RANDOMIZED CONTROLLED PILOT TRIAL OF DAPAGLIFLOZIN IN ALZHEIMER'S DISEASE

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Sponsor : KU ADC

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PRÉCIS
Study Title: Randomized Controlled Pilot Trial of Dapagliflozin in Alzheimer's Disease

Objectives

1. Examine the effect of 12 weeks of 10mg dapagliflozin once daily on cerebral n-acetyl aspartate (NAA) levels using magnetic resonance spectroscopy (MRS).
2. Explore the effect of dapagliflozin on
    a. Systemic NAA levels in blood and urine.
    b. Cerebral metabolism (fluorodeoxyglucose [FDG] PET).
    c. Systemic metabolic biomarkers that indicate and quantify secondary metabolic effects.
    d. Cognition
3. Examine the safety and tolerability of dapagliflozin in individuals with AD including Mild Cognitive Impairment due to AD).

Design and Outcomes

This is a double-blind, randomized, placebo-controlled, parallel group, 12-week study performed at a single site (KU ADC) to investigate the effect of dapagliflozin in participants with probable AD or MCI due to AD. A total of 48 participants will be enrolled with 2:1 randomization to 10mg dapagliflozin once daily (n=32) for 12 weeks vs matching placebo (n=16).

The primary objective of the study is to assess the effect of 12 weeks of 10mg dapagliflozin once daily on cerebral NAA (a proxy measure of mitochondrial mass) in participants with AD or MCI due to AD.

Exploratory outcomes will be assessed with phlebotomy, urine collection, MRI/MRS, FDG-PET, cognitive testing, DEXA scanning, and indirect calorimetry at baseline and 12 weeks. These procedures will allow us to assess:

- N Acetyl-Aspartate (NAA)
    - Cerebral NAA (as measured by MRS)
    - Systemic NAA levels (in blood and urine)
- Cerebral metabolism (by FDG PET)
- Systemic metabolic effects
    - Lipids (total cholesterol, LDL, HDL)
    - Plasma beta-hydroxybutyrate
    - Insulin resistance (Hemoglobin A1c, glucose and insulin during tolerance testing)
    - Catabolic/Anabolic state [activated AKT and MTOR]
    - Mitochondrial function measures [platelet cytochrome oxidase and citrate synthase]
    - Inflammatory mechanisms [MCP-1, eotaxin, TNF alpha, CRP]
    - Body composition (DEXA scanning for fat and lean mass)
    - Resting metabolic rate (indirect calorimetry).
- Cognitive effects will be assessed at baseline and week 12 using the Alzheimer's Disease Assessment Scale-Cognitive Subscale 14 (ADAS-Cog14) and individual tests of Logical Memory I and II, Trailmaking A and B, and Stroop Word Color Test.
- 12 participants will be enrolled in an optional MRI/MRS sub-study with repeat MRI/MRS prior to randomization to assess scan-rescan reliability of the NAA measure.

Safety and tolerability of dapagliflozin (10mg daily) will be monitored throughout the study and formally at every study visit by assessing for any adverse events (AE's), measuring vital signs and body weight, and performing safety labs (including a comprehensive metabolic panel [CMP] and complete blood count [CBC] with differential). In addition, we will conduct physical and neurological examinations at screening and at end of treatment (EOT). We will examine the incidence and severity of AEs and the rate of discontinuations due to AEs.

Interventions and Duration
Participants will be randomized (2:1 ratio) to either dapagliflozin 10mg a day vs matching placebo for a treatment duration of 12 weeks. The total study duration for participants will be will be up to 18 weeks. In-person screening and baseline assessments will occur within a 4-week time-window prior to randomization. Study drug (or placebo) will begin at randomization (time 0) and in person assessments will occur at weeks 1, 4, 8, and 12 weeks (when study drug is stopped) and an end of study visit at 14 weeks.

Sample Size and Population
A total of 48 participants with probable AD or MCI due to AD will be enrolled in the study with 2:1 randomization to 10mg dapagliflozin (n=32) vs matching placebo (n=16) for 12 weeks. To minimize potential risks that may associate with weight loss we will only enroll participants whose BMI is greater than or equal to 23.0.

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1 STUDY OBJECTIVES

1.1 Primary Objective

The primary objective of the study is to assess the effect of 12 weeks of 10mg dapagliflozin once daily on cerebral NAA (a proxy measure of mitochondrial mass) in participants with AD and MCI due to AD.

1.2 Exploratory Objectives

The exploratory objectives are to evaluate the effect of 12 weeks of 10mg dapagliflozin once daily on systemic measures of NAA, metabolic biomarkers in the brain and periphery, and cognition in participants with AD and MCI due to AD.

Specifically we will test the effects of dapagliflozin on

a. Systemic NAA levels in blood and urine.
b. Cerebral metabolism (FDG-PET)
c. Systemic metabolic biomarkers that indicate and quantify secondary metabolic effects (lipids, ketones, insulin resistance, anabolic/catabolic response measures, mitochondrial function, inflammatory markers, body composition, and resting metabolic rate).
d. Cognition

1.3 Safety Objective

Examine the safety and tolerability of 12 weeks of 10mg dapagliflozin once daily in participants with AD and MCI due to AD.

2 BACKGROUND AND RATIONALE

2.1 Background on Condition, Disease, or Other Primary Study Focus

2.1.1 Alzheimer's Disease

Increased life expectancy in the US has fueled an unprecedented growth in the prevalence of AD, with over 5 million Americans affected and nearly 15 million projected to have the disease by 2050. AD is now America's most expensive disease (~$259 billion), more costly than cancer and heart disease.[1] Treatment options are limited. FDA-approved therapies are available but have modest and limited symptomatic benefits.

Drug development efforts are largely focused on disease-modifying approaches, with the majority of these efforts aimed at interfering with a hallmark neuropathological change of AD, amyloid plaques. These efforts to target and disrupt amyloid have not yet produced evidence of clinical benefit. These failures have led, in part, to a diversifying portfolio of approaches that includes targeting neurofibrillary tangles, inflammation, synaptic activity, vascular function, and mitochondrial and metabolic function.

2.1.2 AD and Energy Metabolism

Abundant evidence demonstrates aberrant energy metabolism occurs in AD.[2-26] Energy metabolism research constitutes a major component of our federally supported University of Kansas Alzheimer's Disease Center (KU ADC) (P30AG035982), with investigators focused on defining the nature, causes, and consequence of altered energy metabolism in AD. The KU ADC supports an Alzheimer's Treatment Program (ATP) that conceptualizes, translates, and tests approaches that target energy metabolism for AD treatment and prevention.[27-33]

Fluorodeoxyglucose positron emission tomography (FDG PET) and mitochondria studies demonstrate perturbed energy metabolism in AD.[34] Brain glucose utilization declines with advancing age[35-38] and this decline extends in magnitude and distribution in those with AD.[20, 21, 39-43] Direct measures of metabolism in brain tissue homogenates from individuals with AD show less glucose consumption than control homogenates.[44] Electron microscopy reveals perturbed mitochondrial structures[6, 45-47] and increased mitochondrial fission.[48-50] Several mitochondria-localized enzymes, including pyruvate dehydrogenase complex,[51] α-ketoglutarate dehydrogenase complex,[14] and cytochrome c oxidase (COX) demonstrate reduced activities.[12, 13, 52-60] The AD COX defect, interestingly, also exists outside the brain[12, 53-55, 60-62] and at least partly depends on mitochondrial DNA (mtDNA).[34, 63, 64] Additionally, asymptomatic individuals at higher risk of AD (both due to the presence of an APOE4 allele or an AD-effected mother) also show AD-like glucose utilization patterns on FDG-PET.[23, 24, 65] Although neuron loss and synaptic degradation could contribute to reduced brain glucose utilization in AD, the presence of metabolic changes in preclinical and asymptomatic at-risk individuals suggests primary bioenergetic brain changes play an important role in the AD pathophysiological process.

