Trial of Oxaloacetate in Alzheimer's Disease (TOAD)
IND # 125788

Amendment 5

08/31/2017

Russell H. Swerdlow, MD
Principal Investigator
Professor of Neurology

Neurology
University of Kansas Medical Center
3901 Rainbow Blvd
Kansas City, KS 66160
Phone: 913-945-6632
Fax: 913-945-5035
Email: rswerdlow@kumc.edu

Contents

1.0 INTRODUCTION 3
2.0 OBJECTIVES 3
3.0 BACKGROUND 4
4.0 PARTICIPANT SELECTION 5
5.0 TREATMENT PLAN 6
6.0 EXPECTED TOXICITIES AND DOSING DELAY/DOSING MODIFICATIONS 11
7.0 DRUG FORMULATION AND ADMINISTRATION 11
8.0 CORRELATIVE STUDIES 13
9.0 STUDY CALENDAR 15
10.0 MEASUREMENT OF EFFECT 16
11.0 ADVERSE EVENT REPORTING REQUIREMENTS 16
11.1 Notifying the FDA 16
12.0 DATA AND SAFETY MONITORING 16
13.0 REGULATORY CONSIDERATION 17
14.0 STATISTICAL CONSIDERATION 18
15.0 PUBLICATION PLAN 18
16.0 REFERENCES 19

1.0 INTRODUCTION

Introductory Statement

Since abnormal brain bioenergetic function may contribute to neurodysfunction and neurodegeneration in Alzheimer's disease (AD), manipulating brain bioenergetics may affect the symptoms or alter the course of this disease. Oxaloacetate (OAA) could offer a potential approach for manipulating brain bioenergetics. There is evidence that OAA, a Krebs cycle and gluconeogenesis intermediate, enhances glycolysis flux, supports oxidative phosphorylation, and favorably modifies bioenergetics-related infrastructures. Our pre-clinical studies have also shown that OAA activates the brain's insulin signaling pathway, reduces neuroinflammation, and activates hippocampal neurogenesis [1]. Based on these preclinical findings, a phase I pharmacokinetic (PK) study in six human AD subjects (the Pharmacokinetics of Oxaloacetate Study, or POX Study; NCT02063308) was performed. The POX Study found AD subjects tolerate 100 mg BID of OAA when administered over a one month period. No adverse events were reported.

The next logical step is to conduct a combination safety and target engagement (phase Ib) study to define an OAA dose that is safe and which engages brain bioenergetic metabolism. This study, the "Trial of OAA in AD" (TOAD) trial, is designed to accomplish these goals. The TOAD trial's primary endpoint is safety, with OAA doses that have not previously been studied in human AD subjects. One of the secondary endpoints is target engagement of brain bioenergetics by OAA. The TOAD trial will use magnetic resonance spectroscopy (MRS) and fluorodeoxyglucose positron emission tomography (FDG PET) to assess brain bioenergetic target engagement. Exploratory target engagement data generated during this study will inform future study design. Another secondary endpoint is establishment of a pharmacokinetic profile of OAA at doses that have not been previously tested.

It is hypothesized that OAA, when orally ingested in adequate amounts, is safe and will alter brain bioenergetics. We further hypothesize that the doubling of the OAA dose will result in a doubling of the plasma OAA concentrations and that plasma OAA levels will correlate with our brain bioenergetics engagement outcomes (FDG PET and MRS). The following objectives are proposed to test these hypotheses.

2.0 OBJECTIVES

Primary Objective

The primary objective of the TOAD trial is to determine the safety and tolerability of OAA at doses up to 2 g/day. A dose escalation strategy will be used, which will involve first testing a 1 g/day OAA dose (500 mg po BID) taken for 4 weeks in 15 AD subjects. If this dose proves to be safe, 2 g/day OAA (1000 mg po BID), taken for 4 weeks in 15 subjects, will be tested. As part of the safety analysis, the following measures will be obtained: pre and post-treatment vital signs, safety labs, physical and neurological examinations, and cognitive testing data. We predict both OAA treatment doses will prove to be safe and well-tolerated.

Secondary Objectives

One of the secondary, exploratory objectives will be to test whether OAA (administered at 500 mg BID or as 1000 mg BID) has an observable effect on brain bioenergetics. Enhancement of brain glucose utilization on FDG PET, and changes in brain metabolism intermediates on

MRS, will be evaluated in both the 1 g/day dose and the 2 g/day dose cohorts. FDG PET and MRS will be measured at pre-treatment (just prior to starting OAA), and at post-treatment (just prior to stopping OAA, after approximately one month of treatment). The pre and post-treatment scans will be quantitatively analyzed and compared. We predict a dose will be found at which the post-treatment FDG PET scans show increased cortical glucose utilization, and that a dose will be found at which the post-treatment MRS scans show increased lactate and possibly other metabolite changes.

Another secondary objective is to define OAA PK properties. Specifically, we will determine plasma OAA levels after 500 mg and 1000 mg doses, to determine if a maximum absorption capacity is reached with these doses. We will also test for correlations between plasma OAA levels and brain bioenergetic biomarker, as revealed by FDG PET and MRS.

3.0 BACKGROUND

Prevalence

The elderly constitute a rapidly growing demographic. This reflects public health initiatives and medical advances that reduce risks and provide treatments for many previously terminal disorders. An increasing population of neurodegenerative disease victims is arguably the biggest downside to this success. For several common neurodegenerative diseases incidence rises with advancing age and prevalence is quite high [2]. Alzheimer's disease (AD), the most common neurodegenerative disease, affects 5.4 million Americans and one in every eight Americans over 65 is estimated to have it [3]. Society is also affected, as families and friends of AD patients provide most day-to-day care and altogether AD now costs our economy $385 billion annually [4].

