**Online Resource for *Drugs* Article**

(Supplementary Information)

# Clinical Efficacy, Safety and Imaging Effects of Oral Valiltramiprosate in APOEε4/ε4 Homozygotes with Early Alzheimer's Disease: Results of Phase 3, Randomized, Double-Blind, Placebo-Controlled, 78-Week APOLLOE4 Trial

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## Methods

### Trial design – Short Summary

The APOLLOE4 phase 3 trial (ALZ-801-AD301; Online Resource Fig. 1) was a randomized, double-blind, placebo-controlled, 2-arm, multi-center, 78-week trial that was conducted at 77 North American and European centers (Online Resource Table 1). The trial enrolled APOE4/4 homozygotes with Early Alzheimer's disease (AD), which included mild cognitive impairment (MCI) and mild AD dementia (Mild AD). Randomization followed a 1:1 ratio to valiltramiprosate or placebo and was stratified by acetylcholine esterase inhibitor (AChEi) use, age (50-65 years or > 65 years), sex, and disease severity at screening based on the Mini-Mental State Examination (MMSE) score (≤ 26 or > 26). Stratification was performed for the overall study population but not for the MCI and Mild AD subgroups because both groups were expected to show similar efficacy. Valiltramiprosate was titrated from a dose of 265 mg/day for 2 weeks to 265 mg twice daily. Details of trial design and clinical measures have been published [1, 2]. The trial desing is consistent with current regulatory guidances for Early AD, and the clinical outcome measures have been used in other Phase 3 trials of anti-amyloid antibodies, including two approved drugs [5-7].

### Participants

Male and female APOE4/4 participants aged 50 to 80 years with MCI or Mild AD were enrolled based on NIA-AA (National Institute on Aging Alzheimer's Association) clinical criteria. At US sites, dedicated efforts were made to screen and enroll participants from underrepresented populations [2]. Participants had a screening MMSE of 22 to 30, inclusive, a CDR – Global score of 0.5 or 1 with memory box score ≥0.5, and a Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) delayed memory index score ≤85 and had to fulfill all the inclusion/exclusion criteria, including satistfactory ECG and laboratory tests (chemistry,

hematology, coagulation tests, TSH, B12 levels). Participants were required to have no other neurodegenerative diseases, major depression or other psychiatric diagnoses. They were required to be healthy or have stable adequately treated medical conditions, a body mass index of 17 to 40 $kg/m^{2,}$ andglomerular filtration rate ≥40 ml/min. Screening MRI findings of ARIA-E, large infarcts, severe white matter disease (Fazekas score > 2) or macrohemorrhages were exclusionary, but any number of microhemorrhages were allowed, and having >3 siderosis lesions required sponsor approval.

A stable dose of AChEi was allowed, but change in AChEi dose was not allowed during the trial. Use of memantine and anticoagulants during the trial was prohibited. Atypical anti-psychotics and anti-epilptic medications were allowed only at low doses. Use of anti-amyloid antibodies within the prior 6 months of randomization or lifetime use of active amyloid vaccines was prohibited.

**MRI Methods**

MRI scans from 1.5/3 Tesla magnets were obtained at baseline and at 26, 52, and 78 weeks for volumetric and safety analyses; the harmonized acquisition protocols were overseen by Clario and volumetric and safety MRI analyses were conducted by Clario Inc. All vMRI data were processed centrally with fully automated methods using FreeSurfer v6.0 for brain segmentation at baseline [3]. Bilateral hippocampal volume (HV, left + right), cortical thickness (CT), and whole brain volume (WBV) were derived. Volume changes at follow-up time points were assessed using boundary shift integral. For CT measure, a composite average thickness of medial temporal areas was derived as the Mayo index, as well as average of whole brain CT. Changes in CT (CT, Mayo index) were analyzed by a Jacobian-based method. HV is presented in mm3, cortical thickness in mm and WBV and ventricular volume (VV) in ml. Volumetric measures

were adjusted for age, years of education, and head size. ARIA was assessed using T2-weighted and gradient echo sequences [4]. ARIA-E finding of any severity required repeat MRIs in 4-6 weeks or till resolution.