Targeting Energy Metabolism. We were among the first to propose using energy metabolism manipulation to treat AD.[66-68] Currently, the KU ADC supports the

development of new energy metabolism interventions. Our bench-to-bedside programs focus on the molecular and clinical impact of exercise and diet,[30, 32, 69-74] the causes and consequences of altered insulin signaling,[75-77] developing new energy metabolism biomarkers,[78] and developing rational new pharmacologic approaches.[27-29, 33, 79, 80]

To date, clinical trials that intentionally target energy metabolism in AD include coenzyme Q and idebenone (to enhance electron transfer through the electron transport chain),[81-84] rosiglitazone (promote mitochondrial biogenesis),[85, 86] and latrepirdine (claimed to stabilize mitochondrial membranes).[87, 88] These interventions showed little to no benefit in clinical trials, which could reflect no physiologic engagement of their intended targets (coenzyme Q, idebenone), concentrations insufficient to alter targets (rosiglitazone), or a profoundly limited understanding of their mitochondrial effects (latrepirdine). This experience underscores why rational bioenergetic medicine design must consider existing mechanistic data and pursue new mechanistic insight.[28]

2.1.3 Dapagliflozin and Metabolic Effects

Dapagliflozin is a sodium-glucose transporter 2 (SGLT2) inhibitor used to treat Type 2 diabetes. The SGLT2 inhibition results in a loss of glucose and its associated energy via the urine. This has prominent systemic metabolic effects in Type 2 diabetes, resulting in reduced HbA1c and body weight. In a 2-year study, dapagliflozin reduced HbA1c by 0.3%, weight by 4.5kg and fat mass by 2.8kg (Bolinder et al 2013). The reduction in body weight is faster over the first few weeks, followed by a more gradual decline that plateaus between 24 and 50 weeks of therapy (Bolinder et al 2012). Insulin sensitivity increased, as measured by a 20% increase in glucose disappearance rate, after 12 weeks of 5mg dapagliflozin (Mudaliar S et al 2014), a finding that was also repeated in a 2 week study (Merovci A et al 2014). Dapagliflozin also results in increased hepatic glucose production, increased fasting glucagon levels, and decreased fasting insulin levels (Merovci A et al 2014). An increased glucagon/insulin ratio is expected to reduce hepatic de novo lipogenesis and in parallel increase hepatic fatty acid oxidation that results in reduced liver fat content.

Additionally, chronic dapagliflozin treatment led to robust increases in systemic measures of NAA, which is synthesized within mitochondria and considered a biomarker of mitochondrial mass (confidential Metabolon data). Inducing mitochondrial biogenesis to mitigate or reverse failing AD bioenergetics is increasingly considered a rational AD therapeutic goal, and dapagliflozin represents a logical agent for achieving this goal.

2.2 Study Rationale

2.2.1 Rationale for Study

This study is an exploratory pilot study investigating safety, tolerability and metabolic effects of dapagliflozin in individuals with AD and MCI due to AD. Dapagliflozin has been shown to have effects on glucose, insulin and the production of ketone bodies. Additionally metabolomic data suggests potential mitochondrial effects. As AD is increasingly recognized as having metabolic manifestations, new therapeutic strategies are targeting metabolic processes. Therefore, this study assesses the effect of dapagliflozin 10mg daily for 12 weeks on cerebral and systemic NAA, secondary metabolic effects (glucose, insulin, ketones, lipids, mitochondrial function, inflammation), cognitive function, and safety and tolerability.

2.2.2 Rationale for Study Design

This is an exploratory pilot study to examine the effects of dapagliflozin in participants with AD and MCI due to AD to provide an estimate of its effect on cerebral NAA (a proxy measure of mitochondrial mass), explore other metabolic effects, and to collect information to further understand and establish its safety and tolerability in our target population.

Participants will be randomized to either 12 weeks of 10mg dapagliflozin once daily vs placebo to examine potential effects on cerebral and systemic NAA (reflective of mitochondrial mass or respiration-related carbon flux) and other potentially beneficial secondary metabolic effects. This study will be conducted in individuals with AD or MCI due to AD with a BMI greater than or equal to 23 to reduce the risk of potentially exacerbating weight loss in more frail individuals.

The study duration of 12 weeks was chosen based on prior studies of dapagliflozin that demonstrated metabolic effects are present before 12 weeks including effects on liver fat, body weight, and insulin sensitivity in those with diabetes. Moreover, 12 weeks is sufficient for expecting that putative effects on cerebral NAA will be present.

2.2.3 Dose Rationale

Dapagliflozin is FDA-approved as an adjunct to diet and exercise to improve glycemic control in patients with type 2 diabetes. The recommended dose is 10 mg orally once daily at any time of the day regardless of meals. There are no dose adjustments required or recommended for individuals with renal impairment, geriatric patients, or those without diabetes. Thus, the dose of dapagliflozin 10mg daily will be used in this study.

2.3 Benefit/Risk and Ethical Assessment

This study will provide important data on the potential role of dapagliflozin in treating individuals with AD or MCI due to AD. Additionally, it will provide data to better understand the role of therapeutically targeting metabolic processes as a treatment strategy for individuals with AD or MCI due to AD. Advancing these scientific themes benefits humankind though it may not provide clear clinical benefits to those enrolled.

There are, however, potential clinical benefits for those enrolled in this study, including potentially beneficial effects on glucose and insulin levels, blood pressure, and body weight.

Establishing the safety and tolerability of dapagliflozin in AD and MCI due to AD is a goal of this study. Dapagliflozin is associated with small increases in AEs including urinary tract and genital infections (dysuria, polyuria, vulvovaginitis, balanitis) and back pain which occurred more commonly in placebo-controlled studies in the dapagliflozin group versus placebo. Other potential AEs include orthostatic hypotension and volume depletion. The risk for hypoglycemia is low even in diabetics treated with mono therapy.

3 STUDY DESIGN

A double-blind randomized, placebo-controlled, parallel group, 12 week study to investigate the effect of 10mg daily dapagliflozin vs. placebo for 12 weeks in participants with probable AD or MCI due to ADwhose BMI is greater than or equal to 23.0.

3.1 Primary Objective

The primary objective of the study is to estimate the effect of 12 weeks of 10mg dapagliflozin once daily on cerebral NAA (a proxy measure of mitochondrial mass) in participants with AD or MCI due to AD.

3.2 Exploratory Objectives

The exploratory objectives are to evaluate the effect of 12 weeks of 10mg dapagliflozin once daily on systemic measures of NAA (a proxy measure of mitochondrial mass), metabolic biomarkers in the brain and periphery, and cognition in participants with AD and MCI due to AD. Specific exploratory outcomes are:

- Systemic NAA levels in blood and urine.
- Cerebral metabolism (FDG-PET)
- Systemic metabolic biomarkers that indicate and quantify secondary metabolic effects (lipids, ketones, insulin resistance, anabolic/catabolic response measures, mitochondrial function, inflammatory markers, body composition, and resting metabolic rate).
- Cognition

2. 12 participants will be enrolled in an optional MRI/MRS sub-study with repeat MRI/MRS prior to randomization to assess scan-rescan reliability of the NAA measure.

3.3 Safety Objective

Examine the safety and tolerability of 12 weeks of 10mg dapagliflozin once daily in participants with AD and MCI due to AD.

4 SELECTION AND ENROLLMENT OF PARTICIPANTS

4.1 Inclusion Criteria

For inclusion in the study, participants must fulfil the following criteria:

1. Provision of informed consent prior to any study specific procedures.
2. Have a diagnosis of probable AD, or MCI due to AD per McKhann et al. criteria
3. Have a body mass index (BMI) ≥23
4. Age 50-85
5. Have a reliable and competent study partner who is willing to accompany the participant to all study visits, monitor compliance of study medication administration, and observe/report any changes in the participant's health throughout the study duration
6. Are on stable doses of concurrent medications for at least 4 weeks prior to the screening visit
7. Speaks English as his/her primary language.

