

Tramiprosate in mild-to-moderate Alzheimer's disease – a randomized, double-blind, placebo-controlled, multi-centre study (the Alphase Study)

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Abstract

Introduction: The aim of the study was to assess the clinical efficacy, safety, and disease-modification effects of tramiprosate (homotaurine, ALZHEMED™) in mild-to-moderate Alzheimer's disease (AD).

Material and methods: Double-blind, placebo-controlled, randomized trial in 67 clinical centres across North America. Patients aged ≥ 50 years, with mild-to-moderate AD (Mini-Mental State Examination score between 16 and 26) and on stable doses of cholinesterase inhibitors, alone or with memantine. Intervention: 78-week treatment with placebo, tramiprosate 100 mg or tramiprosate 150 mg BID. Measurements: Alzheimer Disease Assessment Scale – cognitive subscale (ADAS-cog) and Clinical Dementia Rating – Sum of Boxes (CDR-SB) assessments were performed at baseline and every 13 weeks. Baseline and 78-week magnetic resonance imaging (MRI) hippocampus volume (HV) measurements were conducted in a subgroup of patients.

Results: A total of 1,052 patients were enrolled and 790 (75.1%) completed the 78-week trial. Patient discontinuation and reasons for withdrawal were similar across groups. Planned analyses did not reveal statistically significant between-group differences. Lack of adequate statistical validity of the planned analysis models led to the development of revised predictive models. These adjusted models showed a trend toward a treatment effect for ADAS-cog ($P = 0.098$) and indicated significantly less HV loss for tramiprosate 100 mg ($P = 0.035$) and 150 mg ($P = 0.009$) compared to placebo. The incidence of adverse events was similar across treatment groups.

Conclusions: The primary planned analyses did not show a significant treatment effect, but were confounded by unexplained variance. Post-hoc analyses showed a significant treatment-related reduction in HV loss. However, there was only a trend towards slowing of decline on the ADAS-cog and no slowing of decline on the CDR-SB. These results must be interpreted in consideration of the limitations of clinical and disease-modification outcome measures and their relationship, the heterogeneity of the disease and the impact of confounding demographic and clinical variables.

Key words: tramiprosate, Alzheimer's disease, amyloid, disease-modification, volumetric MRI.

Introduction

Current therapies for Alzheimer's disease (AD) are symptomatic with limited impact on the disease itself. Treatment that slows or stops disease progression remains an unmet need. Accumulation of the amyloid β ($A\beta$) peptide is a pivotal process in the natural history of AD. Amyloid β is generated by enzymatic cleavage of the amyloid precursor protein (APP) with subsequent formation of toxic $A\beta$ oligomers and amyloid fibrils, which are ultimately deposited as plaques in the brain [1]. This process is hypothesized to induce neurotoxic events that lead to neurovascular uncoupling, synaptic dysfunction and neuronal loss [2, 3]. Ensuing structural damage in some brain areas such as the hippocampus, as revealed by magnetic resonance imaging, may be associated with, or even precede, clinical symptoms [3]. Interventions that protect against $A\beta$ -induced neurotoxicity may have therapeutic value for the treatment of AD [4].

Tramiprosate (homotaurine, ALZHEMEDTM) is a small, orally-administered compound that binds to soluble $A\beta$ and reduces amyloid aggregation and subsequent deposition [5]. *In vitro*, tramiprosate provides neuroprotection against $A\beta$ -induced neurotoxicity in neuronal and mouse organotypic hippocampal cultures, and reverses $A\beta$ -induced long-term potentiation (LTP) inhibition in rat hippocampus [6], in part, through activation of β -aminobutyric acid A (GABA-A) receptors [7]. *In vivo*, tramiprosate produced dose-dependent reductions of $A\beta$ in the brain of transgenic mice (hAPP-TgCRND8) [6]. Clinical studies [8] showed that tramiprosate was safe and tolerable. In mild-to-moderate AD patients [8] tramiprosate also reduced $A\beta_{42}$ levels in CSF.