**DTI Imaging**

At approximately 50 sites, MRI testing included single-shell diffusion tensor imaging (DTI) and provided mean water diffusivity and fractional anisotropy measures in the grey matter and white matter. All DTI analyses were performed by Clario Inc.

**Plasma PK**

Plasma for pharmacokinetics and biomarkers was collected at each visit. The PK and plasma biomarker samples were batched for analyses using assays validated for clinical research use.

**Statistical Analysis**

The sample size estimation was based on data from the APOE4/4 mild AD subgroup of the tramiprosate Phase 3 study that showed ~ 4.5 benefit versus placebo at 78 weeks on the ADAS-Cog outcome [1]. A more conservative estimate was that ADAS-Cog13 would show a difference in change from baseline between the active and placebo arm of 2.0 to 2.5 points, with standard deviations of 8.1 and 5.6, respectively. A sample size of 125 participants per arm provided 80% to 90% power to show this difference at a 2-sided $\alpha = 0.05$. Assuming an early termination rate of 22%, 320 enrolled and 125 completers/arm provided this power.

The primary efficacy population was the Full Analysis Set, which included participants with a baseline and postbaseline efficacy measure. The primary analysis used a mixed-effect model repeated measure (MMRM) model with fixed-class effect terms for sex, age group, treatment group, disease severity (screening MMSE), use of concomitant AD medications, visit, treatment-

by-visit interaction, and baseline ADAS-Cog13 by treatment-by-visit interaction as covariates. The other endpoints included the measure's baseline score. Model diagnostics were performed to select either a linear or quadratic baseline term for the interaction between baseline, treatment, and visit.

The model presented used the prespecified covariates in the Statistical Analysis Plan (SAP). Interactions between the prespecified covariates were examined during model-checking and these interactions were included because the model assumption of homogeneity of effect across disease severity was violated. This is supported by the corrected Akaike information criteria, which indicates that without these interactions between the prespecified covariates the estimated variance of the model was unreliable. The inclusion of these interactions is reinforced by the significance of these terms, indicating differences in treatment effect based on severity, as had been observed in previous trials of amyloid-targeting drugs. The clinical outcomes required a quadratic baseline term. The vMRI measures followed a linear baseline relationship. Other analysis populations and prespecified sensitivity analyses were detailed in the SAP.

Considering the limited study sample size and the enrollment timeline, no interim analyses were planned or conducted. The final SAP and hierarchy of outcomes was updated and discussed with the US FDA in early 2024, several months prior to database lock.

**Online Resource Table 1** List of participating countries and number of participants

| **Country** | **No. of Participants** |
|---|---|
| United States | 152 |
| United Kingdom | 71 |
| Canada | 35 |
| Czech Republic | 5 |
| France | 24 |
| Germany | 3 |
| Iceland | 8 |
| Netherlands | 10 |
| Spain | 17 |
| **Total** | **325** |

**Online Resource Table 2** Demographics and baseline characteristics of participants by disease stage at screening (prespecified MCI and Mild AD)