8. Females of child-bearing potential (i.e., pre-menopausal) must have a negative urine pregnancy test at the screening visit and must agree to use of contraception throughout the trial and for 30 days after the last dose of study medication. The approved methods of contraception are abstinence, the consistent use of an approved oral contraceptive (birth control pill or "the pill"), an intrauterine device (IUD), hormonal implants, contraceptive injection, double barrier method (diaphragm with spermicidal gel or condom with contraceptive foam).

4.2 Exclusion Criteria

Participants will not be enrolled if any of the following exclusion criteria are met:

1. Received an investigational product in another clinical study during the last 4 weeks prior to screening
2. Diagnosis of Type 1 diabetes
3. Diagnosis of Type 2 diabetes treated with insulin, sulfonylureas, glucagon like peptide1 receptor agonists (GLP-1), thiazolidinedione (TZD) or SGLT2 inhibitors (metformin monotherapy is allowed).
4. Estimated Glomerular Filtration Rate (eGFR; MDRD) <45 mL/min/1.73m2 at screening or unstable renal disease.
5. Any condition when MRI is contraindicated such as, but not limited to, having a pacemaker or claustrophobia.
6. Severe hepatic injury and/or significant abnormal liver function defined as aspartate aminotransferase (AST) >3x upper limit of normal (ULN) and/or alanine aminotransferase (ALT) >3x ULN. Total bilirubin >2.0 mg/dL (34.2 µmol/L)
7. Intolerance or allergy to dapaglifozin or any other SGLT2 inhibitor or any other substance in the tablets.
8. Dementia or cognitive impairment due to causes other than AD
9. History of recurrent urinary tract infection
10. Active mycotic genital infection
11. History of diabetic ketoacidosis
12. Potentially confounding, serious, or unstable medical conditions such as:
    a. cancer within the past 3 years (except basal cell, squamous cell, or localized prostate cancer)
    b. a recent cardiac event (i.e. heart attack, angioplasty, etc. within the 3 months prior to screening visit)
    c. other conditions that pose a potential safety risk or confounding factor in the investigator's opinion
13. Any abnormal physical examination assessment or vital sign assessment at the screening visit that is deemed to be clinically significant by the principal investigator.
14. Any abnormal clinical laboratory test result at the screening visit that is deemed to be clinically significant by the principal investigator.

4.3 Study Enrollment Procedures

4.3.1 Recruitment

Participants for this trial will be recruited through the KU ADC's Recruitment program. Methods include referrals from KU Memory Disorders Clinic, referrals from outside physicians, regular community outreach / education seminars about KU ADC currently enrolling trials, and posted IRB-approved advertisements on social or other traditional media.

Once an individual contacts the KU ADC indicating interest in the trial, a full intake of information for recruitment consideration is obtained through the KU ADC Recruitment and Eligibility program, with the potential participant's consent. During the initial pre-screening process, if it is determined that medical records are needed to assist in making an initial eligibility determination, the potential participant (or their legally authorized representative) is asked to complete a Release of Medical Information form and records from the potential participant's applicable physicians are requested and obtained. If the potential participant appears to be a good candidate for the trial, their recruitment intake information is provided to the study team for further review and discussion with the potential participant and his/her study partner.

4.3.2 Informed Consent, Screening, and Enrollment

The potential participant and his/her study partner are provided with the informed consent form (ICF) for their review prior to scheduling a study screening visit. Any initial questions they may have are addressed. It is explained to potential participants and their study partners that, although some eligibility criteria (such as age and medical conditions) can be determined ahead of time, many eligibility criteria cannot be determined until after all screening procedures take place.

It is also explained to the participant that, due to the nature of this trial enrolling individuals with known cognitive impairment, there may be a need for the participant's legally authorized representative to actually provide the consent for their participation using a surrogate consent form, with the participant providing his/her assent. The assessment and determination of whether the participant has sufficient capacity to provide his/her own consent is made during the consenting process at the first study visit by the delegated study team member. For this reason, it is established with the potential participant prior to the screening visit who has legal authority to serve as his/her representative per the state of Kansas, the state in which all study procedures take place. If any legal documents, such as durable power of attorney, are available, these will be requested to be brought with the participant to the first study visit.

Following the screening visit, the results of all eligibility testing are provided to the principal investigator for his review. The investigator documents review and whether the participant met all inclusion criteria and did not meet any exclusion criteria. A clear statement of the investigator's determination for the participant's suitability to be enrolled in the trial is documented prior to any subsequent baseline procedures taking place.

Participants who fail to meet the eligibility criteria will be excluded. Participants who are enrolled, but subsequently found not to meet all the eligibility criteria will be withdrawn from the study.

A participant screening log will document all participants who sign informed consent for this trial and will list their final disposition, including whether they were determined to be ineligible/screen failed, were enrolled and completed the study, and whether they were enrolled but withdrew or their participation was stopped early. The reason for screen failure and for withdrawal/early termination will also be recorded.

Eligible participants will be randomized in a 2:1 ratio to either 10 mg dapaglifozin (n=32) or matching placebo (n=16). Randomization will be completed by an unblinded staff member using a randomization table to create the order of dispensing active vs. placebo. This unblinded staff member will use the table to create code numbers for the bottles of study medication and will be responsible for labeling the bottles in a manner to ensure blinding of all other study staff. A second unblinded staff member will assist in labeling of the bottles to provided second verification of correct labeling and will also serve as a back-up to provide randomization bottle numbers to blinded staff if needed.

When each participant has been determined to be eligible for the trial, the blinded staff member will submit a request to the unblinded staff member for randomization. The unblinded staff member will provide the blinded staff member with a document listing only the participant identification number and the bottle numbers to be dispensed.

4.3.3 Randomization

The Lead Statistician will generate a randomization schedule upon study approval using a statistical software package. The Lead Statistician will send the schedule directly to the site pharmacy for package labeling, and will be stored in an electronic, password-protected file to maintain appropriate levels of blinding for other members of the study team during the conduct of this trial. Randomization codes will be assigned to eligible, consented participants on a strictly sequentially basis.

5 STUDY INTERVENTIONS

5.1 Interventions, Administration, and Duration

Dapagliflozin 10mg po daily or placebo for 12 weeks. Study drug or placebo will be provided to the participants at baseline, week 4, and week 8. Pill compliance assessments will be completed at week 4, week 8, and week 12.

5.2 Handling of Study Drug

Identity of investigational product(s)

Investigational product	Dosage form and strength	Manufacturer
Dapagliflozin	10 mg, Green, plain, diamond shaped, film coated tablet (orally)	AstraZeneca
Matching placebo for dapagliflozin	Green, plain, diamond shaped, film coated tablet (orally). Does not contain active ingredient	AstraZeneca

Primary packaging of the Investigational Medicinal product (IMP) will be carried out by AstraZeneca or their designee in accordance with Good Manufacturing Practice (GMP). Unlabeled bottles (identical) containing 35 tablets of each IMP will be provided by AstraZeneca.

The tablets contain lactose, which may cause discomfort in lactose-intolerant individuals. Label, storage and distribution are a sponsor's responsibility.

Preparation and labelling of Investigational Medicinal Product
The sponsor or their designee (will issue labels, label the bottles and release package study drug/IMP) will label the IMP in accordance with Good Manufacturing Practice (GMP) and local regulatory guidelines for labelling. The labels will fulfill GMP Annex 13 requirements for labelling.

Drug accountability logs will be maintained throughout the study and will document study drug dispensation, return, and destruction. Unused study drugs will be destroyed locally per the site's Standard Operating Procedure.

Storage
All study drugs should be kept in a secure place under appropriate storage conditions. The IMP label on the bottle specifies the appropriate storage.

The study treatment is double blind for participants, investigators and study personnel (other than the Lead statistician) and will remain so throughout the entire study unless the blind must be broken for safety concerns. To ensure blinding of the treatments, the active and dose-matched placebo tablets will be identical in appearance and in identical packaging and labelling.