The objectives of the Alphase, Phase III, North-American multi-centre, randomized, double-blind, placebo-controlled study was to assess the clinical efficacy, safety, and disease-modification effects of tramiprosate in patients with mild-to-moderate AD.

Material and methods

Patients

Participants (men and women ≥ 50 years) had a diagnosis of probable AD (DSM-IV-TR [9] and NINCDS-ADRDA [10]) and a Mini-Mental State Examination (MMSE) [11] score between 16 and 26. Laboratory assessments, electrocardiograms (ECG) and CT/MRI results were compatible with probable AD. Patients were required to be 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 screening) of anxiolytics, sedatives, hypnotics, antidepressants,

antipsychotics, anticonvulsants, oestrogens, statins, and vitamin E (≤ 2050 IU/day) were allowed. Patients with any other causes of dementia were excluded, as were those with a body mass index < 19 or > 28 , a life expectancy < 2 years, or a clinically significant and uncontrolled medical disease. The study protocol was approved by the ethics review board of each site. Written informed consent was obtained from each patient or legally authorized representative prior to study entry.

Study design

This was a randomized, double-blind, placebo-controlled, parallel-group study conducted at 67 study centres across the United States and Canada. Patients were randomized to tramiprosate 100 mg BID, tramiprosate 150 mg BID, or placebo BID for 78 consecutive weeks, including an 8-week dose escalation phase. Treatment was allocated according to a randomization list issued by an independent biostatistician, using a computer random number generator, and balanced to ensure a ratio of 1 : 1 : 1 across groups. Study medication consisted of modified-release coated tablets, identical in external appearance and containing 50 mg of tramiprosate or placebo. All doses were administered orally as three tablets.

Procedure

Clinical efficacy measures were Alzheimer's Disease Assessment Scale-cognitive subscale (ADAS-cog) [12], Clinical Dementia Rating-Sum of Boxes (CDR-SB) [13], MMSE [11], Clinical Interview Based Impression of Change-plus caregiver interview (CIBIC-plus) [14], Neuropsychiatric Inventory (NPI) [15], and Disability Assessment of Dementia (DAD) [16] scores. Disease-modification measures were volumetric magnetic resonance imaging (vMRI) of the hippocampus, whole brain and entorhinal cortex, plasma/CSF/urine $A\beta$, and CSF tau. Safety measures included Adverse Event (AE) reporting (MedDRA 9.0), vital signs, clinical laboratory parameters, ECG, and physical examinations.

Clinical and safety assessments were conducted at Baseline and Weeks 13, 26, 39, 52, 65 and 78. HV was measured at Baseline and at Week 78 in a subgroup of patients at 51 sites. The complete description of HV measurements is provided elsewhere [17]. Briefly, centralized assessment of HV was derived from a 1.5T T1-weighted 3-dimensional volumetric MR sequence (TR = 10/2160/9.5 ms, TE = 6.8/3.9/4 ms, $192 \times 256/192 \times 256/256 \times 256$ matrix, 260/260/258 mm FOV, flip angle = $20^\circ/10^\circ/8^\circ$, slice thickness = 1.6/1.6/1.6 mm for GE/Siemens/Philips, respectively). HV was computed using a semi-automated atlas-based warping method [18].

Statistical analyses

In this report, only the ADAS-cog, CDR-SB, and HV results will be reported as they constituted the primary clinical efficacy and disease-modification endpoints. Primary clinical efficacy endpoints were the changes from Baseline to Week 78 in ADAS-cog and CDR-SB scores. Secondary efficacy endpoints were the changes from Baseline to Weeks 13, 26, 39, and 52 in ADAS-cog and CDR-SB. Primary disease-modification endpoints were the changes from Baseline to Week 78 in vMRI of the hippocampus.