| | **Disease Stage at Screening** | | | | | |
|---|---|---|---|---|---|---|
| | **MMSE 22-26 (N=195)** | | | **MMSE 27-30 (N=125)** | | |
| **Characteristic** | **Placebo (N=100)** | **ALZ-801 (N=95)** | **Overall (N=195)** | **Placebo (N=58)** | **ALZ-801 (N=67)** | **Overall (N=125)** |
| Sex, n (%) | | | | | | |
| Female | 49 (49.0) | 48 (50.5) | 97 (49.7) | 29 (50.0) | 36 (53.7) | 65 (52.0) |
| Age (years) | | | | | | |
| Mean (SD) | 68.7 (6.0) | 69.5 (6.0) | 69.1 (6.0) | 68.3 (6.0) | 66.9 (6.6) | 67.6 (6.4) |
| Race, n (%) | | | | | | |
| White | 89 (89.0) | 83 (87.4) | 172 (88.2) | 54 (93.1) | 60 (89.6) | 114 (91.2) |
| Ethnicity, n (%) | | | | | | |
| Not Hispanic or Latino | 84 (84.0) | 86 (90.5) | 170 (87.2) | 53 (91.4) | 61 (91.0) | 114 (91.2) |
| BMI ($kg/m^2$) | | | | | | |
| Mean (SD) | 25.3 (4.78) | 25.6 (3.83) | 25.5 (4.33) | 24.9 (3.98) | 26.2 (4.20) | 25.6 (4.13) |
| AchEI use, n (%) | 42 (42.0) | 41 (43.2) | 83 (42.6) | 18 (31.0) | 13 (19.4) | 31 (24.8) |
| MMSE total score | | | | | | |
| Mean (SD) | 23.7 (2.17) | 23.8 (2.04) | 23.7 (2.11) | 27.7 (1.35) | 27.9 (1.16) | 27.8 (1.25) |
| ADAS-Cog13 | | | | | | |
| Mean (SD) | 27.2 (8.94) | 27.4 (7.11) | 27.3 (8.08) | 19.3 (5.94) | 18.2 (6.69) | 18.7 (6.35) |
| CDR-SB total score | | | | | | |
| Mean (SD) | 3.36 (1.43) | 3.58 (1.43) | 3.47 (1.43) | 2.30 (1.21) | 2.28 (1.36) | 2.29 (1.28) |
| Hippocampal volume, bilateral (μL) | | | | | | |
| Mean (SD) | 6850 (958.1) | 6805 (946.0) | 6827 (950.1) | 7341 (916.1) | 7426 (1053) | 7387 (990.0) |
| Cortical thickness, bilateral whole brain (mm) | | | | | | |
| Mean (SD) | 2.39 (0.12) | 2.37 (0.09) | 2.38 (0.11) | 2.43 (0.10) | 2.46 (0.10) | 2.45 (0.10) |
| Whole brain volume (μL) | | | | | | |
| Mean (SD) | 1063344 (110274) | 1061492 (107897) | 1061923 (108813) | 1087795 (101003) | 1099196 (131117) | 1093970 (117931) |
| Baseline ventricular volume, bilateral (μL) | | | | | | |
| Mean (SD) | 45208 (21862) | 45586 (24543) | 45395 (23165) | 39176 (21497) | 32636 (13160) | 35634 (17707) |

AchEi, acetylcholine esterase inhibitor; ADAS-Cog13, Alzheimer's Disease Assessment Scale-Cognitive Subscale-13 item; CDR-SB, Clinical Dementia Rating Sum of Boxes; MMSE, Mini-Mental State Examination; SD, standard deviation. AD, Alzheimer's disease; MCI: mild cognitive impairment. Note: volume units are in μL ($mm^3$)

**Online Resource Table 3** Spearman's correlations of valiltramiprosate/ALZ-801 effects on vMRI outcomes at 78 weeks with clinical outcomes at 78 weeks (prespecified MCI population)

| **Clinical Outcome** | **Bilateral Hippocampal Volume Correlation (*P*-value)** | **Whole Brain Cortical Thickness Correlation (*P*-value)** |
|---|---|---|
| ADAS-Cog13 (N = 50) | **-0.3964 (0.0044)*** | -0.3436 (0.0146)* |
| CDR-SB (N = 49) | **-0.4448 (0.0014)*** | **-0.4890 (0.0004)*** |
| DAD (N = 50) | 0.3335 (0.0179)* | **0.3956 (0.0045)*** |