5.3 Concomitant Interventions

All current medications taken by the participant will be recorded at the first study visit. All changes in medications during the study will also be recorded.

- The participants should not use or have not used dapagliflozin or any other SGLT2 inhibitors within 4 weeks of visit 1
- The participants should not use or have not used insulin, GLP-1 or GLP-1 analogues, and sulfonylureas within 4 weeks of visit 1
- Oral antidiabetic therapies other than the pre-specified use of metformin and TZDs should not be used or have not been used within 4 weeks of visit 1
- Other medication other than that described above, which is considered necessary for the participant's safety and well-being, may be given at the discretion of the Investigator and recorded in the appropriate sections of the Case Report Form.

5.4 Adherence Assessment

Study medication adherence will be monitored throughout the trial. The study partner will oversee administration of the study mediation in the participant's home. The participant and study partner will be instructed to return all used and unused study medication containers to the study staff at specified visits. At these visits, medication compliance will be measured by pill count and a percent compliance will be calculated based on the number of pills taken (not returned) divided by the number of pills expected to have been taken. Accountability for pills dropped/lost will be recorded and factored into the calculation.

If compliance is less than 90% or greater than 100%, participants and their study partners will be counseled on the importance of accurate compliance and methods to help improve compliance will be discussed. Only study drug compliance of less 80% compliance or greater than 110% will be considered a protocol deviation. Participants will not be removed from the study for non-compliance.

6 STUDY PROCEDURES

6.0 Schedule of Evaluations

Procedure	**Screening** (-30 to 0 days)	**Baseline** (-30 to 0 days)	**Week 1** (+/- 3 days)	**Week 4** (+/- 7 days)	**Week 8** (+/- 7 days)	**Week 12** (+/- 7 days)	**Week 14 End-of-Study** (+/- 7 days)
Visit Number	**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**	**Visit 5**	**Visit 6**	**Visit 7**
Informed Consent	X						
Medical History Review	X						
Adverse Event Review	X	X	X	X	X	X	X
Concurrent Medication Review	X	X	X	X	X	X	X
Vital Signs, including weight	X	X	X	X	X	X	X
Blood (8 ml) and Urine Collection for Safety Labs*	X	X	X	X	X	X	X
Neurological Exam	X					X	
Physical Exam	X	X	X	X	X	X	X
Urine Pregnancy Test, As Needed	X						
MMSE	X					X	
Review of Inclusion/Exclusion Criteria	X						
Blood Collection (56 ml) for Biomarker Outcome Measures**		X				X	
Blood Collection (5ml) for ApoE genotype		X					
Urine collection for NAA**		X				X	
Cognitive Testing***		X				X	
FDG PET		X				X	
MRI/MRS		X				X	
Resting Metabolic Rate (Indirect Calorimetry)		X				X	
Oral Glucose Tolerance Test, including blood draws at 7 timepoints for each test		X				X	
DEXA		X				X	
Dispense Study Medications		X		X	X		
Compliance Pill Count				X	X	X	

* Comprehensive Metabolic Panel and Complete Blood Count
** Outcome measures include Lipids, Ketones, HbA1c, AKT, mTOR, Platelet Cytochrome Oxidase and Citrate Synthase, MCP-1, Eotaxin, TNFalpha, CRP**, & NAA**
*** Cognitive testing: ADAS Cog-14, Logical Memory I and II, Stroop Color Word Test, Trailmaking A and B

6.1 Description of Evaluations

The study will consist of 7 study visits. The more intensive baseline and week 12 visits will require 3 to 5 trips to different locations. Primary study visits will be conducted at the Clinical and Translational Science Unit (CTSU), 2 MRI sessions will be conducted at the Hoglund Brain Imaging Center to acquire MRI/MRS data, and 2 FDG PET sessions will be conducted at the University of Kansas Hospital. The visits and detailed study procedures are outlined below.

6.2.1 Screening Period (Visit 1)

The initial screening visit will occur in the Clinical and Translational Science Unit (CTSU) as a single visit to complete the following procedures:

- Informed consent
- Medical history
- Review of concurrent medications
- Mini Mental Status Examination (MMSE) administered by a trained psychometrician
- Blood and urine collection for safety labs
- Vital signs
- Height measurement using a standard stadiometer
- Body weight measurement using a calibrated scale
- A physical and neurological examination.
- If the participant is a female of childbearing potential, a urine pregnancy test will be conducted.
- Adverse event (AE) review/check
- Review of inclusion / exclusion criteria

The screening period lasts up to 4 weeks and includes the time from signing of the consent form until enrollment. At or before Visit 1, the study details will be explained to the participant (or his or her legally authorized representative) and the study partner. Before any study procedures take place, full informed consent will be obtained and documented.

6.2.1.1 Description of Screening Procedures

Review of Medical History: A complete medical history will be obtained, including a history of cognitive impairment and diagnosis of Alzheimer's disease.

Review of Concurrent Medications: Ongoing concurrent medications taken by the participant (including prescription and over-the-counter/vitamins/herbal supplements) and any medications taken within 30 days prior to the screening visit (Visit 1) will be recorded.

Mini Mental Status Exam (MMSE): The MMSE will be completed at screening and Week 12 (Visit 6). The MMSE is a brief, 30-item questionnaire that is widely used to provide an estimate of the severity of cognitive impairment at any given point in time. Lower scores indicate greater impairment.

Vital Signs: Body temperature, sitting and standing blood pressure, respiratory rate, heart rate, and body weight will be measured at every study visit. At the screening visit, height will also be measured.

Physical and Neurological Examination: The study clinician will conduct a physical examination at screening and all visits, of the participant's skin, head, neck, nose, throat, eyes, chest, lungs, heart, abdomen, and muscles to check for signs of disease. During the neurological examination, the study clinician will check the participant's mental status, cranial nerve function, motor system, sensory system, reflexes, coordination, gait, posture, and stability. If any abnormal signs are detected in these examinations that the investigator determines to be clinically significant, the participant will not be eligible for the trial.

Clinical Safety Urine and Laboratory Tests: Approximately 8 ml of blood will be drawn at Screening Visit 1 and all subsequent visits to assess a comprehensive metabolic panel (CMP) and complete blood count with differential (CBC) in the fasting or non-fasting state. Fasting state will be recorded at time of collection. Baseline and Week 12 blood draws will be done in the fasting state at the same time as the collection of blood for the biomarker outcome assessments. A urine sample will also be collected for routine safety tests at all subsequent visits.

On screening labs, if eGFR is <45 mL/min/$1.73m2$, the participant will not be eligible to enroll in this trial. If AST is >3x upper limit of normal (ULN) and/or ALT is >3x ULNand/or total bilirubin is >2.0 mg/dL (34.2 µmol/L), the participant will not be eligible for this trial. If any other clinical laboratory findings from this screening visit are abnormal and determined to be clinically significant, the participant will not be eligible for this trial.

Urine Pregnancy Test: Female participants who are still of child-bearing potential must undergo a urine pregnancy test at the screening visit. Any participant with a positive pregnancy test will not be eligible for the trial.

Review of Inclusion/ Exclusion Criteria: Once all screening visit procedures are complete, the principal investigator, or his/her delegate in his absence, will review the findings to make a determination of whether the participant has met all inclusion criteria and not met any exclusion criteria and whether the participant is suitable to enroll in the trial. This review will be conducted before any Visit 2 / Baseline procedures are conducted.

6.2.2 Baseline and Enrollment Visit (Visit 2)

Baseline procedures may begin anytime following completion of all screening procedures and determination that the participant meets entry criteria. Baseline measures may be completed in any order to accommodate the participant and scheduling limitations of ancillary support facilities with the exception that the dispensation of the study medication occurs last. This final baseline procedure must be completed within 30 days of Visit 1/ Informed Consent.