Sample size calculations were based on the assumptions that after 78 weeks, patients on placebo would decline by a mean (SD) of 10 (8.0) points on the ADAS-cog and 3.0 (2.3) points on the CDR-SB [19]. Assuming a 25% effect size, the study was powered to detect a difference of 2.5 points on the ADAS-cog and 0.75 point on the CDR-SB with 90% power at a two-tailed 5% significance. Adjusting for an approximate 30% dropout rate, an enrolment of 315 patients per group was planned. For HV, sample size estimate was based on an untreated mean (SD) 5.2% (2.5%) annual change in HV [20-25]. In order to detect a 20% difference in the HV change between active and placebo with 80% power, at a 5% two-tailed significance, and assuming an approximate 30% patient discontinuation rate, 150 patients per group were to be enrolled in this sub-study.

Planned models

Changes from Baseline in ADAS-cog and CDR-SB scores were assessed using mixed-effect repeated-measures analyses of covariance (ANCOVA). These models tested for the main effects of Group (treatment arm), Visit (treatment duration) and Group \times Visit ($G \times V$) interaction, adjusted for Baseline values and Site as covariates. The $G \times V$ interaction reflected between-group differences in least-square (LS) mean changes over time. Between-group differences were determined using planned contrasts comparing each active treatment group to placebo with respect to the changes in ADAS-cog and CDR-SB from Baseline to Week 78 (primary clinical outcome) and from Baseline to Weeks 13, 26, 39, 52, and 65 (secondary clinical outcomes). Change in HV was calculated by subtracting the final visit volume from the Baseline volume using the Last Observation Carried Forward approach for withdrawn patients. Differences in changes over time in HV between treatment arms were evaluated with an ANCOVA in which the dependent variable was the change in HV over the 78 Weeks of the study and the independent variables were treatment group with three levels (tramiprosate 150 mg BID, tramiprosate 100 mg BID, and Placebo BID), duration of follow-up (in months), Baseline HV and Site. Between-group differences in the change in HV were

assessed with the overall F-test for treatment group while differences between each active group and placebo were assessed using planned contrasts.

Adjusted models

For all study outcomes, unanticipated and highly significant Site effects ($P < 0.001$) were detected in the planned ANCOVA models. This suggested that either a significant amount of the variability was due to differences between sites, or that "Site" was concealing confounding variables that were not accounted for in the model, contributing to poor model fit. Post-hoc analyses aimed at reducing Site effects and improving model fits were conducted. Adjusted mixed-effects multivariate models were developed using aggregate data. Additional covariates were selected using a four-step process: 1) identification of cross-sectional and longitudinal patterns in the data, 2) identification of variables which were predictors of outcome based on a statistical trend defined as a $P < 0.15$, 3) selection of variables that were significant independent predictors of outcome, with a $P < 0.15$ tolerance, and 4) assessment of the clinical relevance of the identified variables by an independent scientific advisory committee. The fixed effect covariates in the planned models were removed based on likelihood ratio test and information criteria improvement (Akaike and Bayesian). Once the final adjusted models were developed, treatment variables were included in a three-level linear mixed-effect model with random site-specific intercepts and slopes for ADAS-cog and CDR-SB, and a two-level linear mixed model with random site-specific intercepts for HV. In the adjusted models, contrasts comparing each active group to Placebo at each visit were exploratory and aimed at documenting possible trends. No adjustments for multiple comparisons were thus required. Statistical trends ($P < 0.15$) are reported.

Both planned and adjusted models were based on observed cases. Efficacy analyses were conducted in the Intent-to-Treat (ITT) population, defined as all treated patients who had a Baseline ADAS-cog or CDR-SB assessment and at least one corresponding post-Baseline assessment. Disease-modification analyses were based on the MRI-ITT population, defined as ITT patients with a Baseline and on-treatment MRI that was valid for evaluating at least one of the targeted brain structures. Sites that recruited fewer than six patients were pooled. Twelve patients were excluded due to GCP non-compliance. Statistical analyses were performed using SAS version 9.12.

Results

Patient disposition and baseline characteristics.