Rationale for Use

Mitochondrial dysfunction occurs in AD and is felt by many to be disease-relevant and a promising therapeutic target [5]. In AD brains various mitochondria-localized enzymes show reduced activity, and in most neurons intact mitochondria are numerically reduced [5]. Glucose utilization, as demonstrated by FDG PET, is also reduced in the AD brain [6]. For these reasons we believe that for AD, increasing mitochondrial mass, mitochondrial respiration, and glucose utilization are all well-justified mitochondrial medicine approaches and we are currently exploring ways to accomplish these endpoints [7]. Our efforts are informed by caloric restriction, which promotes liver mitochondrial biogenesis in some but not all studies, and exercise, which promotes muscle mitochondrial biogenesis. While we and others find caloric restriction and exercise do affect the brain in specific ways, and may even induce some degree of brain mitochondrial biogenesis, our impression is that these interventions at best affect brain mitochondrial biogenesis less robustly than they affect liver and muscle. We therefore feel approaches that directly and robustly enhance bioenergetic fluxes (glycolysis and respiration-related) and brain mitochondrial biogenesis pathways are needed, and predict such approaches will benefit persons with AD.

The ideal agent would be systemically safe, cross the blood brain barrier, access neurons and astrocytes, activate mitochondrial biogenesis, increase respiratory capacity, and increase glycolysis capacity. OAA putatively meets these criteria (see preliminary data in section 8.0 CORRELATIVE STUDIES).

Description of Study Agent

OAA is a Krebs cycle and gluconeogenesis intermediate. It forms a redox pair with malate and is reduced to malate in a reaction that consumes NADH and generates NAD+. This would predictably shift cell redox balances towards more oxidized states, an effect reported to initiate mitochondrial biogenesis and mediate at least some health benefits of caloric restriction and exercise. OAA, following its reduction to malate, can also access mitochondria and thereby supply carbon to the Krebs cycle and respiratory chain. Finally, OAA is converted to phosphoenolpyruvate (PEP) by PEP carboxykinase (PEPCK), a reaction that comprises part of the gluconeogenesis and glyceroneogenesis pathways. Muscle PEPCK overexpression, possibly due to enhanced glyceroneogenesis, induces a profound muscle mitochondrial biogenesis, enhances respiratory fitness, and robustly extends lifespan [8]. Currently, OAA is commercially available and marketed as a nutritional supplement.

4.0 PARTICIPANT SELECTION

Subjects will be recruited through the University of Kansas Alzheimer's Disease Center's (KU ADC) Clinical Core Cohort/ Registry program. As part of their participation in the KU ADC Clinical Core Cohort/Registry, individuals receive clinical dementia rating (CDR) and Uniform Data Set (UDS) evaluations. Participant diagnoses, which are primarily based on CDR and UDS data, are determined through a consensus conference that includes subspecialty-trained cognitive neurologists and an expert neuropsychologist. Participants diagnosed with AD further meet current McKhann el al. criteria for that diagnosis [9].

The TOAD trial will enroll participants with mild AD as determined at the last KU ADC Clinical Core/ Registry evaluation. Each participant must have a study partner. The study partner will be someone who knows the participant well (typically a spouse, relative, or friend of the participant) and is able to answer questions about the participant's changes in health and/or behaviors over the course of the study. In addition, the study partner will administer the study medication and agree to accompany the participant to all study visits.

Enrollment Information

The TOAD trial will enroll a total of up to 36 participants, to ensure 15 participants in each dose cohort, 1 g/day and 2 g/day, complete the full intervention. No participant will be enrolled into the 2 g/day cohort until all 15 participants have completed the 1 g per day dose and it is established that the maximum acceptable dose-limiting toxicity (DLT) event rate has not been exceeded and it is safe to test the higher dose (See Safety section). Participants who complete the 1 g/day dose study may be invited to participate in the 2 g/day dose study, if still eligible and the principal investigator deems safe to do so.

Inclusion Criteria

Individuals are eligible if they meet the following criteria:

1. Age 50-85;
2. Have a diagnosis of probable AD per McKhann et al. criteria [9];

3. Have a clinical dementia rating (CDR) score of 0.5 or 1 AD at time of their last KU ADC assessment;
4. Have a Mini Mental Status Exam (MMSE) score of 15-28 *at the TOAD screening visit*;
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 TOAD screening visit*; and
7. Speaks English as his/her primary language.
8. If female of child-bearing potential, must have a negative urine pregnancy test *at TOAD screening visit* (and must agree to use of contraception throughout the trial)

Exclusion Criteria

Individuals are ineligible if they meet any of the following criteria:

1. Dementia due to causes other than AD;
2. Potentially confounding, serious, or unstable medical conditions such as:
   - insulin-dependent diabetes mellitus
   - cancer within the past 3 years (except basal cell, squamous cell, or localized prostate cancer)
   - a recent cardiac event (i.e. heart attack, angioplasty, etc. within the 6 months prior to screening visit)
   - other conditions that pose a potential safety risk or confounding factor in the investigator's opinion;
3. Any abnormal physical examination assessment or vital sign assessment *at TOAD screening visit* that is deemed to be clinically significant by the principal investigator;
4. Any abnormal clinical laboratory test result *at TOAD screening visit* that is deemed to be clinically significant by the principal investigator.
5. Any contraindication for undergoing MRS, such as the presence of metal implants, a cardiac pacemaker that is not compatible with MRS, or severe claustrophobia

5.0 TREATMENT PLAN

Study Design

The TOAD protocol is designed as a phase Ib study that will provide information needed to plan a phase II study, or else indicate additional development work that is required before pursuing a phase II study. A dose escalation strategy will be utilized to contribute knowledge to

the safety profile of OAA and to establish an effective target engagement dose. The primary outcome measure is the safety of currently untested OAA doses (1 g and 2 g per day) in AD subjects, as defined by safety laboratory tests, vital signs, physical and neurological examinations, cognitive changes, and reports of adverse events. Our secondary exploratory measures will determine a) whether these OAA doses have an observable effect on brain glucose utilization, as determined by FDG PET, and on levels of metabolism-relevant neurochemicals, as demonstrated by MRS and b) the PK properties of OAA at 500 mg and 1000 mg doses and whether OAA plasma levels at these doses correlate with FDG PET and MRS biomarker data.