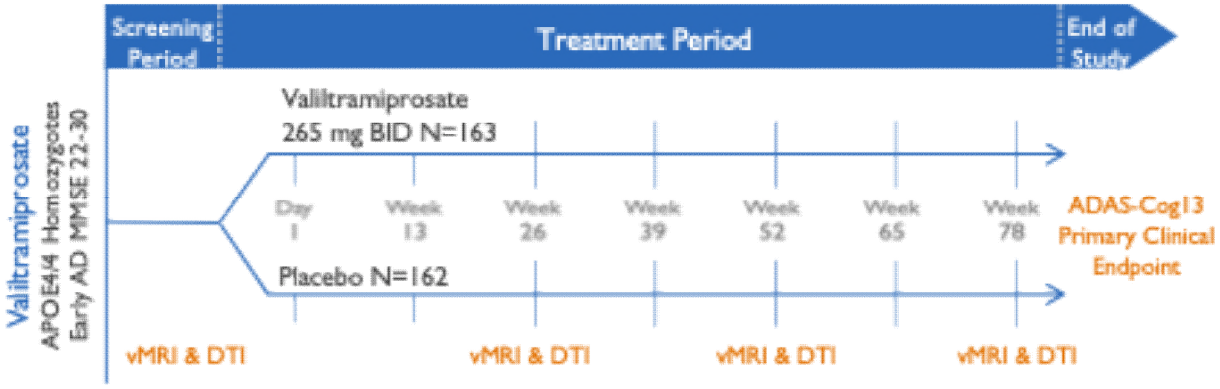
Spearman's correlations of valiltramiprosate (active arm) effects on the vMRI outcomes at 78 weeks to the effects on ADAS-Cog13, CDR-SB, and DAD at 78 weeks in the MCI population (MMRM data). Correlations between clinical and biomarker outcomes >0.30 are considered meaningful in Alzheimer's disease trials. N = number of participants who had both the vMRI and clinical measure at baseline and 78 weeks, allowing CBL correlations. ADAS-Cog13, Alzheimer's Disease Assessment Scale-Cognitive Subscale; CBL, change from baseline; CDR-SB, Clinical Dementia Rating – Sum of Boxes; DAD, Disability Assessment for Dementia; MCI, mild cognitive impairment; vMRI, volumetric magnetic resonance imaging. *Indicate statistically significant correlations, $p < 0.05$. Bolded correlations are those that achieved $p < 0.01$.

**Online Resource Table 4** Pearson's sequential correlations of plasma neurofilament light to hippocampal volume in ALZ-801/valiltramiprosate and placebo (prespecified MCI population)

