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EFFECT OF TRAMIPROSATE IN PATIENTS WITH MILD-TO-MODERATE ALZHEIMER'S DISEASE: EXPLORATORY ANALYSES OF THE MRI SUB-GROUP OF THE ALPHASE STUDY

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Abstract: *Objectives:* The efficacy, safety and disease-modification of tramiprosate (homotaurine)were investigated in a recently completed large-scale Phase III clinical study in patients with mild to moderate Alzheimer's disease (AD), the Alphase study. Disease-modification was assessed using longitudinal volumetric MRI (vMRI) measurements of the hippocampus in a subgroup of patients. The present study describes the vMRI, cognitive and clinical results obtained in this subgroup. *Design:* Multi-center, double-blind, randomized, placebo-controlled study in a subset of the 1052 patients of the Alphase study. *Setting:* 51 vMRI investigative sites in the United States and Canada. *Participants:* A total of 508 patients underwent vMRI scanning. Of these, 312 provided scan pairs for assessing hippocampus volume changes and were included in the analyses. *Interventions:* Patients were randomized to receive Placebo BID (n = 109), tramiprosate 100 mg BID (n = 103), or tramiprosate 150 mg BID (n = 100) for 78 weeks. *Measurements:* Alzheimer's Disease Assessment Scale-cognitive subscale (ADAS-cog) and Clinical Dementia Rating–Sum-of-boxes CDR-SB assessments were conducted at Baseline and at Weeks 13, 26, 39, 52, 65 and 78. Exploratory analyses were performed using similar First and Final mixed-effects repeated-measures models that were used for the analysis of the entire patient dataset. *Results:* Psychometric score results showed numerical trends in favour of tramiprosate that did not reach statistical significance. While there were no statistically significant group differences in hippocampus volume using the First modeling approach, a significant dose–response reduction in hippocampus volume change was found in the Final models. Moreover, there was a marginally significant overall treatment main effect and a significant slope difference in favour of tramiprosate according to the Final model analysis of the ADAS-cog scores. ADAS-cog scores analyzed according to this model also revealed differences in favor of the tramiprosate 150 mg group at weeks 26 and 52, with marginally significant differences at Weeks 13 and 39. Slope analyses of ADAS-cog score changes showed significant differences in favor of the 150 mg BID group, and when both active groups were combined, in comparison to the placebo group. No between-group differences with respect to changes to each visit in the CDR-SB were observed with either modeling approach. Although there was a similar dose–response relationship observed in the hippocampus volume and ADAS-cog Final model analyses, the overall changes in psychometric scores and hippocampus volume were not significantly correlated. *Conclusion:* Exploratory analysis of the vMRI subgroup suggests that tramiprosate slows hippocampal atrophy, and reveals some evidence of a beneficial effect on cognition. The clinical validity of the vMRI biomarker is discussed.

Key words: Tramiprosate, homotaurine, Alzheimer's disease, amyloid, MRI, hippocampus.

Introduction

The pathogenesis of AD is hypothesized to involve the amyloid β (Aβ) peptide as one of the pivotal events in the disease process. Polymerisation of the Aβ peptide has been shown to induce a number of neurotoxic events that may lead to functional and structural neuronal alterations, including brain atrophy (1). Rates of volume loss in the hippocampus, as assessed by magnetic resonance imaging (MRI) are sensitive predictors of AD neuropathology (2, 3), and progression (3-13). More importantly, changes in hippocampus volume have been related to the degree of memory decline in AD patients (14-16). Drugs that can protect against Aβ-induced neurotoxicity, alter changes in hippocampus volume, and slow cognitive decline should have therapeutic value in the treatment of AD (17).

Tramiprosate (homotaurine) is a small, orally-administered amyloid β antagonist that binds to soluble Aβ peptides and reduces amyloid aggregation and subsequent deposition in animals (18). In vitro, tramiprosate has been shown to provide neuroprotection against Aβ-induced neurotoxicity in rodent neuronal and organotypic hippocampus cultures and to reverse Aβ-induced inhibition of long-term potentiation (LTP) in rat hippocampal slices (19), partly through its binding to β-aminobutyric acid (GABA) type A receptors (20). In vivo, tramiprosate produced dose-dependent reductions of both

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soluble and insoluble $A\beta_{40}$ and $A\beta_{42}$ in the brains of transgenic mice (TgCRND8) (19). In humans, administration of tramiprosate in healthy young, elderly, and AD subjects was found to be safe and tolerable. Tramiprosate has been shown to reduce $A\beta_{42}$ levels in cerebral spinal fluid of AD patients (21), demonstrating a potential to target the pathology of AD and to modify the course of the disease.