Safety Outcomes

1 g/day and 2 g/day doses were selected for the following reasons. The POX Study has already indicated 100 mg BID is well-tolerated in AD subjects. Higher doses have not been specifically tested in AD subjects, but in a 1968 OAA clinical study (in diabetics) OAA dosed to 1 g/day for up to 44 days was found to be safe [10]. Also, we are aware that humans with glioblastoma have tolerated 2-3 grams per day for extended periods (in 2012, OAA was given "orphan drug status" for the treatment of gliomas). Ranging our study between 200 mg/day and 2 g/day, therefore, seems justified, especially since we are using a dose escalation approach in which safety at the 1 g/day dose determines whether we attempt the 2 g/day dose.

Safety will be assessed through pre-and post-treatment collection of: safety labs, physical and neurological exams, cognitive measures, and study partner reports of changes in the participant's health (new medical conditions, symptoms, or changes in functioning). Clinical safety labs will consist of complete blood count, serum electrolytes, serum liver function tests, and fasting glucose testing. To ensure our clinical intervention does not exacerbate cognitive decline, we will include pre and post-treatment cognitive testing (ADAS-Cog, MMSE, immediate and delayed Logical Memory, and Stroop Color Word Test). At the time of enrollment the participant's medical history and baseline of current symptoms will be collected from the participant (with study partner input). This symptom checklist will be repeated at the end of the ~4 week treatment period, and again ~4 weeks after the treatment is discontinued as a structured method to probe for any adverse events. A study clinician will conduct a pre and post-treatment physical and neurological examination to observe for any change in status.

Adverse events will be collected from the time the informed consent document is signed until approximately 4 weeks following the last dose of study medication. The frequency and severity of adverse events (AE's) will be examined and their potential causal relationship to the study drug (as determined by the PI) will be assessed at the time of data collection.

Any adverse event that results in the study medication dose being stopped or permanently reduced will be considered a dose limiting toxicity (DLT). The maximum acceptable dose-limiting toxicity (DLT) event rate will be capped at 13.4% in each cohort. Based on a sample size of 15 subjects per cohort, a binomial distribution analysis indicates if the true probability of a DLT event is 5% there is a 99% chance of concluding the dose is safe, with a 10% true probability of a DLT event this chance falls to 94%, and with a 25% true probability of a DLT event this chance falls to 46%. Thus, if more than 13.4% of currently enrolled or completed participants in a dose cohort experiences a DLT, that dose will be discontinued. The dose cohort will be considered safe if, at its completion, 2 or fewer of the total 15 participants experience a DLT.

The maximum number of subjects in an arm who at any time are at risk of experiencing a dose limiting toxicity event will be capped at 3; at no time will the number of subjects that can potentially experience a dose limiting toxicity event in an arm exceed 3. This limitation will be achieved through curtailment. If any 2 among the first 6 subjects experiences a dose limiting toxicity we will discontinue that arm at that time. For a given arm, therefore, the risk to our subjects will be no greater than the risk of a traditional 3+3 design. The occurrence of a dose limiting toxicity event in 3 subjects in an arm terminates that arm, or 2 dose limiting toxicity events if among the first six. The appearance of even 1 non-serious dose limiting toxicity event will put a hold on further enrollment pending review by our Data Safety Monitoring Committee, while the appearance of any serious adverse event will require placing the entire study on hold pending review by the Data Safety Monitoring Committee, our Institutional Review Board, the FDA, and the PI. In order to advance to the higher dose arm, only up to 1 of the first 6 subjects (16.7%) or up to 2 of the planned 15 subjects (13.4%) can experience a dose limiting toxicity; the occurrence of dose limited toxicity events in 2 of the first 6 subjects or 3 of the planned 15 subjects terminates the arm.

No participants will be enrolled into the 2 g/day dose cohort until the 1 g/day cohort is completed within the acceptable DLT range. If the 1 g/day dose is discontinued due to an unacceptable rate of DLT events, instead of testing a higher dose we will instead test a lower dose (500 mg/day, given as 250 mg BID). If the 1 g/day cohort is found to be safe but the 2 g/day cohort is not, remaining subjects will be switched to (if already in the study) or placed on (if not already in the study) 750 mg/day (375 mg BID).

Biomarker Outcomes

The TOAD study will utilize FDG PET and MRS as target engagement biomarkers. FDG PET and MRS data interpretation, however, is complicated by the fact that different interpretation strategies can be utilized and obviously with MRS there are multiple potential outcome measures. For these reasons, FDG PET and MRS data will be treated as secondary outcomes. Changes in these outcomes will nevertheless greatly inform the design of future OAA clinical trials.

In our preclinical mouse MRS studies, pre and post-treatment scans performed on 8 mice were sufficient to show treatment-associated lactate differences [1]. Increased lactate levels in the brains of mice receiving daily 2 g/kg intraperitoneal (IP) OAA injections could potentially reflect an OAA-mediated increase in glucose utilization. If so, FDG PET may provide a reasonably sensitive way to detect increased glucose utilization. Further, considering MRS and FDG PET data together could provide novel insight into bioenergetic fluxes present within the brains of AD subjects.

Fifteen participants per cohort is a reasonable enrollment goal to examine this secondary objective. In our preliminary mouse MRS studies, pre and post-treatment scans performed on 8 mice were sufficient to show treatment-associated lactate differences. This is not to imply 15 subjects per group is “conservatively powered”, since there is at best a very limited ability to extrapolate data from mice receiving 2 g/kg IP OAA and scanned by a 9.4T magnet to humans receiving 500 or 1000 mg po BID and scanned by a 3T magnet.