Baseline measures to be completed include:

- Review of concurrent medications
- Review of adverse events
- Vital signs
- Weight
- Blood and urine collection for clinical safety laboratory tests
- Blood collection for blood biomarker outcome measures
- Urine collection for NAA testing

- Physical examination
- Cognitive battery
- MRI/MRS
- FDG-PET
- Metabolic assessments
- Study medication dispensation and instruction

Description of Baseline Procedures

Prior to administering any study medication, the following baseline measures will be completed:

Blood Biomarker Outcome Measures: Blood will be collected in the AM (after an overnight fast to assess biomarker outcomes at Baseline (Visit 2) and at Week 12 (Visit 6). Approximately 70 mL of blood will be collected to conduct the following biomarker assessments. Specific blood collection and processing instructions will be defined in a separate laboratory manual.

- Apolipoprotein E genotyping (at Baseline only) using a validated allelic discrimination PCR assay (Swerdlow Lab).
- NAA using UPLC-MS/MS method sent to Reed Laboratory to quantify.
- Lipids (LDL, HDL, triglycerides) at the KU Clinical Lab
- Serum β-hydroxybutyrate levels at the KU Clinical Lab
- HgbA1c at the KU Clinical Lab
- Mitochondrial, inflammatory, catabolic/anabolic state biomarkers and banking of additional fluid for future potential purposes will be collected and sent to the Swerdlow lab for processing.
    - The platelet fraction is used for platelet mitochondrial COX activity and CS measurements.
        - COX Vmax activity is determined as a pseudo first order-rate constant (sec^{-1}/mg protein) by measuring the oxidation of reduced cytochrome *c* at 550 nm.[12, 89-91]
        - The CS Vmax assay is performed spectrophotometrically by following the formation of 5-thio-2-nitrobenzoate (412 nm) following the addition of 100 μM oxaloacetate at 30°C,[89, 91, 92] and when referenced to protein yields a value in nmol/min/mg protein.
    - The lymphocytes are used to prepare protein lysates that will be immunochemically analyzed to assess the catabolic/anabolic state by measuring activated AKT and mTOR phosphorylation.
    - The platelet free plasma will be used to perform ELISA measurements of the inflammatory biomarkers: Monocyte chemotactic protein 1(MCP-1; also called CCL2), eotaxin-1 (also called CCL11), and plasma tumor necrosis factor α (TNFα), and C-reactive protein.
    - Samples will be banked for future potential uses (metabolomics, exosome analyses, etc).

Urine n-acetyl aspartate (NAA): A urine sample will be collected at Baseline (Visit 2) and at Week 12 (Visit 6) to assess NAA in the Reed Laboratory.

Metabolic Assessments: This 3-hour visit will occur in the CTSU after a 8hour overnight fast and the following metabolic assessments will be completed:

Indirect Calorimetry: Resting metabolic rate will be assessed using indirect calorimetry which measures CO2 production and O2 consumption to calculate total energy produced. The subject will rest quietly for 10 minutes in an isolated room with the temperature controlled to 18-22 degrees centigrade. Subsequently, an Oro-Nasal Mask (Hans Rudolph- 7450 Series Silicone V2) will be placed over the subject's nose and mouth. The subject will continue to rest quietly while the measurements are taken for up to 30 minutes. Expired gases will be collected with a Parvo Medics TrueOne 2400 system. Criteria for a valid metabolic rate is a minimum of 15 minutes of steady state with steady state determined as less than 10% fluctuation in minute ventilation and oxygen consumption and < 5% fluctuation in respiratory quotient. RMR will be calculated using the Weir equations.[215]

Dual Energy Xray Absorptiometry (DEXA): Body composition will be assessed using dual energy x-ray absorptiometry (GE Lunar iDEXA) to determine fat-free mass, fat mass, and percent body fat at baseline, and week 12. DEXA uses very low X-ray doses (0.02mREM) to detect changes in body composition on the order of 1.6-3.8%.[177, 178]

Oral Glucose Tolerance Test: An Oral Glucose Tolerance test (OGTT) will be conducted in the CTSU to determine glucose tolerance, insulin secretion and insulin sensitivity. Participants will be provided a standard 75g oral glucose bolus followed by blood draws at 0, 10, 20, 30, 60, 90, and 120min, a time course that allows for accurate estimation of both insulin secretion and action.[93] Glucose will be assayed with a YSI 2300 Analyzer. Whole blood will be collected into EDTA-plasma Vacutainer tubes for analysis of insulin using plasma ELISA assay (Alpco). We will calculate insulin secretion and insulin sensitivity.

MRI / MRS: MRI/MRS scans will be obtained during a 1.5-hour visit to the KUMC Hoglund Brain Imaging Center. Given the theoretical issue of diurnal variations in cerebral NAA, we will obtain longitudinal MRI / MRS scans at approximately the same time of day (+/-) 2 hours at baseline and week 12. MRI/ MRS data is obtained using a 3T system (Skyra, Siemens, Erlangen, Germany) equipped with a 32-channel receiver head coil.

After positioning the participant supine in the magnet, three-plane scout MR images are acquired followed by sagittal MPRAGE MRI. We will acquire MRS data from a chemical shift imaging (CSI) slab oriented oblique-axial, parallel to the AC-PC line, in the fronto-parietal area, superior to the lateral ventricles. From this CSI slab, we will determine concentrations of NAA, creatine, choline, and myo-inositol, and glutathione (GSH) from the same location using a multiple-quantum filtered CSI sequence developed by Dr. Choi (Co-Inv). Both NAA (the study's primary outcome measures) and GSH are known to represent mitochondrial and neuronal integrity. MR spectra will be analyzed using the LCModel package and in-house MR data processing pipeline to correct for tissue fraction in each voxel using the segmented MPRAGE MRI corresponding to the CSI slab.

Optional MRS Sub-study: A total of 12 participants will be enrolled into an optional MRI/MRS sub-study. These participants will undergo an additional MRS scan during the baseline period. The scan will be conducted within 2 weeks of the original baseline

scan, at approximately the same time of day (+/-) 2 hours. This optional scan will take place prior to any study drug dosing. This second scanning session will be used to assess scan-rescan reliability of the NAA measure compared to their baseline measure.

FDG PET: FDG PET images will be obtained during a 1.5-hour visit to a satellite location of the University of Kansas Hospital at Baseline (Visit 2) and at Week 12 (Visit 6). The scanner is accredited by the American College of Radiology (ACR), and our physicists perform annual required testing by scanning an Esser PET phantom with 18F to assess SUV range, contrast resolution, spatial resolution, and uniformity. In addition to the ACR annual testing, the nuclear medicine department routinely performs quality control procedures on a daily, weekly, and quarterly basis. Subjects will arrive for FDG PET after having fasted for a minimum of 6 hours and will have a catheter placed for IV administration of FDG. Subjects will receive a single IV bolus of FDG. After the appropriate amount of time has elapsed, frames are reconstructed to a single PET image in native space. Adverse events will be continuously monitored during the imaging session. The radiation dose from the two FDG PET scans our subjects will receive will not exceed 2100 mrem. While this amount of radioactivity obviously exceeds the amount obtained through normal background exposure, it is still considered by Radiation Safety to be well below the risk levels acceptable in research and medical practice.

Cognitive Battery: A cognitive battery will be conducted at Baseline (Visit 2) and at Week 12 (Visit 6). The battery will include the ADAS-Cog 14, Stroop Color Word test, Trailmaking test (A and B) and Logical Memory I and II. It will take approximately 1.5 hours to complete these measures. The ADAS-Cog is a measure of cognition that is widely used in clinical trials. The 14-item version includes measures of delayed recall, attention, and executive function and is considered more sensitive to change than the original 11-item version. We are also performing additional individual tests of verbal memory (Logical Memory I and II) and executive function (Stroop Color-Word test and Trailmaking).

Study Medication Dispensation and Instruction: The final baseline procedure to be completed will be dispensation of the study medication. The participant will receive a one-month supply of study medication. Both the participant and the study partner will be instructed in study medication dosing, as the study partner will oversee that the medication is taken per instruction. The participant will be instructed to take one 10 mg pill of study medication every day, starting at the baseline visit. The study medication can be taken anytime during the day and with or without meals but should be taken at about the same time every day.