The efficacy, safety and disease modification effects of tramiprosate were investigated in a recently completed large-scale Phase III clinical study involving 1052 patients with mild-to-moderate AD followed over an 18-month period (22). Efficacy outcomes included the Alzheimer's Disease Assessment Scale-cognitive subscale (ADAS-cog) and Clinical Dementia Rating–Sum-of-boxes (CDR-SB) scale, while disease modification was assessed using longitudinal volumetric MRI (vMRI) measurements of the hippocampus in a subgroup of patients (the MRI sub-study). The planned analyses of the psychometric data did not show statistically significant between-group differences, while the vMRI data revealed a trend towards greater volume reduction in treated patients. However, both the psychometric and hippocampus volume analysis models were confounded by a site effect and affected by high within- and between-patient variance. Post-hoc analyses aimed at reducing the site effect and improving statistical model fit were conducted by developing mixed-effects multivariate repeated-measures models that included additional covariates. These analyses showed some trends toward a lower ADAS-cog decline and a dose-related reduction in hippocampus volume loss in tramiprosate-treated patients.

The objective of the current paper is to present the hippocampus volume and psychometric results in the subgroup of patients that underwent vMRI in the Alphase study (22).

Methods

Men and women (50 years and older) with a diagnosis of probable AD by standard criteria (DSM-IV-TR (23) and NINCDS-ADRDA (24)) and a mild to moderate level of severity (Mini-Mental State Examination [MMSE] (25) between 16 and 26 inclusively) participated in this study. All patients were on a stable dose of a cholinesterase inhibitor (ChEI), which may have been combined with memantine, for a minimum of four months prior to the screening visit. Stable doses (≥ 1 month prior to the screening visit) of the following medications were allowed: anxiolytics, sedatives, hypnotics, antidepressants, antipsychotics, anticonvulsants, estrogens and statins, vitamin E (not exceeding > 2050 IU/day). Patients were required to have a Geriatric Depression Scale (GDS) (26) score ≤ 10. Patients with any other causes of dementia as determined by medical history, physical or neurological examination were excluded. Also excluded were patients with a body mass index less than 19 or greater than 28, a life expectancy less than 2 years, or a clinically significant and uncontrolled medical disease. The study protocol was approved by the ethics review board of each of site. Written informed consent was obtained from each patient or legally authorized representative prior to study entry.

This was a randomized, double-blind, placebo-controlled, parallel-design study, in which a total of 1052 patients were enrolled at 67 study centres across the US and Canada and randomized at a 1:1:1: ratio to receive tramiprosate 100 mg BID (n = 352), tramiprosate 150 mg BID (n = 347) or placebo BID (n = 353) for 70 consecutive weeks, preceded by an 8-week dose titration period. Clinical assessments were conducted at Baseline and at Weeks 13, 26, 39, 52, 65 and 78. Volumetric MRI assessments took place at Baseline and at Week 78 in a subset of patients. Primary measures included the ADAS-cog and CDR-SB for clinical efficacy, and vMRI of the hippocampus for disease modification. Results for the safety, clinical and vMRI measures for the entire cohort are described elsewhere (22).

The vMRI sub-group included 508 patients who volunteered to enroll at 51 MRI sites. Each vMRI subject was scheduled to undergo a Baseline scan, conducted within the four-week period prior to randomization, and a Week 78 scan, conducted at Week 78 or early termination. Early termination scans were conducted only for patients who had been participating in the study for more than 6 months, but had failed to complete the Week 78 visit. MRI (three-dimensional T1 weighted and axial proton density/T2-weighted turbo spin echo) scans were used to measure hippocampus volume. The absolute change in hippocampus volume was determined from both scans (screening and Week 78 [or early termination]) after totaling the voxel volumes of left and right hippocampus at each visit. Additional details regarding the vMRI procedures are presented elsewhere (22).

All analyses were based upon observed cases in the Intent-to-Treat (ITT) population, as defined by subjects who had an ADAS-cog or CDR-SB assessment performed at Baseline, and at least at one follow up visit. Psychometric data obtained from the vMRI subgroup were analyzed according to the two modeling approaches used for the entire cohort dataset: one model was developed for the ADAS-cog data, while a second model was developed for the CDR-SB data (22). In the first approach (First models), between-group differences in change scores to each visit were submitted to repeated-measures analysis of covariance (ANCOVA) models, with treatment group as the independent variable and visit as the within-patient repeated-measures factor. In these models, within-patient variance was modeled using a compound symmetry covariance pattern, with the standard error for the parameter estimates adjusted using the "sandwich" covariance estimator. For each psychometric model, baseline psychometric values and site (study centre) were entered as covariates. These models tested for main effects of group and visit, as well as for groups by visit interactions. Additional comparisons included between-group differences in the changes from Baseline to each follow-up visit in the ADAS-cog and the CDR-SB, using planned

contrasts within the ANCOVA models. Planned contrasts were also used to compare slopes of score changes across treatment groups.