More applicable are FDG PET data previously reported by Keller et al. [11]. That study was able to demonstrate an increase in the FDG PET-determined cerebral metabolic rate of glucose (CMRglu) in 13 AD subjects placed on galantamine and subsequently maintained on the drug for

12 months. If OAA at the doses in the current TOAD study is able to influence glucose utilization as robustly as galantamine, an n=15 per group should provide a reasonable chance of detecting a treatment-induced change if one exists, while providing a small cushion in the event of subject-drop out or if, as is possible, occasional individual scans prove to be of limited quality. Moreover, the short study duration (4 weeks) will ideally minimize drop-outs. In this respect, to our knowledge this will be the first study to compare pre and post-treatment PET FDG data at a 4 week time point. This is justified given our preliminary data, which indicate OAA rapidly affects brain bioenergetics [1].

Assuming safety outcomes are met for a particular cohort, lack of change on both the MRS and FDG PET target engagement biomarkers would argue that rather than progressing to a more advanced clinical trial phase, we will need to acquire additional very-early stage test data, such as the PK of 500 mg and 1000 mg doses to see if there is a linear relationship, and ideally to test the safety of higher OAA doses. If there is a more robust degree of target engagement with 2 g/day than the 1 g/day dose, further studies will examine safety and target engagement biomarkers at doses above 2 g/day to see if the higher doses provide more robust target engagement. If equivalent target engagement biomarker changes are seen at 1 g/day and 2 g/day OAA, the 2 g/day dose will move forward in subsequent studies. Ultimately, future studies will utilize the data from the TOAD study to define a specific target engagement biomarker analysis. Specifically, if positive MRS data emerge, results from this study will inform which metabolites are informative. Positive FDG PET data will tell us which brain regions are most informative.

Pharmacokinetics (PK)

We recently conducted an OAA PK-Safety study in AD subjects (see section 8.0 Correlative Studies / Clinical Studies). To measure PK in this study, plasma was prepared and analyzed for OAA concentration using a commercially-available coupled enzyme assay kit. Our data indicate that following oral administration the Tmax for OAA occurs between 1 and 1.5 hours.

We have recently developed a liquid chromatography-tandem mass spectroscopy (LC-MS/MS) based assay for OAA quantification that will provide sensitivity greater than what can be obtained through the coupled enzyme method. For the TOAD-PK analyses, we will use the LC-MS/MS-based assay to quantify OAA across a broader range of plasma concentrations.

Study Procedures

At the initial screening visit (V1), participants will undergo the following procedures: informed consent, medical history and symptom inventory, review of concurrent medications, Mini Mental Status Examination (MMSE), fasting safety labs (to include complete blood count, serum electrolytes, serum liver function tests, glucose) vital signs, and a physical and neurological examination. If the participant is a female of childbearing potential, a urine pregnancy test must be conducted. Once eligibility for the study is confirmed and within 14 days of this, participants will complete the baseline visit (V2). The baseline visit will include FDG PET, MRS, and the safety cognitive battery which consists of the ADAS Cog, Logical Memory I and II, and the Stroop Color Word Test. After all other baseline procedures are completed, study medication will be dispensed.

Participants (and their study partners) will be instructed to take the study medication two times a day in both the 1 g/day and the 2 g/day cohorts. In the 1 g/day dose cohort, participants will take 500 mg OAA BID for 4 weeks. The 2 g/day cohort will take 1000 mg OAA BID for 4

weeks. Participants will take their first dose of study medication in the clinic in each cohort and blood samples will be drawn just prior to administration of study medication, at 60 minutes post ingestion, and at 90 minutes post ingestion.

At the end of weeks 1, 2, and 3 of OAA administration we will place a phone call to enrolled subjects and their caregivers to ascertain side effects that could indicate emerging toxicity. Approximately 4 weeks (+/- 7 days) after starting study medication, participants will be asked to complete V3, which will include a post-treatment FDG PET, MRS, a repeat cognitive battery (including MMSE), review of symptoms (to check for adverse events), review of concurrent medications, fasting safety labs, vital signs, and physical/neurological examination. All used and unused study medication will be returned and study medication compliance will be calculated. The V3 FDG PET and MRS will be conducted while still taking study medication but just prior (within 7 days) to stopping.

A final phone interview (V4) will be conducted approximately 4 weeks (+/- 7 days) after stopping study medication to review the symptom inventory and determine if any adverse events were experienced in this time period.

Description of FDG PET Procedures

FDG PET images will be obtained at a satellite location of the University of Kansas Hospital. 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.

Description of MRS Procedures

MRS scans will be obtained using a 3T system (Skyra, Siemens, Erlangen, Germany) fitted with a 12-channel receiver head coil. After positioning the participant supine in the magnet, three-plane scout MR images are acquired followed by an oblique-axial MP-RAGE sequence oriented parallel to the AC-PC line. We will collect several MRS sequences to sample a large spectroscopic imaging slab at the same angle, superior to the lateral ventricles. From this we will determine concentrations of several neurochemicals including lactate, glutamine, and glutamate which are of specific relevance to the current proposal. We will also quantify glutathione from the same locations using a double-quantum filtered sequence developed at the Hoglund Brain Imaging Center. In addition, based on our animal results we will also acquire a single voxel spectrum that includes the hippocampus. Scan time will be approximately 1.5 hours, which from our experience is feasible in this subject group. Spectra will be analyzed using LCModel and corrected for tissue fraction in each voxel using the segmented MP-RAGE images corresponding to the spectroscopic imaging slab, and using existing laboratory routines.

Description of Pharmacokinetic Procedures

In each cohort (500 mg OAA bid and 1000 mg OAA bid), the participant will consume the first dose of study medication in the clinic, after having fasted for a minimum of 8 hours. Just prior to administration of this first dose, a blood PK sample (approximately 4 mL of blood) will be drawn to provide a baseline measurement. A second PK sample will be drawn at 60 minutes post ingestion and a third PK sample will be drawn at 90 minutes post ingestion. Each PK sample will immediately be placed on ice, and will be processed into plasma within 10 minutes of collection. Aliquots of plasma (approximately 0.5 mL each) will be frozen and stored at -80°C immediately until analyses. Quantitation of OAA in plasma will be performed using the LC-MC/MS based assay described above.