The study took place between August 2004 and February 2007. A total of 1,432 patients were

screened, of whom 1,052 were randomized (placebo BID: $n = 353$, tramiprosate 100 mg BID: $n = 352$, tramiprosate 150 mg BID: $n = 347$) and 790 (75.1%) completed the trial. Patient discontinuation and reasons for withdrawal were similar across groups. Adverse event (48.1%) and withdrawal of consent (30.5%) were the most frequent reasons for early discontinuation. Patient disposition is presented in Figure 1. There were no statistical differences between groups with regards to baseline and demographic characteristics (Table I).

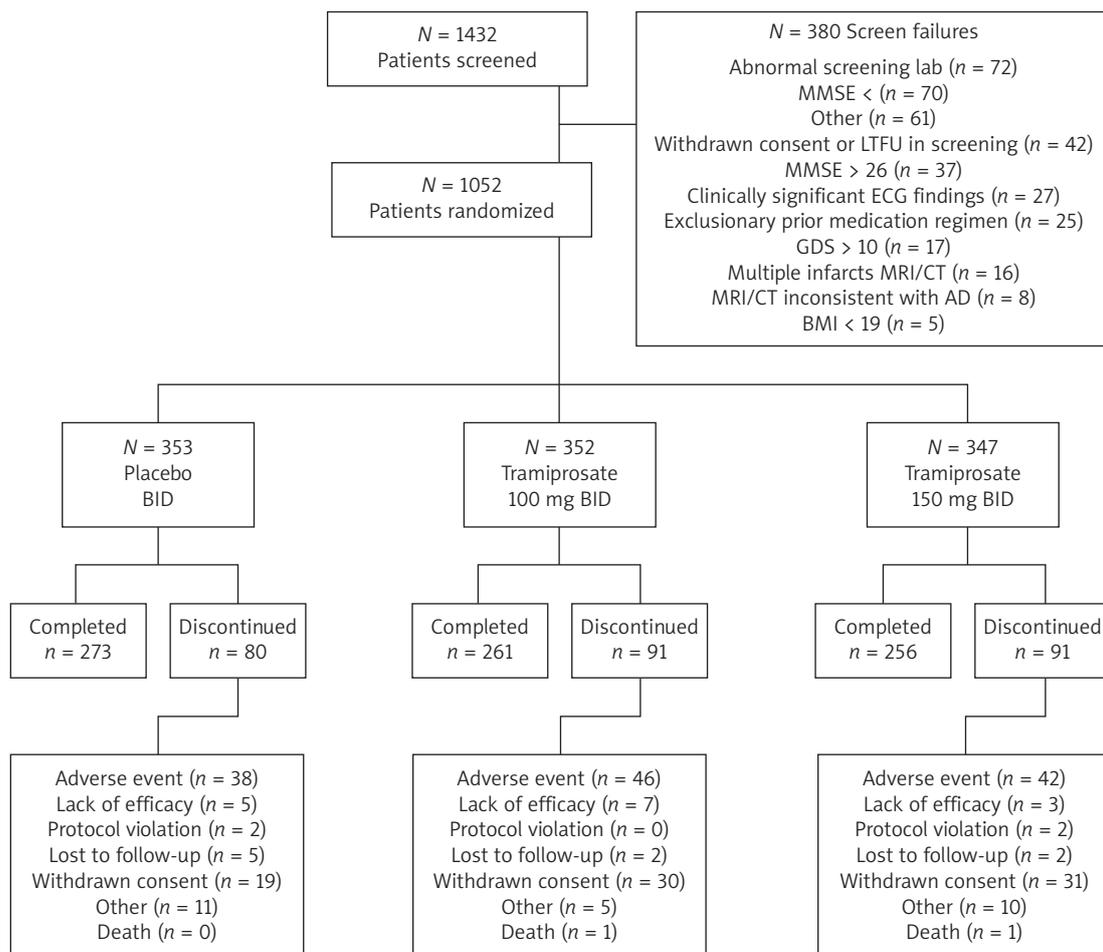
Planned analyses

Table II summarizes results using the planned analyses. All three groups experienced an increase from Baseline to each visit in clinical scores, indicating deterioration. The planned ANCOVA model for ADAS-cog did not reveal a significant $G \times V$ interaction ($P = 0.152$) although planned contrast for the primary ADAS-cog outcome revealed numerically less decline at Week 78 in the 100 mg versus placebo (-0.70 points). Neither the