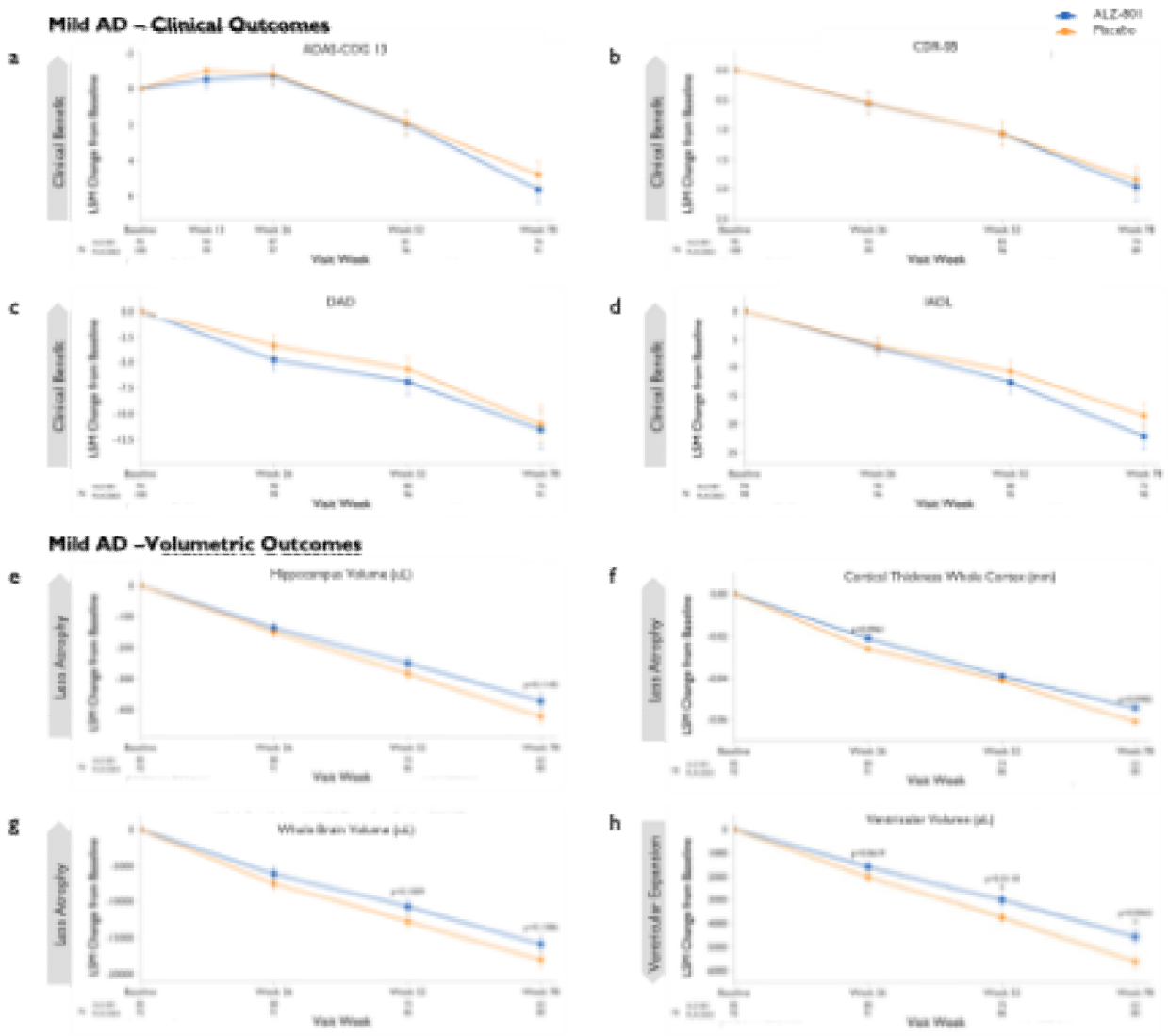
| **NfL and HV CBL Correlation** | **HV at 26 weeks** | **HV at 52 weeks** | **HV at 78 weeks** |
|---|---|---|---|
| **Valiltramiprosate Arm - MCI** | | | |
| NfL at 26 weeks | -0.15, $p$ = 0.27<br>N = 54 | -0.22, $p$ = 0.12<br>N = 51 | 0.07, $p$ = 0.64<br>N = 49 |
| NfL at 52 weeks | | **-0.45, $p$ = 0.0008***<br>**N = 51** | **-0.41, $p$ = 0.003***<br>**N = 50** |
| NfL at 78 weeks | | | -0.28, $P$ = 0.047*<br>N = 50 |
| | | | |
| **Placebo Arm - MCI** | | | |
| NfL at 26 weeks | -0.26, $p$ = 0.07<br>N = 49 | 0.08, $p$ = 0.57<br>N = 48 | 0.16, $p$ = 0.28<br>N = 47 |
| NfL at 52 weeks | | 0.08, $p$ = 0.59<br>N = 47 | 0.18, $p$ = 0.24<br>N = 45 |
| NfL at 78 weeks | | | 0.18, $p$ = 0.23<br>N = 45 |

Pearson's correlations between plasma NfL drug effects at the early 26-week and 52-week timepoints and the 78-week endpoint to HV outcomes at the 78-week endpoint. N = number of participants in each trial arm who had both HV and plasma NfL at baseline and 78 weeks, allowing CBL correlations. Since plasma NfL is a measure of ongoing neurodegeneration, these significant correlations in the valiltramiprosate arm (but not in placebo arm) suggest that the slowing of HV atrophy on treatment represents stabilization of neurodegeneration in this APOE4/4 MCI population. AD, Alzheimer's disease; APOE, apolipoprotein E; APOE4/4, APOE ε4 allele homozygotes; CBL, change from baseline; HV, hippocampal volume; NfL, neurofilament light; MCI, mild cognitive impairment; vMRI, volumetric magnetic imaging. *Indicate statistically significant correlations, $p < 0.05$. Bolded correlations are those that achieved $p < 0.01$.