Procedure Timeline

The timing of study procedures/schedule of events are summarized in the STUDY CALENDAR in Section 9.0

6.0 EXPECTED TOXICITIES

There are no expected toxicities.

7.0 DRUG FORMULATION AND ADMINISTRATION

Drug Production Site

OAA will be purchased from Terra Biological LLC, which is based in San Diego, CA. OAA sold by Terra is produced according to good manufacturing practices.

Drug Transportation

Study medication will be shipped to the study site by express mail.

Drug Formulation

Terra markets OAA as a nutritional supplement in capsules that contain 100 mg of OAA and 150 mg of ascorbic acid. To prevent ascorbic acid from complicating interpretations, Terra will provide the study site with capsules containing 250 or 500 mg OAA and no ascorbate. This will limit the number of capsules participants must ingest.

Drug Quality Assurance

Ten of our 250 or 500 mg capsules, from the same lot being dispensed at that time, will be randomly selected each month for the duration of our study and will be analyzed according to the protocol summarized in the Table below.

Monthly analysis and quality parameters of our 250/500 mg oxaloacetate capsules.	
Method	**Specification**
HPLC Oxaloacetic Acid Assay	Greater than 95%

If the average oxaloacetate content in ten analyzed oxaloacetate capsules at any point falls below 95%, or if any other parameter does not meet our specifications, a new set of capsules will be synthesized and reanalyzed. If meeting this standard requires synthesizing a new batch of oxaloacetate, the new batch will also be fully analyzed by the protocol summarized below before being packaged into capsules. The new batch will then be dispensed to participants to ensure all participants are taking the proper dosage of oxaloacetate above 95%.

Monthly analysis and quality parameters of our 250 mg oxaloacetate capsules.	
Method	**Specification**
HPLC Oxaloacetic Acid Assay	Greater than 95%
Appearance	White to Off-White Crystalline Powder
Sulfated Ash	Max: 0.5%
Loss on Drying	Max: 1.0%
Water (Karl Fisher)	Max: 1.0%
Solution (10% in Water)	Clear
Heavy Metals as Lead (USP <231>	Max: 0.001%
Identification (IR)	Consistent with Structure

Monthly analysis and quality parameters of our 500 mg oxaloacetate capsules.	
Method	**Specification**
HPLC Oxaloacetic Acid Assay	Greater than 95%
Appearance	White to Off-White Crystalline Powder
Sulfated Ash	Max: 0.5%
Water (Karl Fisher)	Max: 1.0%
Heavy Metals as Lead (USP <231>	Max: 0.001%
Identification (IR)	Consistent with Structure

For the 500 mg capsules, microcrystalline cellulose (MC) has been added to the capsules to assist with packing the OAA in the capsules. With the 500 mg capsules, we allow for cloudiness in the solution because the presence of MC renders the solution cloudy, therefore the Solution test is not used as a parameter for the 500 mg capsules. Also with the 500 mg capsules, we will define acceptability of the capsules using the Karl Fisher test, as this test is more sensitive to water content than the Loss on Drying test. Levels less than 1.0% on the Karl Fisher test are considered acceptable.

The oxaloacetate capsules we use in these studies will be stored in plastic bottles containing a desiccant. The bottles will be kept in a locked cabinet in the KU Alzheimer's Disease Center which is monitored for temperature changes or in a secure -20 degree freezer in the University of Kansas Clinical Research Center building. Every month 10 capsules from the same lot used for dispensing will be delivered to the company that performs the analysis. The company that will perform the analysis is Expert Chemical Analysis (ECA), which is located in San Diego, CA. ECA is registered and audited by the FDA (www.ecalab.com). There is a possibility that loss of oxaloacetate integrity could occur as an artifact of the shipping process (when the capsules are briefly outside of our controlled environment), but this approach will ensure that the quality of the oxaloacetate distributed to our trial subjects meets or exceeds the quality of the assayed oxaloacetate capsules.

Drug Administration

Participants in the 1 g/day dose cohort will take 2- 250 mg OAA capsules in the morning and 2-250 mg OAA capsules in the evening for approximately 4 weeks. Participants in the 2 g/day dose cohort will take 2-500 mg OAA capsules in the morning and 2-500 capsules in the evening for approximately 4 weeks.

8.0 CORRELATIVE STUDIES

Pre-Clinical Studies

In one recent study using C. elegans, OAA extended median and maximum lifespan through AMP kinase (AMPK) and forkhead O-box (FOXO) transcription factor-dependent pathways [12]. In mammals, it can be inferred that systemically administered OAA accesses the brain. This inference is based on rodent studies that report OAA prevents kainate-induced seizures and neuron damage, decreases ischemia-induced stroke volume, decreases cortical impact-induced traumatic brain injury, and slows glioma growth rates [13-16].

Over the past five years we have completed our own preclinical target validation studies. Initially, we evaluated the effects of OAA on cell culture bioenergetic fluxes and found that in undifferentiated and differentiated human neuronal SH-SY5Y cells OAA increased the maximum glycolysis capacity, spare glycolysis capacity, enhanced the mitochondrial respiratory flux, and blunted the Crabtree effect. To extend our studies to animals, we next administered OAA to mice in order to test its impact on genes, proteins, and pathways that monitor, regulate, and mediate brain bioenergetic fluxes. We also ascertained the effect of OAA on brain insulin signaling, inflammation, and hippocampal neurogenesis as relationships exist between these parameters and bioenergetics. We further probed for changes in metabolic fluxes by using MRS to measure in vivo levels of detectable neurochemicals.