A highly significant site effect, large between- and within-patient variation and poor fit of the data rendered the analyses produced from the First models inconclusive and a second modelling approach was implemented (22). The aim of the second approach was to minimize the site effect and improve model fit and validity by adding additional explanatory covariates. This was done by developing two additional statistical models (Final models), one for each of the psychometric and the vMRI datasets. These models were developed using aggregate data without including the treatment group variable, which allowed for an empirical selection of covariates, while minimizing model selection bias. Covariates to be included in the models were selected in a six-step process. First, an exploratory analysis was conducted by graphing individual and group profiles, identifying cross-sectional and longitudinal patterns in the data. Second, bivariate analyses were used to identify variables which were predictors of outcome based on a statistical trend defined as a $P < 0.15$. These included both baseline (e.g. education level) and on-study (e.g. vitamin E dose) variables. Third, a stepwise procedure, with a $P < 0.15$ tolerance, was used to select, among the variables that were identified in the second step, those that were significant independent predictors of the outcome. However, some variables which did not have a highly significant association with the change in psychometric scores were nonetheless retained in the model if they improved model fit in the presence of the other covariates. Fourth, the variables identified were reviewed for face validity by an independent scientific advisory committee to assess their clinical relevance. Fifth, the unnecessary fixed effects covariates in the model were removed based on likelihood ratio test and information criteria improvement (Akaike and Bayesian). A restricted maximum-likelihood estimation method was used in the selection of the covariance structure and the maximum-likelihood estimation in the selection of the fixed effects (27, 30). Once the final model was developed, the treatment variable was included as the final step with the covariates in a three-level linear mixed-effect model with random site-specific intercepts and slopes for ADAS-cog and CDR-SB. Only patients reaching Week 78 were included in the HV model because inclusion of those withdrawn with HV data prior to week 78 was introducing problems regarding the validity of the model caused by extreme residuals, convergence, and poor fit. All multiple comparisons were adjusted for multiplicity with the Simulation test (31), which provides a family-wise error rate protection. The same procedure for developing the Final models for the psychometric data was used for developing a model for the vMRI data.

The present paper reports the baseline and demographic characteristics, changes from Baseline to each visit in ADAS-cog and CDR-SB scores, and changes in vMRI of the vMRI subgroup (reported elsewhere (22)). The analyses used both the First and the Final statistical models. Slope analyses are also presented to further quantify trends in rates of decline among treatment groups. Finally, correlations between changes in vMRI of the hippocampus and changes in psychometric scores at Week 78 are presented.

Irrespective of the significance of the main effects or interactions, contrasts comparing each active treatment group to Placebo with respect to the changes in psychometric or vMRI outcome measures were conducted in order to document trends in the effects of tramiprosate.

Results

Baseline and follow-up scan pairs were obtained from 388 of the 508 patients who underwent MRI assessments. Of these, 312 scan pairs were of adequate quality to assess hippocampus volume change and were included in the first vMRI sub-group analysis. There were 11 patients who provided early termination scans at follow-up. The mean (median) delay between the baseline and follow-up scans was as follows: Placebo: 546.2 (546) days; homotaurine 100 mg BID: 537.5 (546) days; homotaurine 150 mg BID: 537.1 (546) days. For the Final model analysis, 47 scan pairs were excluded from the analysis because subjects either had terminated early or had missing covariate information (n=36) that was required to compute the Final model. Table 1 summarizes the demographic and baseline characteristics for the vMRI ITT population. The mean age of patients in the vMRI sub-group was approximately 72 years, 53% were female, and 62% had one APOE-4 allele. Among these patients 47% were treated with memantine for a mean duration of approximately 12 months. Baseline mean scores on the ADAS-cog and CDR-SB scales were 21.1 and 5.5 respectively with a mean value for hippocampus volume of 3324.3 mm^3. The three treatment groups were similar with respect to demographics and baseline characteristics.

