Sample Size Calculation

The main goal of the pilot study is to provide effect sizes of the outcomes to guide future larger trials. The target sample size of 50 participants (25 per arm) was chosen in accordance with the "stepped rules of thumb" for pilot trials proposed by Whitehead et al. (2016), which indicate that this sample size is mathematically optimal for a pilot study intended to inform the design of a future trial fully powered at 90% to detect a small standardized effect size (Cohen's d=0.2). In addition, this sample size yields a 95% confidence interval for the between-group difference with an approximate margin of error of 0.57 standard deviations, providing sufficient precision to estimate both the treatment effect and the population variance needed to plan a large trial.

Statistics

Baseline variables will be summarized by treatment arms using means and standard deviations for continuous variables and counts and percentages for categorical variables. Analyses will include all participants according to the assigned treatment (intention to treat analyses).

Linear regression analyses will be conducted to evaluate treatment effects on change in imaging outcomes ($^{18}F^{-}$Florbetapir PET SUVR, MRI-based hippocampal volume, MRI-based cortical thinning and plasma p-tau217) from baseline to 52 weeks, adjusting for baseline value of the imaging measure, age, sex and apolipoprotein E e4 positive status. Linear mixed effects models (LMM) will be used to assess the treatment effects on cognitive and functional outcomes (PACC, ADCS-ADL-PI, MMSE and CDR sum of boxes). Change score from baseline will be specified as the dependent variable, with treatment group, study time point (categorical), and their interaction included as predictors, adjusting for the baseline value of the outcome, age, sex, and apolipoprotein E ε4 carrier status. Subject-specific random effects will be included to

account for within-subject correlation. Each model incorporates all available follow-up assessments at 12, 26, and 52 weeks.

Adverse events by treatment group will be summarized by frequencies of occurrence. Plasma acyclovir concentrations obtained at 52 weeks will be summarized using means and standard deviations. All statistical analyses will be conducted using R version 4.3.3. As this is a pilot study, statistical significance will be assessed using a two-sided alpha level of 0.05, with no adjustment for multiple comparisons.