Anti-viral Therapy in Alzheimer's Disease

NCT03282916

July 14, 2025

July 14th, 2025

Statistical Analysis Plan for "Antiviral therapy: Valacyclovir Treatment of Alzheimer's Disease (VALAD) Clinical Trial"

1. Introduction.

The aim of the study was to evaluate the efficacy of Valacyclovir treatment in a 78-weeks, multicenter, randomized, double-blind, placebo-controlled, parallel-group treatment trial in participants with early Alzheimer's Disease (AD) and positive serum antibodies (IgG or IgM) to HSV1 or HSV2.

This statistical analysis plan provides more detailed descriptions of the statistical analyses conducted in the paper.

2. Study Design

One hundred and twenty participants with clinical diagnosis of probable AD or mild cognitive impairment (MCI), confirmed by a positive amyloid PET or FDG PET scan or a CSF AD profile, were randomized to Valacyclovir or placebo at 1:1 ratio. Block randomization with varying block sizes (2 and 4) was used to reduce the risk of treatment allocation prediction by clinicians. Participants were evaluated at five scheduled visits (weeks 0, 12, 26, 52, and 78). As a proof-of-concept study, the target dose of Valacyclovir was 4 g per day -- at the higher end of the usual oral dosing range. Treatment began at 2 g per day (1 g twice daily), with the dose increased by 1 g per day every two weeks until reaching either 4 g per day or the participant's maximum tolerated dose.

3. Outcome Measures.

1) Cognitive and functional measures:

- **Alzheimer's Disease Assessment Scale – Cognitive Subscale 11 (ADAS-Cog 11)**, assessed at weeks 0, 12, 26, 52, 78; scoring range 0-70, higher scores indicate greater cognitive impairment. (Primary)
- **Alzheimer's Disease Cooperative Study-Activities of Daily Living Scale (ADCS-ADL),** assessed at weeks 0, 12, 26, 52, 78; scoring range 0-78, higher scores indicate better daily functioning. (Secondary)
- **Craft story delayed verbatim recall**, assessed at weeks 0, 52, 78; scoring range 0-44, higher scores indicating better memory. (Secondary)
- **Montreal Cognitive Assessment (MoCA),** assessed at weeks 0, 52, 78; scoring range 0 to 30, higher scores indicating better global cognition. (Secondary)

For all cognitive and functional measures, the outcome of interest is the change from baseline at each follow-up time point. The primary endpoint is the change from baseline to 78 weeks.

2) Imaging measures:

- **^{18F}Florbetapir PET SUVR:** Mean standardized uptake value ratio (SUVR) from the medial orbitofrontal cortex, anterior cingulate, parietal lobe, posterior cingulate, temporal lobe, and precuneus, normalized to cerebellar gray matter.
- **^{18F}MK-6240 PET SUVR (Medial Temporal):** SUVR averaged across medial temporal regions including the amygdala, hippocampus, entorhinal cortex, and parahippocampus.
- **^{18F}MK-6240 PET SUVR (Global Mean):** SUVR global mean normalized to cerebellar gray matter.
- **MRI Cortical Thickness:** Mean cortical thickness across nine predefined brain regions.
- **MRI Hippocampal Volume:** Volume of the hippocampus.

All imaging measures were collected at baseline and 78 weeks. The outcomes are defined as the change in each measure from baseline to 78 weeks.

4. Sample Size Calculation.

The sample size calculation was based on the primary outcome: change in ADAS-Cog11 scores from baseline to 78 weeks, using the RMASS program for longitudinal studies. Assuming a within-subject correlation of r=0.3 (moderate correlation) for repeated measures and a uniform dropout rate reaching 15% by 78 weeks, a total sample size of 130 participants (65 per arm) was originally projected to detect Cohen's d of 0.50 with 80% power at 5% significance level. With approval from sponsor (NIA) and DSMB, the recruitment target was reduced to 120 participants due to pandemic-related recruitment delays and required study completion within the extended funding timeline. For n=120, the minimum detectable effect size increased slightly to a Cohen's d of 0.52.

5. Statistical analysis

The analyses were conducted on the Intent-to-treat (ITT) sample, i.e., all randomized participants according to the treatment that they were assigned. All hypotheses were tested at level of significance of 5%. There was no adjustment for multiple statistical comparisons in this trial. All analyses were conducted using R.

We first examined patients' baseline characteristics to ensure that covariates were balanced between treatment and placebo arms. Continuous variables were summarized using means and standard deviations, while categorical variables were summarized using counts and percentages.

Linear mixed effects models were used to evaluate the efficacy of Valacyclovir as compared to placebo on cognitive and functional outcomes. Specifically, for each outcome measure, we considered the following model

$$\Delta Y_{it} = \beta_0 + \beta_1 * Group_i + \boldsymbol{\alpha} * Time_{it} + \boldsymbol{\gamma} * Group_i * Time_{it} + \delta Y_{i0} + b_i + \epsilon_{it}, \qquad (1)$$

where ΔY_{it} is change of the outcome measure (week t minus baseline) for subject i at timepoint t, $Group_i$ is the treatment group indicator for subject i (1= Valacyclovir and 0=placebo), $Time_{it}$ is the visit time point (treated as a categorical variable), Y_{i0} is baseline value of the outcome measure for subject i, b_i is a subject-specific random intercept, and ϵ_{it} is the unexplained

residual error term. $(\beta_0, \beta_1, \boldsymbol{\alpha}, \boldsymbol{\gamma}, \delta)$ are fixed effects parameters. The efficacy of Valacyclovir versus placebo at each time point was tested by forming contrasts of the fitted model. The model was further adjusted for key demographic and genetic variables, including age, sex, and apolipoprotein E ε4 carrier status. Missing data on outcome variables were dealt with by using (longitudinal) linear mixed effects models which do not require complete measurements under the "missing at random" assumption.

For the primary outcome (ADAS-Cog 11), a series of sensitivity analyses were conducted to evaluate the robustness of the findings across key clinical subgroups and under different analytic assumptions. These included: 1) a per-protocol analysis limited to participants who completed the study; 2) a subgroup analysis of participants who received cholinesterase inhibitors or memantine; and 3) a subgroup analysis of participants with a baseline 18F-Florbetapir PET SUVR ≥ 1.15 (amyloid positive for AD).

Linear regression analyses were performed to evaluate the effect of Valacyclovir on changes in imaging outcomes from baseline to 78 weeks. Each model included the baseline value of the corresponding imaging measure as a covariate to control for initial differences. The models were further adjusted for key demographic and genetic variables, including age, sex, and apolipoprotein E ε4 carrier status. To assess the robustness of the findings and address potential bias due to missing data at follow-up, sensitivity analyses were conducted using weighted linear regression. In these models, the weights were calculated as the inverse probability of a subject being a completer, with probabilities estimated using logistic regression with ridge regularization to handle potential collinearity among baseline variables.

To assess safety, adverse events were systematically evaluated. For each type of adverse event, number and proportion of participants who experienced the adverse event was reported and compared by treatment arms using Fisher's exact test. In addition, plasma acyclovir and CSF acyclovir concentrations obtained at 12 weeks and 78 weeks were summarized using means and standard deviations.