SAFETY AND FEASIBILITY OF DASATINIB AND QUERCETIN IN ADULTS AT RISK FOR ALZHEIMER'S DISEASE (STAMINA)

STATISTICAL ANALYSIS PLAN

NCT Number: 05422885
January 23, 2024

Statistical Analysis Plan

Advarra Protocol Number: Pro00053594

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Synopsis. This document provides the statistical analysis plan (SAP) for the Senolytics to Improve Cognition and Mobility in Older Adults at Risk of Alzheimer's Disease (STAMINA) trial (NIA AG073886). This is an open-label single arm trial intended to develop evidence of feasibility of delivery of combination Dasatinib and Quercetin (D+Q) for preservation of cognitive function among older adults at risk of Alzheimer's disease.

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Introduction

Abnormalities in cognition and mobility are common accompaniments of aging that often precede the development of Alzheimer's disease. Among their many etiologies, these abnormalities are associated with alterations in the regulation of cerebral blood flow to frontal regions of the brain that subserve executive functions and gait speed. We have previously shown that treatment with cocoa flavanols can improve blood flow in response to a cognitive task (neurovascular coupling [NVC]), as well as executive function in older people with impaired NVC. These compounds can also reduce the number of senescent cells and their toxic secretory products (SASP) in a variety of tissues. In mice, "senolytic" compounds such as flavanols and tyrosine kinase inhibitors, have been shown to reduce neurofibrillary tangle density, neuron loss, and ventricular enlargement, and in humans with idiopathic pulmonary fibrosis, improve gait speed and other functional abilities. Based on these findings, we hypothesize that the flavanol, Quercetin, and tyrosine kinase inhibitor, Dasatinib, (Q+D) will improve NVC in response to an executive task, reduce circulating SASP components, and in so doing, improve cognition and mobility in older adults who are at risk of Alzheimer's disease.

This single-arm, open label study will be used to demonstrate the feasibility of administration of Q+D among older adults at risk of Alzheimer's Disease, and to develop preliminary evidence of the combination's ability to preserve cognitive function among such individuals.

Design, Objectives, and Hypotheses

This is a single-site, single-arm, open label, 12 week pilot study conducted in 12 older adults aged ≥65 years with slow gait speed (<1.0 m/sec) and Mild Cognitive Impairment. The overall objective is to demonstrate the feasibility and safety of administering intermittent doses of two senolytic compounds Dasatinib and Quercetin (D+Q) in older adults at risk of Alzheimer's disease. Screened and eligible participants will perform baseline physical and cognitive assessments, as well as provide a blood and urine sample. Two weeks after their last dose, participants will complete a follow-up assessment of physical and cognitive function, as well as provide a blood and urine sample. Safety and possible adverse events will be monitored throughout the entire duration of the study as described below. The specific aims are:

1) To determine the feasibility, safety, and recruitment challenges of studying intermittent doses of D+Q in older adults at risk of Alzheimer's disease. We will evaluate the number of volunteers needed to be screened to identify eligible participants, the number of protocol deviations that are necessary to ensure safe and scientifically rigorous human subject participation (reported to the FDA and IRB), the frequency of any adverse drug effects, and compliance with medication administration.

2) To obtain preliminary data on the effect of this D+Q regimen on: a) sitting and standing cerebral blood flow (CBF), and neurovascular coupling (NVC) during an executive task, b) gait speed, c) and executive function. We will measure CBF during sitting and standing, NVC during an N-Back cognitive task, 4 meter gait speed, and executive function via the Trail Making Test (TMT) pre- and post-intervention to estimate the average changes and variability of these measures. Such information will be used to design future, large-scale efficacy trials.

3) To develop preliminary evidence concerning whether D+Q is associated with a) a reduction in biomarkers of senescence in serum and urine and senescent cells in blood, and b) whether reductions in these biomarkers are associated with improvements in NVC, gait speed, and executive function. We will measure biomarkers of senescence in urine and plasma and senescent cells in blood pre- and post-intervention to obtain preliminary evidence of potential mechanisms.

We hypothesize that 12 week co-administration of intermittent doses of D+Q in older adults at risk of Alzheimer's disease will be feasible and safe.

Administration of Intervention

Participants will ingest 100 mg of Dasatinib and 1,250 mg of Quercetin for two consecutive days. Every two weeks, the same cycle will be repeated where participants are asked to take the same dose of study medications for 2 consecutive days. A total of six, 2-day administrations of study medications will take place over a total of 12 weeks.

Outcomes Measures

The primary outcome measure will be the proportion of individuals screened who advance to full enrollment in the trial, defined as 'recruitment rate' in **Appendix**; **Table 1**. Outcomes related to safety will include incidence of adverse events (AE) and serious adverse events (SAE; **Table 1**.) Other endpoints will include measures of physical and executive function (Tables 2 and 3 respectively) and biomarkers of senescence (**Table 4**).