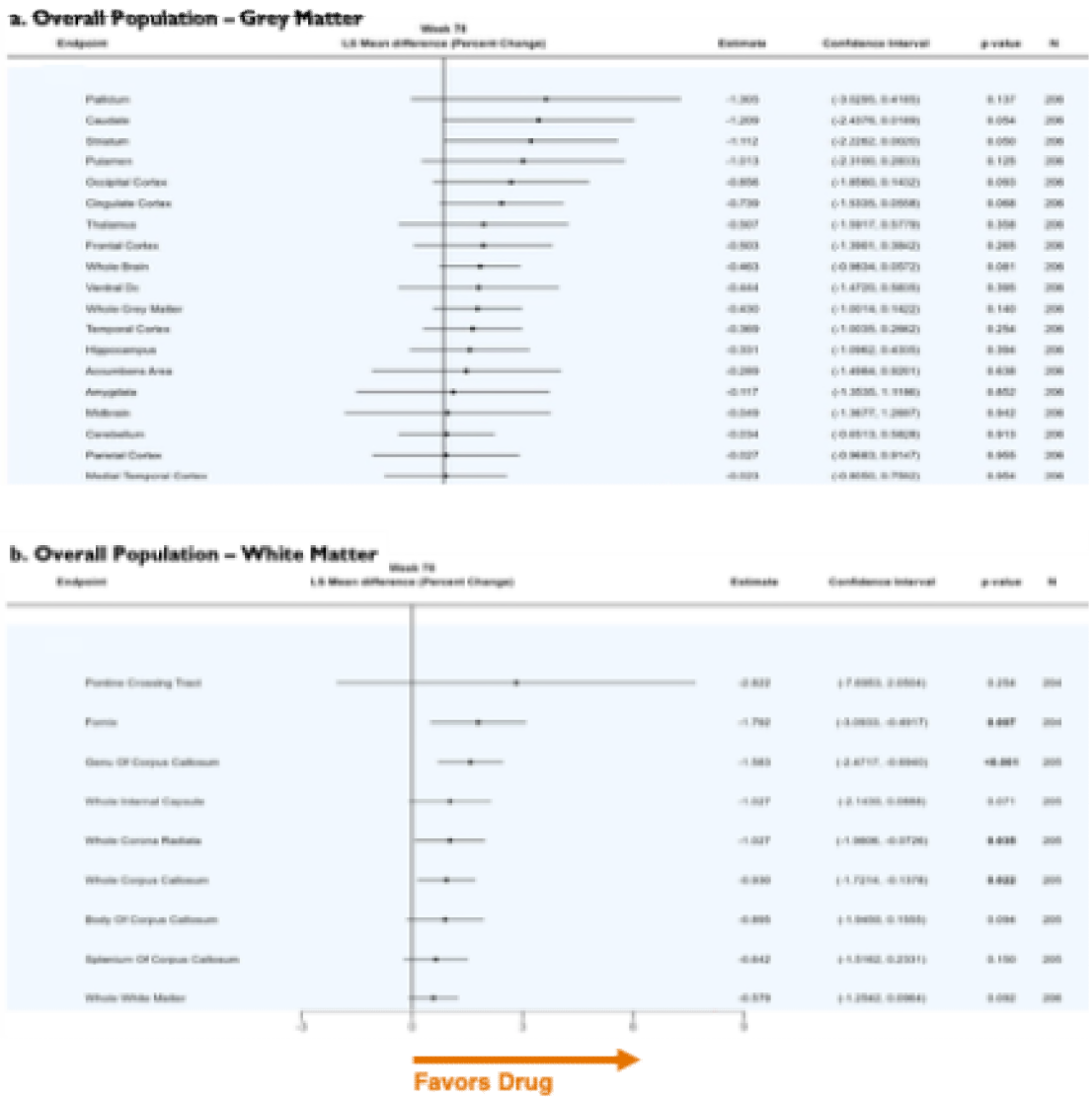
**<Supplementary Figures>**



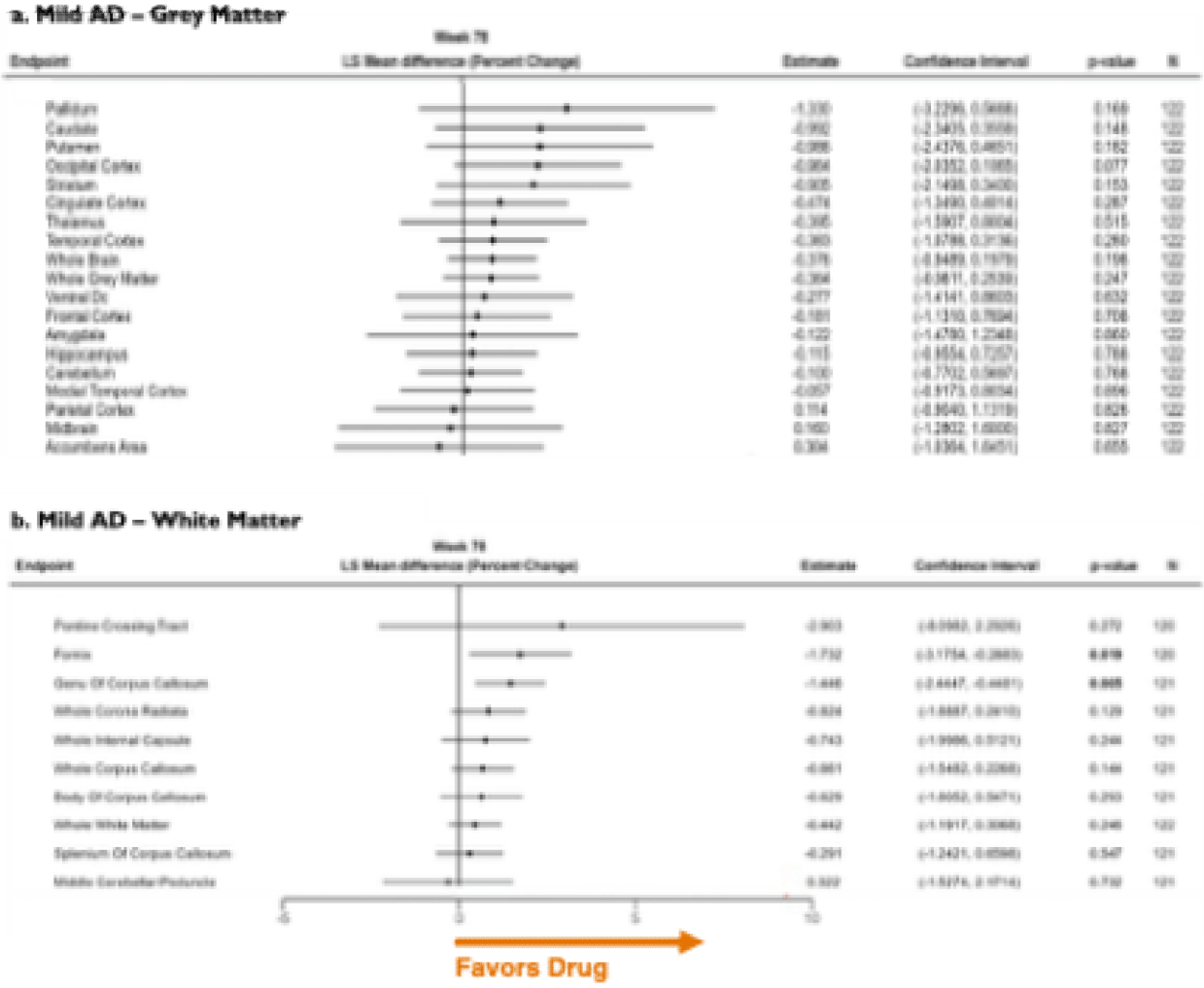

**Online Resource Fig. 1** Schematic diagram of trial visits and assessments – Phase 3 trial of ALZ-801/valiltramiprosate in APOE4/4 participants with Early AD (APOLLOE4). AD, Alzheimer's disease; ADAS-cog 13, 13-item Alzheimer's Disease Assessment Scale-Cognitive Subscale; APOE, apolipoprotein E; APOE4/4, APOE ε4 allele homozygotes; BID, twice daily; MMSE, Mini-Mental State Examination; vMRI, volumetric magnetic resonance imaging; DTI, diffusion tensor imaging.