Table 2 summarizes the results for the changes from Baseline to each visit in psychometric scores, using the First statistical model. Patients in all three groups experienced an increase from Baseline to each visit in ADAS-cog and CDR-SB scores, indicating decline. Although patients in the two tramiprosate groups experienced lower decline in the ADAS-cog scores when compared to the Placebo group throughout the study, the treatment x visit interaction was not statistically significant and none of the differences vs. Placebo at each visit was statistically significant. The adjusted slope analysis showed that the mean (SE) difference in rate of decline in ADAS-cog score during the 18-month follow-up was -7.8 (4.08); 95% CI: (-15.80 to 0.19); $P = 0.056$ for the 100 mg group versus Placebo, -6.90 (4.55); 95% CI: (-15.82 to -2.02); $P = 0.130$ for the 150 mg group versus Placebo, -0.91 (4.23); 95% CI: (-7.39 – 9.20); $P = 0.831$ for the 150 mg versus 100 mg groups and -7.35 (3.76); 95% CI: (-14.73 to -0.03); $P = 0.051$ for combined 150 + 100 mg groups versus Placebo. The changes in the CDR-

Table 1
Baseline Characteristics – MRI sub-group

Baseline Characteristics	Statistics (n=109)	Placebo 100 mg BID (n=103)	tramiprosate 150 mg BID (n=100)	tramiprosate
Age (years)	Mean (SD)	73.4 (8.7)	71.7 (8.4)	70.7 (9.6)
Gender				
Male	n (%)	56 (51.4)	52 (50.5)	39 (39.0)
Female	n (%)	53 (48.6)	51 (49.5)	61 (61.0)
Education (years)	Mean (SD)	13.7 (3.5)	14.1 (3.4)	14.1 (3.3)
Race				
Caucasian	n (%)	108 (99.1)	95 (92.2)	98 (98.0)
Black or African American	n (%)	1 (0.9)	5 (4.9)	2 (2.0)
Asian/Oriental	n (%)	0 (0.0)	1 (1.0)	0 (0.0)
Hispanic	n (%)	0 (0.0)	2 (1.9)	0 (0.0)
At least one APOE-4 allele	n (%)	72 (66.1)	64 (62.1)	57 (57.0)
ChEI use				
Donepezil	n (%)	70 (64.8)	74 (72.6)	67 (68.4)
Galantamine	n (%)	23 (21.3)	19 (18.6)	25 (25.5)
Rivastigmine	n (%)	15 (13.9)	9 (8.8)	6 (6.1)
Duration of ChEI use (months)	Mean (SD)	22.7 (17.4)	25.2 (20.6)	22.2 (15.3)
Memantine use:				
No use	n (%)	67 (61.5)	51 (49.5)	47 (47.0)
Use	n (%)	42 (38.5)	52 (50.5)	53 (53.0)
Duration of Memantine use (months)	Mean (SD)	12.3 (7.5)	12.0 (5.8)	12.2 (7.5)
Total hippocampus volume (mm^3)	Mean (SD)	3294.4 (723.4)	3278.9 (741.2)	3405.9 (702.6)
Psychometric scores				
ADAS-cog score	Mean (SD)	21.1 (8.0)	20.8 (7.5)	21.4 (8.4)
CDR-SB score	Mean (SD)	5.5 (2.4)	5.5 (2.6)	5.4 (2.5)
MMSE score	Mean (SD)	21.3 (3.3)	21.5 (3.0)	21.0 (3.2)

Table 2
Changes in ADAS-Cog and CDR-SB at Each Visit, First Mixed-Effects Repeated-Measures Models, MRI sub-group, Observed Cases