6.2.3 Treatment Period

6.2.3.1 Week 1 (Visit 3), Week 4 (Visit 4), and Week 8 (Visit 5) Safety Assessments

At each of these visits, participants will return to the CTSU to assess for safety and tolerability of the study drug. Participants will undergo a review of concurrent

medications, a physical examination, review/check for any adverse events, vital signs including body weight and clinical safety laboratory tests.

Procedures (described above) completed at these visits:

- Review of concurrent medications
- Review/check for any adverse events
- Vital signs
- Physical examination
- Body weight
- Blood and urine collection for clinical safety laboratory tests
- Study medication compliance check (Week 4 & Week 8 only)
- Dispensation of study medication (Week 4 & Week 8 only)

At weeks 4 and week 8 visits, compliance will be assessed and study medication will be dispensed.

- Study Medication Compliance Check: The participant will be asked to return all used and unused study medication containers for an assessment of study medication compliance. A percentage compliance will be calculated for each of these time points.
- Study Medication Dispensation: (Week 4 & Week 8) At these visits, another 30-day supply of study medication will be dispensed.

6.2.3.2 Week 12 (Visit 6) Safety and Other Outcome Assessments

Safety assessments and outcome measures will be completed after 12 weeks (Visit 6) of intervention, including the following:

- Review of concurrent medications
- Review/check for any adverse events
- Vital signs
- Body weight
- Physical and neurological examination
- Blood and urine collection for clinical safety laboratory tests and biomarker outcomes
- Urine collection for NAA levels
- Cognitive battery
- MRI/MRS
- FDG PET
- Metabolic assessments
- Study medication compliance check

The Week 12 (Visit 6) procedures may be completed in any order depending on ancillary facility scheduling availability and participant convenience, but all Week 12 procedures will be completed while the participant continues to take the study drug (+/- 7 days of the 12-week time point). As a result, participants may take the study drug for up to 13 weeks in duration.

A description of each procedure listed can be found above under Screening or Baseline Visit procedures.

6.2.4 Follow Up Period: End of Study Visit (Visit 7)

At week 14, two weeks after stopping study drug, participants will return for a final visit to the CTSU to assess for a final safety check.
The following procedures will be conducted:

- Review of concurrent medications
- Review of adverse events
- Vital signs, including body weight
- Physical examination
- Blood and urine collection for clinical safety laboratory tests (fasting not required, but is acceptable. Fasting status will be recorded))

7 SAFETY ASSESSMENTS

7.1 Dapagliflozin Safety Profile

Randomized clinical trials and post-marketing data have found the dapagliflozin is associated with potential adverse effects including hypoglycemia, laboratory increases in hematocrit, serum phosphorus and LDL-C, ketoacidosis, urinary tract infections, genital infections, and weight loss. When used as monotherapy, the risk for hypoglycemia was low and similar to those taking placebo. Higher rates of hypoglycemia were seen when dapagliflozin was used in combination with sulfonylurea or insulin therapy. A small reduction in blood pressure has been seen in patients treated with dapagliflozin.

Hypoglycemia: Dapagliflozin promotes glycosuria. Hypoglycemia has been reported with dapagliflozin, especially with dapagliflozin as an add-on to sulfonylureas or insulin. In studies with dapagliflozin used as monotherapy, or as add-on to metformin or pioglitazone there were no reported major episodes of hypoglycemia in up to 102 weeks (ref).

Increase in hematocrit and serum phosphorus: Other AEs reported with dapagliflozin include an increased hematocrit (+2.3% compared to Baseline versus -0.33% in the placebo group) and serum inorganic phosphorus (+0.13 mg/dL vs -0.04 mg/dL in the placebo group).

Ketoacidosis: Ketoacidosis has been reported in post-marketing studies in patients with type 1 and type 2 diabetes.
Infections: Treatment with dapagliflozin increases the risk for urinary tract and genital infections. The most frequently reported genital infections are vulvovaginal mycotic infections in females, and balanitis in males. Most patients responded to an initial course of standard treatment, and these infections rarely caused discontinuation from the study.

Weight loss: Urinary glucose excretion induced by dapagliflozin is associate with caloric loss and reduction in weight. The majority of weight reduction is body-fat loss (rather than lean tissue) and fluid loss. In a 24 week placebo controlled study in type 2 diabetes comparing dapagliflozin in combination with antidiabetic agents, body weight dropped 2.26 kg in the dapagliflozin group vs 0.72kg in placebo. Though weight loss is beneficial in diabetes patients, weight loss in patients with AD is often a poor prognostic sign and

associated with greater clinical progression. No studies have demonstrated that the loss of body fat rather than lean tissue is detrimental.

7.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

Patients on insulin and sulfonylureas have a higher risk of hypoglycemia with dapagliflozin and GLP-1 agonists may influence cognitive outcomes and will be excluded. Ketoacidosis has been reported with dapagliflozin in type 1 diabetes and patients with type 1 diabetes will be excluded from the study. Since the efficacy of dapagliflozin is dependent on renal function, we will exclude individuals with an eGFR below 45 ml/min/1.73m2.

In addition, participant safety will be ensured through education, clinical assessment, and laboratory testing.

Education: Dapagliflozin has been associated with reduced blood volume.. Participants will thus be educated on symptoms of volume depletion such as lethargy, fatigue or dizziness. Before initiating dapagliflozin, volume status will be assessed and suspected hypovolemia will be corrected through increased oral intake.

For early diagnosis of infectious AEs associated with dapagliflozin, participants will also be educated on the signs and symptoms of urinary tract infections, vaginal yeast infections, and balanitis, such as dysuria, hematuria, increased urinary frequency, tenderness, pruritus or erythema in the genital areas.

Clinical assessment: Assessment of fluid status with vitals and physical exam will be performed at baseline visit before initiating dapagliflozin. Symptoms of hypovolemia will be assessed at all visits during therapy. Sitting and standing blood pressure and body weight will be assessed at every visit. At each visit, participants will be evaluated and provide a urine sample for signs and symptom of urinary tract or genital infections, and treated appropriately as indicated. Participants will be encouraged to increase fluid intake in those with evidence of hypovolemia.

Laboratory testing: Safety laboratory testing will be conducted at baseline, week 1, week 4, week 8, week 12, and week 14 to monitor for acute kidney injury, hypoglycemia, dehydration, infections, ketoacidosis, and hypo- or hyperphosphatemia. Laboratory tests included will be: complete blood count with differential (hemoglobin, hematocrit, red blood cell count, white blood cell count, platelet count, neutrophil count, lymphocyte count, monocyte count, eosinophil count, basophil count), comprehensive metabolic panel (blood urea nitrogen, creatinine, eGFR, albumin, calcium, bicarbonate, glucose, potassium, sodium, total bilirubin, total protein, alanine aminotransferase, alkaline phosphatase, and aspartate aminotransferase) and serum phosphorus. Fasting lipid panel will be checked at baseline and week 12 to monitor cholesterol including LDL-C.

In addition to these labs, a safety urinalysis will be performed at every visit.

Weight Loss: Body weight will be assessed at baseline, week 1, week 4, week 8, week 12, and week 14/end-of-study. If total weight loss reaches 5% of total body weight compared to baseline and is associated with any concomitant clinical symptoms (fatigue, weakness, reports of cognitive worsening, etc), the study drug will be held or stopped per the judgment of the investigator.

Temporary interruption or cessation of the investigational product (IP) will be considered for individuals per the judgment of the investigator for those who develop orthostatic hypotension or other symptoms of volume depletion, renal impairment, weight loss greater than 5% total body weight at baseline, ketoacidosis or other AEs judged to be related to the study drug.

Overdose: An overdose is defined as a patient receiving a dose of IP in excess of that specified in the Investigator's Brochure, unless otherwise specified in this protocol.