In these studies [1], male C57Bl/6 mice received IP OAA, 1-2 g/kg once per day for 1-2 weeks. Both doses induced brain changes; we did not determine a minimum effective dose. Observed changes were consistent with an activation of mitochondrial biogenesis pathways. Brain peroxisome proliferator activated receptor γ coactivator 1α (PGC1α) and peroxisome related coactivator (PRC) mRNA levels increased, and brain PGC1α and PRC protein within cells redistributed such that their nucleus:cytoplasm ratios increased. mRNA levels of nuclear

respiratory factor 1 (NRF1) and transcription factor A of the mitochondria (TFAM) increased. OAA treatment also increased cytochrome oxidase subunit 4 isoform 1 (COX4I1) mRNA and protein. AMPK Thr172 phosphorylation, p38 MAPK phosphorylation, and cAMP-response element binding (CREB) Ser133 phosphorylation each increased.

OAA treatment enhanced the brain insulin signaling pathway activation state. Akt Ser473, mTOR Ser2448, and P70S6K Thr389 phosphorylation increased. OAA treatment also reduced markers of neuroinflammation. Hippocampal C-C motif chemokine 11 (CCL11) mRNA, hemisphere nuclear factor κB (NFκB) protein levels, and NFκB nucleus-to-cytoplasm ratios were lower than in control mice.

It was previously reported in mice that CCL11 retards subgranular layer neurogenesis [17]. Because CCL11 gene expression was reduced in the hippocampus of OAA-treated mice, and because Akt and mTOR activation promotes cell growth, we evaluated hippocampal neurogenesis and found enhanced hippocampal neurogenesis. Hippocampal vascular endothelial growth factor A (VEGF) mRNA levels increased. Hippocampal mRNA and protein levels of doublecortin (DCX), which is commonly used as a surrogate measure of new neuron formation, increased. The number of intensely DCX-positive neurons increased. Relative to the control group, DCX-positive neurites were longer in the OAA-treated mice.

^{1}H-MRS was performed on mice before receiving any treatment, and on the same mice after they received 2 g/kg/day OAA IP for one week (the 1 g/kg/day dose was not studied). OAA induced changes in several neurochemicals. After one week of OAA, brain lactate, GABA, and glutathione (GSH) levels were respectively 21%, 15%, and 27% higher than they were on the pre-treatment scans.

Clinical Studies

Prior to our own recent OAA studies, the literature reported only one human study of OAA, a 1968 study that evaluated its potential anti-hyperglycemic effects. This study, performed in human diabetics, reported OAA was well-tolerated and also limited PK data [10]. Levels were not increased 30 minutes but were increased 60 minutes after 200 mg were orally administered to three subjects. On average, OAA levels rose from 0 ug/100 ml to 2.3 ug/100 ml.

We recently conducted an OAA PK-safety study in AD subjects. OAA 100 mg capsules (obtained from Terra, Inc., which markets OAA as a nutritional supplement) were administered to 6 subjects recruited from the University of Kansas Alzheimer's Disease Center (KU ADC) clinical cohort. The first 100 mg capsule was administered at the University of Kansas Clinical Translational Science Award (KU CTSA) Clinical Trials Unit (CTU), and over a four hour period PK blood samples were obtained at 15, 30, or 60 minute intervals. Basic safety labs (CBC, chemistries) and cognitive tests (MMSE, ADAS-Cog) were performed. Subjects continued on 100 mg BID OAA for one month, and then returned to the CTU where blood samples were again taken for PK determinations, safety labs and cognitive tests were repeated, and a formal side effect query were completed. There were no drop-outs, pre and post treatment cognitive scores were comparable, safety labs were unremarkable, and no side effects were reported.

Further human experience data are available from the case series of Kesari. In the Kesari case series human subjects with gliomas were administered oxaloacetate at doses far greater than 500 mg BID. This case series includes 8 subjects whose single dose amount was 1000 mg and 6 subjects whose single dose amount was 2000 mg (500 mg doses were not used in this case

series). Among the subjects maintained at these high doses a single side effect was reported to be oxaloacetate-related, and included a single subject who experienced nausea on the 2000 mg dose. Neither discontinuation nor a reduction in dosage was required. Oxaloacetate was administered TID in this case series, and the mean treatment duration for the group was over 2 months. Therefore, well over 2000 doses of over 500 mg have been administered to humans without incurring a dose limiting toxicity event.

In the Kesari case series, the 8 subjects dosed with 1000 mg TID were followed for an average of 2.3 months (minimum duration=1 month, maximum duration=8 months). The average cumulative dose for these 8 subjects was therefore ~210,000 mg (minimum=90,000 mg, maximum=720,000 mg). The 6 subjects dosed with 2000 mg TID were followed for an average of 4.2 months (minimum duration=1 month, maximum duration=8 months). The only reported side effect in this series was one reported case of nausea in the 2000 mg TID group, and this did not require discontinuation or a change in dosing. The average cumulative dose for these 6 subjects was therefore ~756,000 mg (minimum=180,000 mg, maximum=1,440,000 mg); the cumulative ~1 month amount at 500 mg BID would be ~30,000 mg.

It is important to note that oxaloacetate has been available for years as a supplement in the United States and other countries, and no toxicity has been reported. This is consistent with the fact that marketed oxaloacetate is produced from tartaric acid, a GRAS compound. As it is produced from a GRAS compound, oxaloacetate itself could also technically be considered to meet GRAS status.

9.0 STUDY CALENDAR

Trial of Oxaloacetate in Alzheimer's Disease (TOAD) Schedule of Events

Procedure	Visit 1 (Screening) -14 to 0 days	Visit 2 (Baseline)[c] Day 0	Visit 3[f**] Week 4 (+/- 7 days)	Visit 4 4 weeks post V3 End Date (+/- 7 days)
Informed Consent	X			
Medical History Review	X			
Symptom Checklist/ Adverse Event Review	X	X	X	X
Concurrent Medication Review	X	X	X	X
Vital signs	X	X	X	
Physical & Neuro Exam	X		X	
Safety Labs (fasting)	X		X	
Pharmacokinetic labs (fasting)		X		
Urine pregnancy test*	X			
MMSE	X		X	
Review of Inclusion / Exclusion Criteria	X			
ADAS Cog		X	X	
Logical Memory I & II		X	X	
Stroop Color Word Test		X	X	
FDG PET[a]		X	X	
MRS[b]		X	X	
Dispense Study Medication/Begin Study Medication[d]		X		
Stop Study Medication/ Medication Compliance Check[e]			X	

[a]FDG PET=fluorodeoxyglucose positron emission tomography. At V2, FDG PET may be performed any time after participant is deemed eligible for study but must be done prior to administration of first dose of study medication. At V3, FDG PET must be completed prior to stopping study medication.