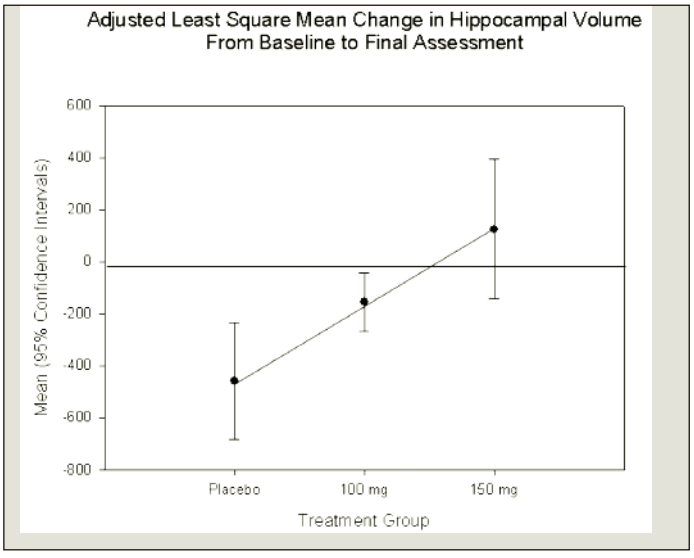
Visit	Statistic	ADAS-Cog Placebo (n = 109)	ADAS-Cog 100 mg BID (n = 103)	ADAS-Cog 150 mg BID (n = 100)	CDR-SB Placebo (n = 109)	CDR-SB 100 mg BID (n = 103)	CDR-SB 150 mg BID (n = 100)
13	n	107	101	100	109	102	99
	LS Mean (SE) Change	1.7 (0.5)	0.7 (0.5)	0.4 (0.5)	0.1 (0.1)	0.1 (0.2)	0.3 (0.1)
	95% CI	0.8; 2.7	-0.3; 1.8	-0.8; 1.3	-0.2; 0.2	-0.2; 0.4	-0.0; 0.5
	Diff. (%) vs. Placebo	NA	-1.0 (-58.8)	-1.3 (-76.5)	NA	0.0 (0.0)	0.2 (+200.0)
26	n	108	102	100	109	102	98
	LS Mean (SE) Change	3 (0.5)	1.1 (0.5)	1.4 (0.6)	0.4 (0.1)	0.5 (0.2)	0.7 (0.2)
	95% CI	1.9; 4.0	0.0; 2.1	0.1; 2.4	0.1; 0.6	0.2; 0.8	0.3; 1.0
	Diff. (%) vs. Placebo	NA	-1.9 (-63.3)	-1.6 (-53.3)	NA	0.1 (+25.0)	0.3 (+75)
39	n	109	101	99	109	99	99
	LS Mean (SE) Change	3.7 (0.5)	2.6 (0.6)	2.4 (0.6)	0.8 (0.2)	0.8 (0.2)	1.1 (0.2)
	95% CI	2.7; 4.6	1.5; 3.7	1.1; 3.4	0.4; 0.9	0.5; 1.2	0.6; 1.4
	Diff. (%) vs. Placebo	NA	-1.1 (-29.7)	-1.3 (-35.1)	NA	0.0 (0.0)	0.3 (+37.5)
52	n	109	99	97	109	97	97
	LS Mean (SE) Change	5.1 (0.7)	3.4 (0.6)	3.9 (0.8)	1.5 (0.2)	1.2 (0.2)	1.6 (0.2)
	95% CI	3.8; 6.3	2.2; 4.4	2.1; 5.2	1.0; 1.7	0.9; 1.7	1.0; 2.0
	Diff. (%) vs. Placebo	NA	-1.7 (-33.3)	-1.2 (-23.5)	NA	-0.3 (-20.0)	0.1 (+6.7)
65	n	109	100	95	108	100	95
	LS Mean (SE) Change	5.6 (0.7)	4.8 (0.6)	5.3 (0.8)	1.6 (0.2)	1.6 (0.2)	2 (0.3)
	95% CI	4.1; 6.9	3.6; 6.0	3.7; 6.7	1.1; 1.9	1.2; 2.0	1.5; 2.5
	Diff. (%) vs. Placebo	NA	-0.8 (-14.3)	-0.3 (-5.4)	NA	0.0 (0.0)	0.4 (+25.0)
78	n	107	98	95	109	98	95
	LS Mean (SE) Change	7.8 (0.8)	6.2 (0.7)	7.1 (0.8)	2.0 (0.2)	2.2 (0.2)	2.5 (0.3)
	95% CI	6.1; 9.2	4.9; 7.6	5.5; 8.6	1.5; 2.3	1.8; 2.7	2.0; 3.1
	Diff. (%) vs. Placebo	NA	-1.6 (-20.5)	-0.7 (-9.0)	NA	0.2 (+10.0)	0.5 (+25.0)

Note: ADAS-cog scores range from 0 to 70 and CDR scores range from 0 to 18. Higher scores indicate greater impairment and LS mean changes from Baseline > 0 indicate deterioration.

SB at each visit were similar for the three study groups, with no statistically significant treatment x visit interaction, no significant between-group differences in slope, and no statistical differences between each treatment group vs. Placebo at each visit.

The covariates included in the Final statistical models for the changes in psychometric scores and hippocampus volume are listed in Table 3. These results show that for the ADAS-cog significant independent associations were identified with Visit, Age and Disease Severity (as measured by the MMSE), and Race. The interactions of visit, representing time on treatment, with disease severity, and memantine dose were also important predictors that were retained in the model. Other variables (Anti-depressant use, Vitamin E dose, Genotype, Type of ChEI used, Visit by Age, and Treatment by Visit) did not have a highly significant association with the change in ADAS-cog but were retained in the model because they contributed to improving model fit. With respect to the CDR-SB, Visit, Disease Severity, Presence of cardiovascular disease, Type of ChEI used, and the interaction of Visit with Disease severity were all significant predictors of change from baseline. In this model Genotype, Years of education, Anti-depressant use, Memantine use, and Vitamin E use, as well as the Treatment by Visit interaction, were also retained as important predictors of change although the parameter estimates for these variables were not statistically significant. For the change in hippocampus volume, Genotype, Presence of cardiovascular disease, Race, Intracranial volume at baseline, as well as interactions of Treatment group with Genotype, and Hippocampus volume, Duration of treatment by Genotype, by Cardiovascular disease, by Race, as well as Total intracranial volume, were retained within the final models.

