust WJ, Johnson KA, Knopman DS, Petersen RC. A/T/N: An unbiased descriptive classification scheme for Alzheimer disease biomarkers. Neurology. 2016;87(5):539-47.
5. ONS https://www.ons.gov.uk/peoplepopulationandcommunity/healthandsocialcare/causesofdeath/datasets/leadingcausesofdeathuk
6. Hanseeuw BJ, Betensky RA, Jacobs HIL, et al. Association of Amyloid and Tau With Cognition in Preclinical Alzheimer Disease: A Longitudinal Study. JAMA Neurol. 2019; 76(8):915–924. doi:10.1001/jamaneurol.2019.1424
7. Whittington, Alex, and Roger Gunn. TauIQ-A canonical image based algorithm to quantify tau PET scans. Journal of Nuclear Medicine. 2021.
8. White IR, Royston P, Wood AM. Multiple imputation using chained equations: issues and guidance for practice. Statistics in medicine. 2011; 30(4):377-99.
9. White IR, Horton NJ, Carpenter J, Pocock SJ. Strategy for intention to treat analysis in randomised trials with missing outcome data. BMJ 2011; 342:d40
10. R Development Core Team (2020). R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing. ISBN 3-900051-07-0, http://www.R-project.org.
11. StataCorp. 2017. *Stata Statistical Software: Release 15.* College Station, TX: StataCorp LLC)

9.0 Approval

Date:

Current Version:

Version and Date of SAP Amendments:

Signatures

Trial Role	Name	Signature	Date
Investigator Statistician	Dr Brian Tom	B Tom 23/10/2023 09:40:46	23/10/2023
Chief Investigator	Dr Ivan Koychev	I Koychev 23/10/2023 10:27:42 Ivan Koychev	23/10/2023
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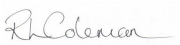
	Professor Rury Holman	R Holman 23/10/2023 09:58:41 Rury Holman	23/10/2023
DMC Chair	Professor Cornelia van Duijn	C V Duijn 23/10/2023 19:36:10 Cornelia van Duijn	23/10/2023
DTU Statistician	Dr Ruth Coleman	R Coleman 23/10/2023 09:24:35 	23/10/2023

10.0 Appendix

10.0 Table of Contents for Output

10.1 Tables

1. Site recruitment by calendar month

Month	Site 1	Site 2	Site 3	Site 4	Site 5	Overall
1st Month						
2nd Month						
3rd Month						
…						
Total						

2. Randomisation by arm

	Arm A N (% total)	Arm B N (% total)	Total
T2DM No Yes			
MCI No Yes			
Site Site 1 Site 2 Site 3 Site 4 Site 5			
Overall			

3. Adherence/Compliance by arm over 52-week follow-up – cumulative by week

Week	Total no. patients	Arm A N (% total)	Arm B N (% total)	% patients that comply from total
Week 1				
Week 2				
Week 3				
Week 4				
Week 5				
Week 6				
…				
Week 51				
Week 52				
Total				

Koychev I, *et al. BMJ Open* 2024; 14:e081401. doi: 10.1136/bmjopen-2023-081401

4. Number of subjects attending follow-up assessment visit by arm

Visit assessment	**Arm A** N (% - no. subject at visit/total no. of subjects randomised to arm)	**Arm B** N (% - no. subject at visit/total no. of subjects randomised to arm)	**Total no. of subjects attending visit**
4 weeks			
8 weeks			
26 weeks			
39 weeks			
52 weeks			

5. Baseline information by arm

Variables	Arm A	Arm B
Age (yrs)	Mean (SD)	Mean (SD)
Gender		
Female	% (n)	% (n)
Male	% (n)	% (n)
Ethnicity		
White	% (n)	% (n)
Non-white	% (n)	% (n)
Medical history	% (n)	% (n)
Concomitant medication	% (n)	% (n)
Blood pressure	Mean (SD)	Mean (SD)
Heart rate	Mean (SD)	Mean (SD)
Blood markers	Mean (SD)	Mean (SD)
Cognitive test scores	Mean (SD)	Mean (SD)
Depression scores	Mean (SD)	Mean (SD)
Quality of life	Mean (SD)	Mean (SD)
Anxiety Scores	Mean (SD)	Mean (SD)
PET tau SUVR	Mean (SD)	Mean (SD)
PET TSPO SUVR	Mean (SD)	Mean (SD)
etc		

6. Annualized change in outcome by arm

Variables	Arm A	Arm B
PET tau SUVR	Mean (SD)	Mean (SD)
PET TSPO SUVR	Mean (SD)	Mean (SD)
Hippocampal Volume	Mean (SD)	Mean (SD)
CES-D	Mean (SD)	Mean (SD)
HAI	Mean (SD)	Mean (SD)
EQ-5D-5L	Mean (SD)	Mean (SD)
etc		

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Signature Fingerprint	79afd372-ff5b-4497-a76f-09e85da24b4c

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