Any overdose of a study patient with the investigational product., with associated AEs/SAEs, is required to be reported within 24 hours of knowledge of the event to the sponsor and AstraZeneca Patient Safety or designee using the designated Safety e-mailbox (see Section 1.6 or contact information). If the overdose results in an AE, the AE must also be recorded as an AE. Overdose does not automatically make an AE serious, but if the consequences of the overdose are serious, for example death or hospitalization, the event is serious and must be recorded and reported as an SAE (see Section 1.6). The investigator will use clinical judgment to treat any overdose.

7.3 Adverse Events and Serious Adverse Events

An **adverse event (AE)** is generally defined as any unfavorable and unintended diagnosis, symptom, sign (including an abnormal laboratory finding), syndrome or disease, which occurs during the study, having been absent at baseline, or if present at baseline, appears to worsen. Adverse events are to be recording regardless of their relationship to the study intervention.

A **serious adverse event (SAE)** is generally defined as any untoward medical occurrence, occurring after the signing of the informed consent through the follow-up period that fulfils one or more of the following criteria: that results in death, is life threatening, requires hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, , an important medical event that may jeopardize the patient or may require medical intervention to prevent one of the outcomes listed above.. or is a congenital anomaly.

All SAEs will be reported, whether or not considered causally related to the IP, or to the study procedure(s). All SAEs will be recorded in the CRF. The causality of SAEs (their relationship to all study treatment/procedures) will be assessed by the investigator(s) and communicated to AstraZeneca.

AEs and SAEs will be collected from the time of informed consent until end of study (week 14).

The following variables will be collected for each AE;

- AE term, using the diagnosis if known, or signs or symptoms when diagnosis is not known
- The date when the AE started and stopped
- Maximum intensity (mild, moderate or severe)
- Whether the AE is serious or not
- Investigator causality rating against the Investigational Product (yes or no)
- Action taken with regard to investigational product
- Outcome.
- In addition, the following variables will be collected for SAEs:

- Date AE met criteria for serious AE
- Date Investigator became aware of serious AE
- Criterion that classifies AE as serious
- Date of hospitalization
- Date of discharge
- Probable cause of death
- Date of death
- Autopsy performed
- Causality assessment in relation to Study procedure(s)
- Description of AE.

7.4 Reporting Procedures

All AEs (related or unrelated to the study intervention) will be reported quarterly to the Safety Officer for review. A summary of these quarterly reports will be provided to the KU IRB on an annual basis. In addition, expedited reporting to the KU IRB will follow IRB guidelines.

Serious Adverse events i.e. Suspected, unexpected, serious, adverse, reaction (SUSAR) will also be reported to the FDA in an expedited fashion, following these guidelines:

- **Within 7 calendar days**
  Any adverse event that is:
  - associated with the use of the study drug
  - unexpected,
  - fatal or life-threatening, and

- **Within 15 calendar days**
  Any adverse event that is:
  - associated with the use of the study drug,
  - unexpected, and
  - serious, but not fatal or life-threatening
  - a previous adverse event that was not initially deemed reportable but is later found to fit the criteria for reporting (reporting within 15 calendar days from when event was deemed reportable).

The investigator must inform the FDA, e.g. via a MedWatch form, of any serious or unexpected adverse events that occur in accordance with the reporting obligations of 21 CFR 312.32. A copy of the MedWatch report must be emailed concurrently to AstraZeneca (TCS vendor) at the time the event is reported to the FDA. It is the responsibility of the sponsor to compile all necessary information and ensure that the FDA receives a report according to the FDA reporting requirement timelines and to ensure that these reports are also submitted to AstraZeneca at the same time.

- Sponsor must also indicate, the causality of events in relation to all study medications and if the SAE is related to disease progression, as determined by the principal investigator.

- Send SAE report and accompanying cover page by way of email to AstraZeneca's designated mailbox: AEMailboxClinicalTrialTCS@astrazeneca.com

- If a non-serious AE becomes serious, this and other relevant follow-up information must also be provided to AstraZeneca and the FDA.

- Serious adverse events that do not require expedited reporting to the FDA still need to be reported to AstraZeneca.

- The investigator will report to AstraZeneca any unblinding that occurs due to critical patient safety concern related to an SAE.

7.5 Follow-up for Adverse Events

All AEs reported during time of study, will be followed by the study team up to 14 days after last dose administration. Any unresolved AEs considered probably study drug related, will be followed up by the study team until the AE has resolved or is considered stable.

7.6 Safety Monitoring

A safety officer with expertise in clinical trials and in use of SGLT2 inhibitors will be appointed to monitor drug safety. The safety monitor will review AE's and study data quarterly and advise the study team on additional monitoring if needed or need for termination of study in case of unexpected serious AE's.

The study may be stopped if, in the judgment of the safety officer, study participants are placed at undue risk because of clinically significant findings that are assessed to be causally related to the study drug.

Safety measures to be reviewed by the safety officer include, laboratory abnormalities, AEs, and discontinuations. These safety measures will be carefully collected and recorded by the study team as they have extensive prior experience and expertise with these procedures.

8 INTERVENTION DISCONTINUATION

Screening failures are participants who do not fulfill the eligibility criteria for the study, and therefore must not be randomized. Participants may be discontinued from investigational product (IP) in the following situations:

- Participant withdrawal of consent. The participant is at any time free to discontinue treatment, without prejudice to further treatment.
- Adverse Event. The investigator may elect to discontinue the participant from treatment if he feels it is in the best interest of the participant.
- Significant noncompliance (<80% medication compliance) on the part of the participant with the study protocol

At any time, participants are free to discontinue IP or withdraw from the study (i.e. IP and assessments), without prejudice to further treatment. A participant that decides to discontinue IP will always be asked about the reason(s) and the presence of any adverse events (AEs). If the participant is discontinued from IP, the participant will be asked to

return to site for an optional safety follow-up visit. The study drugs should be returned by the participant at this visit.

If a participant withdraws from participation in the study, then his/her enrolment/randomization code will not be reused. Withdrawn participants will not be replaced. All data available for the participant at the time of discontinuation of follow-up will be recorded. All reasons for discontinuation of treatment will be documented.

9 STATISTICAL CONSIDERATIONS

9.1 General Design Issues

This study is a double-blind, randomized, placebo-controlled parallel group study. The primary objective is to obtain estimates of the effect of the study drug on cerebral NAA. Secondary and safety and tolerability measures will also be collected and compared between treatment groups.

9.2 Sample Size and Randomization

Forty-eight (48) subjects will be enrolled and randomized 2:1 to either treatment (n=32) or placebo (n=16). Our sample size of 48 subjects was determined consistent with our primary objective of estimation rather than hypothesis testing; the latter (hypothesis testing) we deemed more appropriate for confirmatory studies that we anticipate will follow this initial pilot trial. We anticipate the results of this study will inform the power calculations of those subsequent trials. Thus, we base our sample size justification on providing sufficient precision for estimating our effect with a 95% confidence interval. Assuming 32 subjects in the active group and 16 subjects in the placebo group and allowing for up to 15% attrition our sample size of 48 will enable estimates of the difference in cerebral NAA to be within approximately 0.69 standard deviations for a 95% confidence interval. This magnitude of the half-width of the confidence intervals (0.69 standard deviations) was derived from the confidence interval formula for estimating a difference between two treatment means using quantiles from a t distribution, using the pooled variance formula for a common variance, and post-attrition group sizes of 27 and 13 subjects. From Kalra, Cashman, Genge, and Arnold (1998, *NeuroReport* 9, 1757-61), estimated standard deviations for changes in NAA/Cr both in treated and control conditions were approximately 0.2. For the regression techniques to apply that we described below, we anticipate our results will produce similar sized magnitudes for estimating the standard deviations for this study (i.e., we anticipate our confidence intervals to be within a magnitude of approximately 0.14).