Figure 1
Least-square means of the changes in Hippocampus volume – Final model



Note: Hippocampus Volume (HV) Change = (Week 78 Volume – Screening Volume). Units are in mm^3. Atrophy is indicated by a mean change < 0.

Table 3
Summary of covariates and covariate interaction retained in the Final Mixed-Effects Repeated-Measures Models

Covariate and Covariate interaction terms	P - value ADAS-cog	CDR-SB	Hippocampus Volume
Treatment Group	0.091	0.337	0.011
Treatment Group x Treatment Duration	0.883	0.826	0.019
Visit	<0.001	<0.001	
Age (quartiles)	0.010		
Disease severity	<0.001	0.001	
Antidepressant use	0.266	0.357	0.075
Memantine use		0.246	
Vitamin E use		0.313	0.881
Vitamin E dose	0.159		
Genotype	0.068	0.093	<0.001
Cardio-vascular disease		0.004	0.004
Years of education (quartiles)		0.211	
Race (Caucasian vs. non)	0.044		<0.001
Type of ChEI used	0.179	0.006	0.161
Visit x Age (quartiles)	0.082		
Visit x Disease severity	0.001	0.041	
Visit x Memantine dose	0.042		
Total Intracranial volume at baseline			0.015
Baseline Hippocampus Volume x Treatment			0.045
Whole Brain Volume at Baseline			0.334
Age x Treatment Group			0.196
Genotype x Treatment Group			0.012
Race x Treatment Group			0.114
Treatment Duration x Genotype			<0.001
Treatment Duration x Cardio-Vascular Disease			0.003
Treatment Duration x Race			<0.001
Treatment Duration x Total Intracranial Volume at Baseline			0.018

Table 4 summarizes results from the Final models for the changes from Baseline to each visit in psychometric scores for the MRI sub-group. Results for the change in the ADAS-cog scores showed a marginally significant overall treatment effect (P = 0.084) and a non-significant interaction of treatment with time (P = 0.856). In this model, the adjusted slope analysis showed that the mean (SE) difference in rate of decline in ADAS-cog during the 18-month follow-up was -5.62 (3.65); 95% CI: (-12.80 to 1.57); P = 0.125 for the 100 mg group versus Placebo, -7.77 (3.67); 95% CI: (-14.98 to -0.56); P = 0.035 for the 150 mg group versus Placebo, -2.16 (3.71); 95% CI: (-9.45 – 5.13); P = 0.561 for the 150 mg versus 100 mg groups and -6.69 (3.16); 95% CI: (-12.90 to -0.49); P = 0.035 for combined 150 + 100 mg groups versus Placebo. Significant differences in favor of the 150 mg group (vs. Placebo) were observed at Weeks 26 (P = 0.016) and 52 (P = 0.041), as well as marginally non-significant differences at Weeks 13 (P = 0.071) and 39 (P = 0.064). There were no significant between-group differences with respect to the Final model results for changes in the CDR-SB scores.

Table 4
Change in ADAS-cog and CDR-SB (Final Mixed-Effects Repeated-Measures Models)