Analytic plan

Overall approach

Outcomes measures will be computed as detailed in **Tables 1-4**. We will assess distributional characteristics of each outcome measure, and develop descriptions of each endpoint using tabular and graphical summaries. Following this, descriptive comparisons of pre- and post- intervention measures will be developed as appropriate.

Aim-specific Analyses

Aim 1 is concerned with feasibility and safety of administration of the combination intervention. Recruitment rate will be computed with reference to the number of potential enrollees approached for screening (**Table 1**) and coupled with an 80% confidence interval to assist with planning of future trials.

Total incidence of each AE and SAE will be computed and reported.

Medication compliance will be estimated with a participant-level mean and corresponding 80% confidence interval.

Aim 2 is concerned with physical and executive function, which are measured at three timepoints (**Tables 2 and 3**). Descriptive assessments will be developed at each timepoint. A mixed-effects regression model incorporating random intercepts will be used to develop estimates, using regression contrasts, of within-individual change in each measure at weeks 8 and 14. Estimates of changed will be accompanied by model-based 80% confidence intervals.

Aim 3 is concerned with biomarkers of senescence, which are measured at baseline and 14 weeks (**Table 4**). Descriptive assessments will be developed at each timepoint, and within-participant change scores computed. Point and 80% confidence interval estimates of mean change will be computed for each marker. Correlations between changes in biomarkers of senescence and changes in functional outcomes will be assessed using scatterplot smoothing and summarized using mixed-effects linear or nonlinear regression analyses as appropriate.

Sensitivity Studies

The potential influence of age and sex will be explored via stratification and added variable plotting as appropriate. Participant compliance with intervention will be summarized, and association with other outcomes examined with descriptive analyses as appropriate.

Sample size considerations

This project will enroll 12 individuals who will be assessed before, during, and following the 12-week D+Q intervention. The proposed sample size is motivated by the need to make resource assessments in the design of a subsequent RCT of D+Q. We anticipate screening at least two and possibly many more individuals for every potential participant who is eligible and advances to enrollment. Under conservative assumptions we will be able to estimate the proportion of participants screened who are eligible and advance to enrollment to within 0.20 using an 80% exact binomial confidence interval, the upper bound of which will be used in planning the subsequent trial. We will additionally utilize data obtained in this pilot to similarly estimate the variability of outcomes measures and differences attributable to administration of D+Q; again using an 80% confidence interval, we will be able to estimate the standard deviation of continuous measures including CBF and gait speed to within 0.3 standardized units. The design is not intended to provide sufficient data to test the efficacy or effectiveness of D+Q on endpoints relevant for Aims 2 and 3. However, prior small human studies have demonstrated surprisingly robust relationships. For instance, a recent human study of only 9 subjects by the Kirkland group successfully demonstrated that D+Q reduced adipose tissue senescent cell burden within 11 days after a single 3 day course of treatment. Furthermore, circulating SASP factors were reduced in these 9 subjects. We therefore anticipate that we will see suggestive evidence of an association with SASP factors and inflammatory biomarkers in serum and urine, and senescent cells in blood, sufficient to motivate moving forward to a larger clinical trial.

Statistical Programming

Analyses will be performed using R version 4.2.2 or later (R Foundation for Statistical Computing, Vienna) or SAS version 9.3 or later (SAS Institute, Cary, NC.)

Appendix. Tables: Detailed Description of Outcomes Measures

Table 1: Feasibility- and Safety-Related Outcomes

Outcome	Unit	Type	Timeframe	Brief description
Recruitment Rate	#of participants completed study/# of screened participant	Primary	Throughout the entire study	The number of volunteers needed to be screened to identify eligible participants.
Adverse Events	N of AE's	Primary	Throughout the entire study	The frequency of adverse events – related or possibly related to the study medications.
Serious Adverse Events	N of SAE's	Primary	Throughout the entire study	The frequency of serious adverse drug events.
Medication Compliance	% of intended doses consumed	Primary	Week 2 (Baseline), Week 4, Week 6, Week 8, Week 10, Week 12, Week 14	Compliance with medication administration. Assessed via self-reported intake of the at-home dosage as well as returned pill containers/pills.

Table 2: Physical Function-Related Outcomes

Outcome	Unit	Type	Timeframe	Brief description
Gait Speed	Meters/sec	Primary	Screening, 8, and 14 weeks	This metric assesses the ability to control gait. It is performed without a distracting cognitive task. The faster of two trials will be used.
SPPB: Short Portable Performance Battery	Points	Secondary	Week 2 (Baseline), 8, and 14 weeks	Physical performance, including gait, balance, and strength to perform a chair stand. The total score will be used.
TUG: Timed Up and Go	Meters/sec	Secondary	Week 2 (Baseline), 8, and 14 weeks	This is a timed test of mobility, including standing from a chair, walking 20 feet, and turning. The faster of the two trials will be used.
Grip strength	kg	Secondary	Week 2 (Baseline), 8, and 14 weeks	This test measures grip strength using a hand dynamometer. The maximum of the 3 trials will be used.