[b]MRS=magnetic resonance spectroscopy. At V2, MRS may be performed any time after participant is deemed eligible for study but must be done prior to first dose of study medication prior to administration of first dose of study medication. At V3, MRS must be completed prior to stopping study medication.
[c]All V2 procedures must be completed no later than 14 days after V1.
[d]Dispensing of study medication and administration of first dose may take place on the same day as other V2 study procedures (such as PET or MRS or cognitive testing) but must be the **last** procedure completed at V2.
[e]Stopping study medication/medication compliance check must be the last procedure completed at V3.
[f]V3 procedures may be completed on multiple days, but should begin 4 weeks +/- 7 days from Day 0. Once first V3 procedure is completed, all other V3 procedures must be completed within 7 days.
*For females of childbearing potential only
**Phone call to participant/caregiver will take place at end of Week 1, Week 2, & Week 3 of OAA administrationi to ascertain side effects that could indicate emerging toxicity.

10.0 MEASUREMENT OF EFFECT

The primary and secondary outcomes will be assessed as described under section 5.0 TREATMENT PLAN, Study Design.

11.0 ADVERSE EVENT REPORTING REQUIREMENTS

11.1 Notifying the FDA

The study sponsor will report adverse events in an expedited fashion to the FDA. These written notifications of adverse events (IND safety reports) will follow the following guidelines:

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

- **Within 15 calendar days**
  Any study event that is:
  - associated with the use of the study drug,
  - unexpected, and
  - serious, but not fatal or life-threatening

    -or-
  - 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).

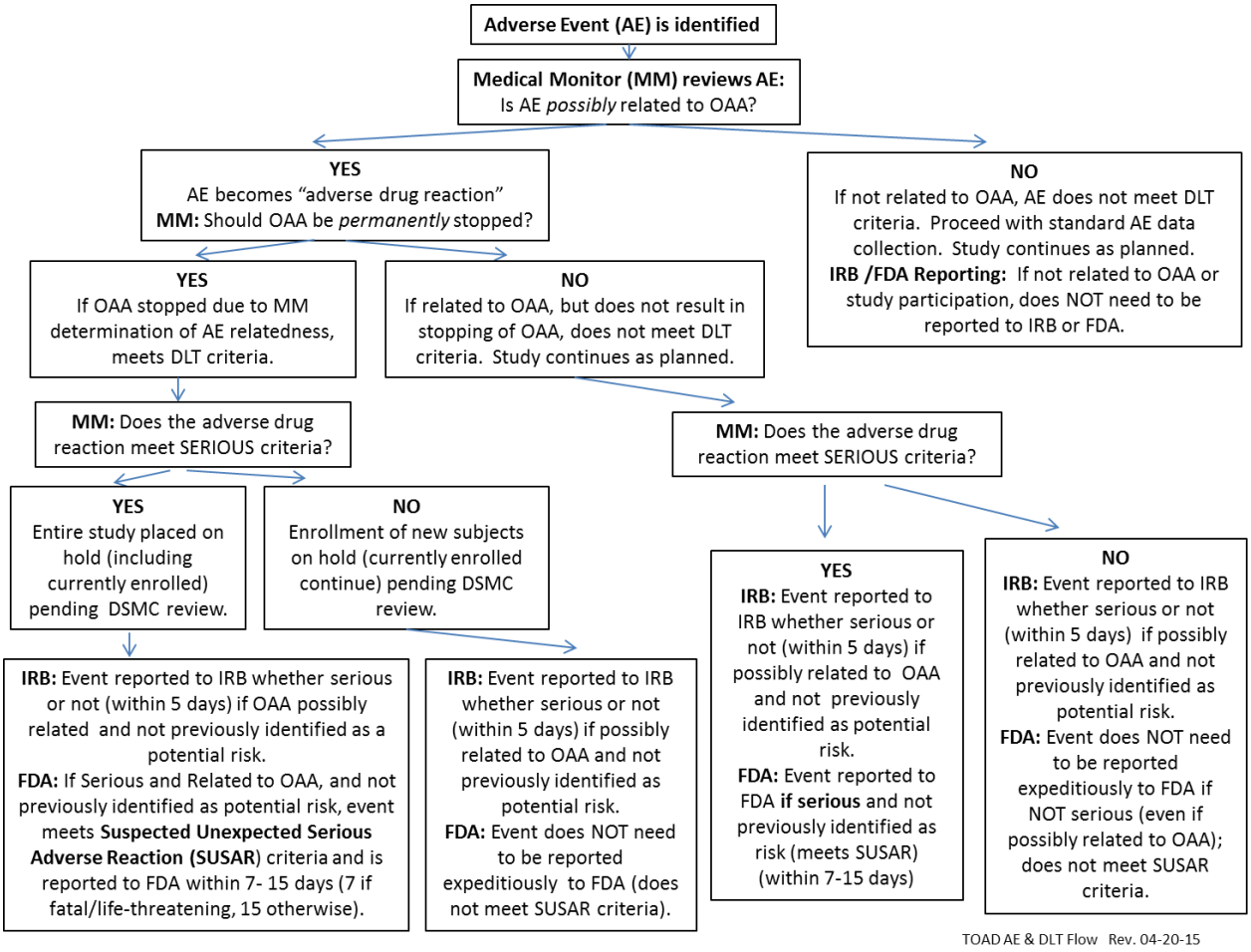
12.0 DATA AND SAFETY MONITORING

Data Collection

The PI will oversee all data collection.