Visit	Statistic	ADAS-Cog Placebo (n = 109)	ADAS-Cog 100 mg BID (n = 103)	ADAS-Cog 150 mg BID (n = 100)	CDR-SB Placebo (n = 109)	CDR-SB 100 mg BID (n = 103)	CDR-SB 150 mg BID (n = 100)
13	n	97	95	96	99	96	95
	LS Mean Change	0.25	-0.30	-1.05	0.02	0.06	0.21
	95% CI	-1.78; 2.27	-2.21; 1.60	-3.12; 1.02	-0.37; 0.42	-0.34; 0.46	-0.20; 0.61
	Diff. (%) vs. Placebo	NA	-0.55 (-220.0)	-1.30 (-520.0)	NA	0.04 (+200.0)	0.19 (+950.0)
	P vs. Placebo	NA	0.442	0.071	NA	0.834	0.338
26	n	99	96	95	100	96	94
	LS Mean Change	1.50	0.35	-0.23	0.38	0.43	0.60
	95% CI	-0.52; 3.53	-1.55; 2.25	-2.29; 1.83	-0.02; 0.77	0.03; 0.84	0.20; 1.01
	Diff. (%) vs. Placebo	NA	-1.15 (-76.7)	-1.73 (-115.3)	NA	0.05 (+13.2)	0.22 (+57.9)
	P vs. Placebo	NA	0.108	0.016	NA	0.771	0.243
39	n	99	94	93	99	93	93
	LS Mean Change	2.64	1.97	0.88	0.73	0.81	0.98
	95% CI	0.44; 4.85	-0.19; 4.13	-1.40; 3.17	0.19; 1.27	0.25; 1.36	0.43; 1.53
	Diff. (%) vs. Placebo	NA	-0.67 (-25.4)	-1.76 (-66.7)	NA	0.08 (+11.0)	0.25 (+32.3)
	P vs. Placebo	NA	0.476	0.064	NA	0.812	0.436
52	n	99	93	93	99	91	93
	LS Mean Change	4.21	3.01	2.26	1.46	1.32	1.54
	95% CI	2.01; 6.42	0.85; 5.17	-0.02; 4.55	0.92; 2.00	0.76; 1.88	0.99; 2.09
	Diff. (%) vs. Placebo	NA	-1.20 (-28.5)	-1.95 (-46.3)	NA	-0.14 (-9.6)	0.08 (5.5)
	P vs. Placebo	NA	0.206	0.041	NA	0.660	0.807
65	n	100	92	90	99	96	95
	LS Mean Change	5.04	4.58	4.18	1.73	1.76	2.18
	95% CI	2.83; 7.25	2.42; 6.74	1.89; 6.48	1.19; 2.28	1.20; 2.32	1.62; 2.73
	Diff. (%) vs. Placebo	NA	-0.46 (-9.1)	-0.86 (-17.1)	NA	0.03 (1.7)	0.45 (+26.0)
	P vs. Placebo	NA	0.626	0.370	NA	0.925	0.168
78	n	97	93	88	97	93	88
	LS Mean Change	7.03	6.23	6.11	2.17	2.46	2.68
	95% CI	4.82; 9.24	4.07; 8.38	3.81; 8.42	1.62; 2.71	1.90; 3.02	2.12; 3.24
	Diff. (%) vs. Placebo	NA	-0.80 (-11.4)	-0.92 (-13.1)	NA	0.29 (+13.4)	0.51 (+23.5)
	P vs. Placebo	NA	0.399	0.342	NA	0.369	0.114

Note: ADAS-cog scores range from 0 to 70 and CDR scores range from 0 to 18. Higher scores indicate greater impairment and LS mean changes from Baseline > 0 indicate deterioration.

The First model for hippocampus volume change did not show any statistical difference between placebo and treatment groups. The LS mean (SE) changes in hippocampus volume for placebo, tramiprosate 100 mg BID and tramiprosate 150 mg BID were -202.2 (22.7), -210.6 (23.4) and -259.7 (23.6) mm^3 respectively. However, after adjusting for important covariates and including site as a random variable, the Final mixed model showed a significant treatment effect in favor of tramiprosate (P = 0.011). Placebo and tramiprosate 100 mg BID had a decrease in mean hippocampus volume that was significantly different than zero (LS Mean [SE]: -419.3 [113.0] mm^3, P < 0.001, and -135.1 [58.0] mm^3, P = 0.021, respectively), with the 150 mg BID group having no difference in change (79.5 [132.9] mm^3, P = 0.550). The least squares mean estimates of hippocampus volume loss was significantly lower when comparing the tramiprosate 100 mg group and 150 mg BID groups to placebo (P = 0.035 and P = 0.009 respectively) (Figure 1).

At Week 78, the overall correlation between the changes in hippocampus volume and in ADAS-cog scores was -0.08 (P = 0.18). The overall correlation between the changes in hippocampus volume and in CDR-SB scores at week 78 was -0.04 (P = 0.44).

Discussion

The present paper reports the hippocampus volume and psychometric results obtained from the MRI subgroup of the Alphase trial. Overall, the demographic and baseline characteristics of the MRI sub-group were similar to those of the entire cohort. Changes from Baseline to each visit in ADAS-cog and CDR-SB scores in patients who had undergone vMRI of the hippocampus have been analyzed using the same two modeling approaches as with the main cohort (22). In the vMRI subgroup, there was numerically less decline from Baseline to each visit in ADAS-cog scores in tramiprosate-treated patients relative to placebo according to the First model, although none of these differences were statistically significant. Results using the Final model revealed statistically significant differences in favor of the tramiprosate 150 mg group at weeks 26 and 52, with a marginally significant difference at weeks 13 and 39. These results are consistent with those obtained from the slope analysis showing decreased rates of ADAS-cog

decline over 18 months in favor of the 150 mg group. No between-group differences with respect to changes in the CDR-SB were observed with either modeling approach.