Table 3: Executive Function Related Outcomes

Outcome	Unit	Type	Timeframe	Brief description
Full MoCA score	Points	Primary	Week 2 (Baseline), 8, and 14 weeks	An evaluation of global cognitive function
2 Trial Avg of Left MCA cerebral blood flow during cognitive task (2BK - BL2BK) – (IDX – BL IDX)	Cm/sec	Primary	Screening, 8, and 14 weeks	Speed of blood flow on the Left MCA to the brain to increase blood flow in response to a cognitive task. Two trials averaged.
2 Trial Avg of Right MCA cerebral blood flow during cognitive task (2BK - BL2BK) – (IDX – BL IDX)	Cm/sec	Primary	Screening, 8, and 14 weeks	Speed of blood flow on the Right MCA to the brain to increase blood flow in response to a cognitive task. Two trials averaged.
1st Trial of Left MCA cerebral blood flow during cognitive task (2BK - BL2BK) – (IDX – BL IDX)	Cm/sec	Primary	Screening, 8, and 14 weeks	Speed of blood flow on the Left MCA to the brain to increase blood flow in response to a cognitive task. 1st trial only.
1st Trial of Right MCA cerebral blood flow during cognitive task (2BK - BL2BK) – (IDX – BL IDX)	Cm/sec	Primary	Screening, 8, and 14 weeks	Speed of blood flow on the Right MCA to the brain to increase blood flow in response to a cognitive task. 1st trial only.
Avg of Left and Right MCA cerebral blood flow during cognitive task (2BK - BL2BK) – (IDX – BL IDX)	Cm/sec	Primary	Screening, 8, and 14 weeks	Speed of blood flow on the Right MCA to the brain to increase blood flow in response to a cognitive task. Two trials on each side (left vs right) averaged.
1st Trial only Avg of Left and Right MCA cerebral blood flow during cognitive task (2BK - BL2BK) – (IDX – BL IDX)	Cm/sec	Primary	Screening, 8, and 14 weeks	Speed of blood flow on the Right MCA to the brain to increase blood flow in response to a cognitive task. 1st trial only on each side (left vs right)
Executive Function (Trails B minus A)	Percentile	Primary	Week 2 (Baseline), 8, and 14 weeks	Executive function corrected for response time
Dual-task cost in gait speed ([$Velocity_{ST}$ - $Velocity_{DT}$] / $Velocity_{ST}$ x 100%)	% difference	Secondary	Week 2 (Baseline), 8, and 14 weeks	Gait speed in response to a cognitive task (DT). Compared to a gait speed without (ST).
Dual-task cost in accuracy([$Accuracy_{ST}$ - $Accuracy_{DT}$] / $Accuracy_{ST}$ x 100%)	% difference	Secondary	Week 2 (Baseline), 8, and 14 weeks	This test measures accuracy of a cognitive task while walking (DT), compared to accuracy while completing the cognitive task while sitting (ST).

Table 4: Biomarkers of Senescence

Outcome	Unit	Type	Timeframe	Brief description
Senescent CD3 cells expressing $p16^{INK4A}$		Secondary	Screening and 14 weeks	This measures the number of senescent CD3 lymphocytes in blood.
Urinary interleukin-1- alpha	picogram/mL	Secondary	Screening and 14 weeks	Assay will measure the senescence associated biomarkers, interleukin-1-alpha (picogram/mL), in urine.
Serum interleukin-1-alpha	picogram/mL	Secondary	Screening and 14 weeks	Assay will measure the senescence associated biomarkers, interleukin-1-alpha (picogram/mL), in serum.
Urinary interleukin-6	picogram/mL	Secondary	Screening and 14 weeks	Assay will measure the senescence associated biomarkers, interleukin-6 (picogram/mL), in urine.
Serum interleukin-6	picogram/mL	Secondary	Screening and 14 weeks	Assay will measure the senescence associated biomarkers, interleukin-6 (picogram/mL), in serum.
Urinary MMP-9	nanogram/mL	Secondary	Screening and 14 weeks	Assay will measure the senescence associated biomarkers, MMP-9 (nanogram/mL), in urine.
Serum MMP-9	nanogram/mL	Secondary	Screening and 14 weeks	Assay will measure the senescence associated biomarkers, MMP-9 (nanogram/mL), in serum.
Urinary MMP-12	nanogram/mL	Secondary	Screening and 14 weeks	Assay will measure the senescence associated biomarkers, MMP-12 (nanogram/mL), in urine.
Serum MMP-12	nanogram/mL	Secondary	Screening and 14 weeks	Assay will measure the senescence associated biomarkers, MMP-12 (nanogram/mL), in serum.