Data and Safety Monitoring

A medical monitor, Jeffrey Burns, MD, will assess adverse events to determine severity and causality. A protocol for handling adverse events is provided in the Figure below. Adverse events will be reported to the local IRB according to IRB reporting policies and to the FDA according to the above requirements. An internal safety committee will meet quarterly to review adverse events and the overall safety of the ongoing trial. The following individuals will serve on this committee: Richard Dubinsky, MD, Michael Abraham, MD, and Kelly Lyons, PhD.



13.0 REGULATORY CONSIDERATION

Protection of Human Subjects and Informed Consent

Regulations in regard to Human Subjects Protection and Informed Consent will be followed in accordance with 21 CFR 50. All institutional, FDA, state, and federal regulations concerning informed consent will be followed.

Institutional Review Board (IRB)

Regulations regarding initial and continuing review of the study by an Institutional Review Board will be followed. The University of Kansas Medical Center has a federally approved

Institutional Review Board, which will be involved in all aspects of study planning and considerations as per 21 CFR 56. The KUMC IRB FWA# is FWA00003411.

Financial Disclosure

There are no financial disclosures to be made.

Obligations of Investigators

The Principal Investigator/Sponsor, Dr. Russell Swerdlow, will be responsible for all elements of 21 CFR 312 subpart D. Dr. Swerdlow will maintain oversight of the clinical trial ensuring relevant regulatory requirements are met and necessary guidelines are followed. The following are co-investigators:

- Jeffrey Burns, MD
- Jonathan Mahnken, PhD
- William Brooks, PhD
- Eric Vidoni, PhD
- Janna Harris, PhD

The Principal Investigator has confirmed all co-investigator credentials, experience, and training. He has also ensured additional personnel will follow the appropriate investigational plans and guidelines as submitted in this IND. All changes to the study, including modifications to this IND, will be communicated by the Principal Investigator, and he will be the primary liaison to the IRB.

All detected and newly discovered adverse effects and risks will be reported by the involved investigator at the time of the event, per institutional guidelines. Each investigator is also responsible for privacy and welfare of his patient. Record-keeping and data is strictly confidential, only to be shared between investigators in the study and only those who are directly involved in the care of the patient.

14.0 STATISTICAL CONSIDERATION

The statistical analysis will be conducted as described under 5.0 TREATMENT PLAN, Study Design.

15.0 PUBLICATION PLAN

Publications resulting from this study will be prepared by the PI. Co-authors will include personnel from the study site who have meaningfully contributed to study execution or development, as well as a project consultant (Dr. Lisa Mosconi, NYU Medical Center). Only co-authors who agree with the final manuscript will be listed as authors on the final manuscript.

16.0 REFERENCES

1. Wilkins, H.M., et al., Oxaloacetate activates brain mitochondrial biogenesis, enhances the insulin pathway, reduces inflammation and stimulates neurogenesis. Hum Mol Genet, 2014. **23**(24): p. 6528-41.
2. Swerdlow, R.H., The neurodegenerative mitochondriopathies. J Alzheimers Dis, 2009. **17**(4): p. 737-51.
3. Hebert, L.E., et al., Alzheimer disease in the US population: prevalence estimates using the 2000 census. Arch Neurol, 2003. **60**(8): p. 1119-22.
4. Alzheimer's Association. 2011 Alzheimer's disease facts and figures. Alzheimer's and Dementia, 2011. **7**: p. 208-244.
5. Swerdlow, R.H., Mitochondria and cell bioenergetics: increasingly recognized components and a possible etiologic cause of Alzheimer's disease. Antioxid Redox Signal, 2012. **16**(12): p. 1434-55.
6. Mosconi, L., et al., FDG-PET changes in brain glucose metabolism from normal cognition to pathologically verified Alzheimer's disease. Eur J Nucl Med Mol Imaging, 2009. **36**(5): p. 811-22.
7. Swerdlow, R.H., Bioenergetic medicine. Br J Pharmacol, 2014. **171**(8): p. 1854-69.
8. Hakimi, P., et al., Overexpression of the cytosolic form of phosphoenolpyruvate carboxykinase (GTP) in skeletal muscle repatterns energy metabolism in the mouse. J Biol Chem, 2007. **282**(45): p. 32844-55.
9. McKhann, G.M., et al., The diagnosis of dementia due to 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**(3): p. 263-9.
10. Yoshikawa, K., Studies on the anti-diabetic effect of sodium oxaloacetate. Tohoku J Exp Med, 1968. **96**(2): p. 127-41.
11. Keller, C., et al., Long-term effects of galantamine treatment on brain functional activities as measured by PET in Alzheimer's disease patients. J Alzheimers Dis, 2011. **24**(1): p. 109-23.
12. Williams, D.S., et al., Oxaloacetate supplementation increases lifespan in Caenorhabditis elegans through an AMPK/FOXO-dependent pathway. Aging Cell, 2009. **8**(6): p. 765-8.
13. Yamamoto, H.A. and P.V. Mohanan, Effect of alpha-ketoglutarate and oxaloacetate on brain mitochondrial DNA damage and seizures induced by kainic acid in mice. Toxicol Lett, 2003. **143**(2): p. 115-22.
14. Campos, F., et al., Oxaloacetate: a novel neuroprotective for acute ischemic stroke. Int J Biochem Cell Biol, 2012. **44**(2): p. 262-5.
15. Ruban, A., et al., Blood glutamate scavengers prolong the survival of rats and mice with brain-implanted gliomas. Invest New Drugs, 2012.
16. Zlotnik, A., et al., Effect of glutamate and blood glutamate scavengers oxaloacetate and pyruvate on neurological outcome and pathohistology of the hippocampus after traumatic brain injury in rats. Anesthesiology, 2012. **116**(1): p. 73-83.
17. Villeda, S.A., et al., The ageing systemic milieu negatively regulates neurogenesis and cognitive function. Nature, 2011. **477**(7362): p. 90-4.