As previously reported (22) the First analysis model did not show statistically significant between-group differences in hippocampus volume change. The results from the Final model however showed a significant treatment effect. More specifically, there was a 68% difference between 100 mg BID tramiprosate and placebo and a 120% difference between tramiprosate 150 mg BID and placebo in the mean reduction of hippocampus volume during the 18 months of treatment in the study. This dose–response relationship observed in the hippocampus volume loss is comparable to the direction of the between treatment group differences observed for the ADAS-cog. This supports the internal consistency of the Final model analyses.

Due to its diverse nature and variable evolution, AD progression and potential response to treatment in clinical trials are difficult to assess by simple statistical analysis, unless excessively large patient cohorts are recruited to compensate for large within- and between-patient variance. For studies of reasonable sample sizes, such as the current one, development of multivariate models is essential. Despite advancement in the field of AD clinical trials, it is highly unlikely that a definitive universal model could be developed that could be used in all settings. Currently, there is a need for regulatory agencies to consider developing guidelines for multivariate model building that avoid statistical bias but allow adequate evaluation of potential treatment effects.

In this analysis, it is worthy to mention that the MRI sub-study was not powered to detect psychometric treatment effects. Despite this, there were signals favouring tramiprosate with respect to the ADAS-cog change scores. Furthermore, while the visit-by-treatment group interactions were not statistically significant for either the First or Final models used in these analyses, statistically significant differences in rates of change (i.e. score slopes) were obtained in the final ADAS-cog model, despite large response variability. Marginally significant between group slope differences were also obtained in the First ADAS-cog model. The lack of statistical significance for the treatment x time interaction term must be interpreted with caution given the high power requirements for a statistically significant time x treatment effect. While both types of analyses (time-by-visit interactions, slopes) are reflective of the patient's clinical experience over the course of the trial, the current findings suggest that slope analyses were more sensitive in detecting significant between-group differences in ADAS-cog score over the course of the trial. This may be because slope based models minimize between-visit variability by estimating score changes across visit according to a best-fit (regression) method. Future studies may therefore benefit from including a slope analysis approach in which clinical progression can be examined after controlling for covariate factors.

Although there was a similar dose–response relationship observed in the hippocampus volume and ADAS-cog Final model analyses, the overall changes in psychometric scores and hippocampus volume were not significantly correlated. The lack of correlation may be due to several reasons. First, it may suggest that the psychometric and vMRI measures do not fully reflect the same disease processes or timing of changes in the natural history of the disease. Indeed, both the ADAS-cog and CDR-SB are global measures encompassing a number of cognitive and clinical domains, including but not limited to memory. Even within a memory specific item, the patient's performance may still depend on other cognitive skills (e.g., attention and language are required to remember a set of instructions in the ADAS-cog). The hippocampus, on the other hand, is primarily implicated in memory and learning functions and its structural integrity would have an impact on these memory specific skills. Differences in the scope of cognitive domains could potentially account for the lack of correlation between these psychometric and hippocampus volume measures. Second, different patients may have similar global change scores, but different patterns of score change across the various test items within the ADAS-cog or CDR-SB, further obscuring their potential correlations with hippocampus volume change. Finally, while changes in psychometric measures and hippocampus volume may reflect changes in similar memory functions, they may do so over different time periods. In particular, the redundancy of neural networks may be such that substantial atrophy may be required before its detrimental effects can be measured at the cognitive or clinical level (32). Thus, while vMRI may offer the potential for demonstrating disease modification effects, future studies may need to include domain-specific neuropsychological tests. Multiple vMRI scans may also be conducted during a trial and back-correlations between later psychometric assessment results with earlier scan acquisitions may be performed to further elucidate this structure/function relationship.

In this vMRI subgroup of the Alphase trial, the analyses of the psychometric outcome measures were conducted using the same modeling approaches as those applied to the entire cohort (22). The final model for assessing hippocampus volume change was also developed using the same approach. While results from the First modelling approach did not reveal between-group differences in psychometric scores, those of the Final modeling analyses of the ADAS-cog revealed between-group differences and trends in favour of tramiprosate. These subgroup results are consistent with those for the entire study population. Taken together, the imaging and psychometric analyses are consistent with the hypothesis that tramiprosate has a disease modifying effect. Study design issues and the complexity of the disease must be considered in the interpretation of these findings and in the design of future studies assessing treatments for Alzheimer's disease.

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