9.2.1 Treatment Assignment Procedures

Subjects will be randomly assigned to treatment or placebo based on the randomization schedule developed by the lead study statistician. This procedure will be balanced within random blocks to increase the balance between treatments over the recruitment period. Treatment order within blocks will be determined by randomly selecting and sorting random draws from a uniform distribution using a pseudo-random number generator on a statistical software package.

9.3 Interim analyses and Stopping Rules

No interim analyses or stopping rules are planned for this trial.

9.4 Outcomes

9.4.1 Primary outcome

MR spectra will be analyzed to determine the concentration of NAA (as a continuous measure) using the LCModel package and in-house MR data processing pipeline to correct for tissue fraction in each voxel using the segmented MPRAGE MRI corresponding to the CSI slab.

9.4.2 Exploratory outcomes

MRS Measures: In addition to NAA, we will assess the concentration (as continuous measures) of several other neurochemicals including glutathione, creatine, choline, myo-inositol, and glutamate+glutamine from the same location using a multiple-quantum filtered CSI sequence.

Cerebral metabolism by FDG PET: PET images will be analyzed using custom software written for Statistical Parametric Mapping (SPM12). Pre- and post-intervention P images will be co-registered. A priori regions of interest masks from the Automated Anatomic Labeling series will be inverse warped to match native space images. The PET images will be standardized to the uptake value of the cerebellum and standardized uptake value ratios (SUVR) will be calculated from native-space ROIs.

Systemic metabolic measures will be continuous measures.

- Systemic NAA levels (in blood and urine)
- Lipids (total cholesterol, LDL, HDL)
- Plasma beta-hydroxybutyrate
- Hemoglobin A1c
- Glucose and insulin Area Under the Curves (tolerance testing)
- Catabolic/Anabolic state [activated AKT and MTOR]
- Mitochondrial function measures [platelet cytochrome oxidase and citrate synthase]
- Inflammatory mechanisms [MCP-1, eotaxin, TNF alpha, CRP]
- Body composition (DEXA scanning for fat and lean mass)
- Resting metabolic rate (indirect calorimetry).

Cognitive performance will be assessed using the ADAS-cog 14 (total score) and individual performance scores on Trailmaking A and B, Stroop Word Color test, and Logical Memory I and II.

9.4.3 Safety Outcomes

We will examine the incidence and severity of AEs and the rate of discontinuations due to AEs. Additionally, we will examine changes in cognitive performance, body weight, and laboratory values observed during the course of the study.

9.5 Data Analyses

Unless otherwise specified, all efficacy analyses will be carried out on all subjects enrolled. Continuous variables will be summarized (using number of participants, mean, standard deviation, and percentiles) by treatment group and visit. For baseline, all end-of-study measurements, the changes, or relative changes from baseline will be presented by treatment group for all efficacy variables to be analyzed. Categorical variables will be summarized, by treatment group, using frequency count and the percentage of

participants in each category. This will facilitate assessment for independence in these baseline measures across treatment groups, which we anticipate given treatment will be allocated at random. (Notably, this inference of independence is equivalent to testing for no difference in proportional distributions across treatment groups.)

The primary objective is estimation, so we will generate 95% confidence intervals for "adjusted" treatment means and their differences based on the formulas analogous to an analysis of covariance (ANCOVA)/ordinary least squares (OLS) regression model hypothesis testing procedure. For these models we will use the baseline measure as a covariate, including assigned treatment as a fixed effect, and change from baseline measure (i.e., follow-up minus baseline) as the response/outcome measure. To deem the adequacy of normal distribution quantiles for confidence interval estimation we will carry out the residual analyses to assess the assumptions of that test for each measure. In the event that the residual diagnostics (including residual vs. predicted value plots, quantile-quantile plots, etc.) do not conform to the underlying normality and other assumptions of this test, we will revert to nonparametric rank-based approach using ANCOVA as described above with ranks used instead for the response to derive model-based p-values. For categorical measures, we will use confidence intervals based on the Wald estimate of the standard error in comparisons of proportions between assigned treatment for dichotomous responses/outcomes if the expected cell counts (under the hypothesis of independence) for corresponding contingency tables are at least five in most (>80%) cells. If the expected cell counts are not sufficiently large, we will instead estimate using exact, Clopper-Pearson confidence intervals.

To assess scan-rescan reliability for NAA on the 12 subjects, we will estimate the variance in the difference scores between each of these 12 subjects' pre-randomization MRI/MRS scans. This will provide information on intra-individual scan-to-scan variation for consideration as we interpret differences (time one minus time two) estimated between treatment groups.

As only pre- and post-treatment measures are obtained, subjects missing post-treatment follow-up measures will be excluded from these analyses.

10 DATA COLLECTION AND QUALITY ASSURANCE

10.1 Data Collection

Data collection will initially be captured on paper case report forms (CRF's) in real time by appropriate study team members who have been adequately trained for their roles.

10.2 Data Management

Data from paper CRF's will be entered into a secure electronic database, accessible only by study team members, housed on an institutional server.

Designated study staff will review the data entered into the eCRFs for completeness and accuracy and make any required corrections or additions.

10.3 Quality Assurance

10.3.1 Training

Study staff will complete Good Clinical Practice and HIPAA training prior entry on the study roster. Training and certification will be completed based on study role as appropriate.

10.3.2 Quality Control Committee

No Quality Control Committee will be appointed for this single-site trial. Quality will be assured through trained staff following Good Clinical Practice standards and appropriate data validation of the eCRF through range limits, data audit trails and appropriate role-based access to forms. The study Statistician will review these metrics quarterly.

10.3.3 Metrics

Missing and out-of-range values will be queried in the eCRF and reported on a quarterly basis to the PI by the study Statistician.

10.3.4 Protocol Deviations

Protocol deviations and adverse events will be captured at all study contacts by study staff, recorded on eCRFs, and reported to the PI. Documentation of the deviation or adverse event will be kept in the participant's paper chart.

10.3.5 Monitoring

The Data Monitoring plan for this trial focuses on monitoring by the PI and the trial coordinator. Before study initiation, the study team will review the protocol and eCRFs. During the study, the study team will employ several methods of ensuring protocol and Good Clinical Practice (GCP) compliance and the quality/integrity of the data. The Investigator will maintain source documents for each participants in the study, consisting of case and visit notes (hospital or clinic medical records) containing demographic and medical information, laboratory data, and the results of any other tests or assessments. All information on the eCRFs will be traceable to these source documents in the participant's file. The Investigator will also keep the original informed consent form signed by the participant (a signed copy is given to the participant). Checks of the consistency of the source data with the eCRFs will be performed using validation tools in the eCRF software. Review of the rate of participant accrual and adherence to inclusion/exclusion criteria will occur during the recruitment phase to assure that participants meet eligibility criteria.

11 PARTICIPANT RIGHTS AND CONFIDENTIALITY

11.1 Institutional Review Board (IRB) Review

This protocol and the informed consent document and any subsequent modifications will be reviewed and approved by the IRB or ethics committee responsible for oversight of the study. The consent form should be separate from the protocol document.

11.2 Informed Consent Forms

A signed consent form will be obtained from each participant. For participants who cannot consent for themselves, such as those with a legal guardian (e.g. person with power of attorney), this individual must sign the consent form. The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy will be given to each participant or legal guardian and this fact will be documented in the participant's record.

11.3 Participant Confidentiality

Any data, specimens, forms, reports, video recordings, and other records that leave the site will be identified only by a participant identification number (Participant ID, PID) to maintain confidentiality. All records will be kept in a locked file cabinet. All computer entry and networking programs will be done using PIDs only. Information will not be released without written permission of the participant, except as necessary for monitoring by IRB, the FDA, the NIA, and the OHRP.

11.4 Study Discontinuation

The study may be discontinued at any time by the IRB, the NIA, the OHRP, the FDA, or other government agencies as part of their duties to ensure that research participants are protected.

12 ETHICAL CONSIDERATIONS

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with ICH/Good Clinical Practice.

13 PUBLICATION OF RESEARCH FINDINGS

Any presentation, abstract, or manuscript will be made available for review by the sponsor prior to submission.

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