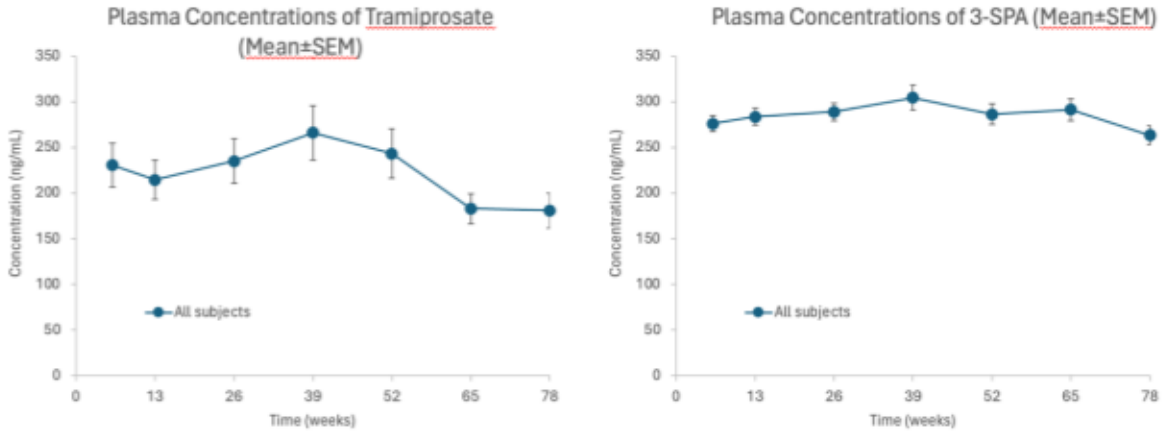

**Online Resource Fig. 2** Effects of ALZ-801/valiltramiprosate on the main clinical and volumetric outcomes in prespecified Mild AD (MMRM analysis). **a-d** clinical outcomes. **e-h** volumetric MRI outcomes. An increase in the ADAS-Cog13, CDR-SB, or IADL score indicates clinical worsening; a decrease in the DAD score indicates clinical worsening. AD: Alzheimer's disease; ADAS-Cog13, 13-item Alzheimer's Disease Assessment Scale-Cognitive Subscale; CDR-SB, Clinical Dementia Rating – Sum of Boxes; DAD, Disability Assessment for Dementia; IADL, Amsterdam Instrumental Activities of Daily Living; LSM, least squares mean; MMRM, mixed-effect model repeated measure.




**Online Resource Fig. 3** Effects of ALZ-801/valiltramiprosate on the DTI endpoints in the overall DTI population (MMRM analysis). **a**, grey matter regions; **b**,white matter tracts. DTI (diffusion tensor imaging mean diffusivity outcomes). LS means difference, least squares mean for drug-placebo difference on percent change from baseline. MMRM, mixed mixed-effect model repeated measure.




**Online Resource Fig. 4** Effects of ALZ-801/valiltramiprosate on the DTI endpoints in prespecified Mild AD (MMRM analysis). **a**, grey matter regions; **b**, white matter tracts. AD: Alzheimer's disease; DTI (diffusion tensor imaging, mean diffusivity outcome); LS means dfference, least squares mean for drug-placebo difference on percent change from baseline. MMRM, mixed mixed-effect model repeated measure.




**Online Resource Fig. 5 Pharmacokinetic Analysis of ALZ-801/valiltramiprosate drug exposures in plasma.** Plasma exposures of tramiprosate and 3-SPA over 78 weeks in the APOLLOE4 Phase 3 trial. The steady-state levels of tramiprosate and 3-sulfopropanoic acid (3-SPA), the active agents in valiltramiprosate, are shown from all participants over 78 weeks, based on sparse sampling at trial visits. The data shown are from single time-point sampling at trough levels at each visit from Week 6 to Week 78. Note that variability is over 2-fold lower for 3-SPA than tramiprosate, 3-SPA is the best estimator of steady state exposure due to its longer half-life . SEM, standard error of the mean.

## References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