planned $G \times V$ interaction ($P = 0.573$) nor the planned contrast for the primary CDR-SB outcome at Week 78 (100 mg vs. Placebo: $P = 0.405$; 150 mg vs. Placebo: $P = 0.837$) revealed a statistically significant treatment effect. Planned contrasts for the secondary ADAS-cog outcomes revealed that, relative to placebo, the 100 mg BID group had a statistically lower change from Baseline ($P = 0.027$) at Week 26. No statistical difference relative to placebo was observed for any of the secondary CDR-SB outcomes. For HV change, the planned model showed a non-significant treatment effect ($P = 0.120$). Compared to Placebo, the 100 mg BID group showed no volume change difference ($P = 0.782$), while a trend toward greater HV decrease was observed in the 150 mg BID group ($P = 0.056$).

Adjusted models

The adjusted models (Table III) improved data fit as indicated by Akaike Information Criteria (AIC) values of 28772.5 for the ADAS-cog and 17355.4 for



MMSE – mini-mental state exam, LTFU – lost to follow-up, GDS – Geriatric Depression Scale, BMI – body mass index

Figure 1. Patient disposition

Table I. Demographic and baseline characteristics – ITT population

	Placebo BID (N = 341)	100 mg BID (N = 335)	150 mg BID (N = 329)	All patients (N = 1005)
Race, Caucasian, n (%)	333 (97.7)	321 (95.8)	318 (96.7)	972 (96.7)
Sex, female, n (%)	182 (53.4)	167 (49.9)	184 (55.9)	533 (53.0)
Age [years], mean (range)	74.2 (51-92)	73.8 (50-92)	73.6 (48-94)	73.9 (48-94)
Education [years], mean (range)	14.1 (0-25)	13.9 (0-25)	14.1 (0-25)	14 (0-25)
¹ APOE4 genotype, at least 1 ε4 allele, n (%)	220 (64.7)	201 (58.6)	206 (60.6)	627(61.3)
² ChEI use [months], mean (range)	23.4 (0.6-125.1)	23.5 (1.3-101.3)	23.1 (1.9-117.9)	23.3 (0.6-125.1)
² Memantine use [months], mean (range)	12.8 (1.5-67.1)	12.4 (1.0-34.1)	11.6 (0.9-42.4)	12.3 (0.9-67.1)
ADAS-cog, mean (range)	22.2 (5.0-52.0)	22.1 (7.7-52.7)	21.7(4.0-48.0)	22.0 (4.0-52.7)
CDR-SB, mean (range)	5.8 (0.5-17.0)	5.7 (0.5-17.0)	5.7 (0.5-14.0)	5.7 (0.5-17.0)
MMSE, mean (range)	21.0 (12.0-26.0)	21.1 (15.0-26.0)	21.1 (15.0-26.0)	21.1 (12.0-26.0)

¹all-enrolled population, ²at randomization, ChEI – cholinesterase inhibitors, comparisons against Placebo based on one-way ANOVAs (continuous variables) and Cochran-Mantel-Haenszel (CMH) tests for general association (categorical variables)

the CDR-SB, compared to 31559.2 and 20030.3, respectively, for the planned models. The ADAS-cog adjusted model revealed a statistical trend for treatment effect in favour of the two tramiprosate groups ($G \times V$ interaction: $P = 0.098$) while post-hoc planned contrasts showed lower changes from Baseline relative to placebo in the 100 mg BID group at Week 26 ($P = 0.065$) and Week 52 ($P = 0.090$). The adjusted model for CDR-SB revealed no significant treatment effect ($G \times V$ interaction: $P = 0.505$).

For HV, the adjusted model showed an improved AIC of 3600.3 compared to 8139.1 for the planned model. The following covariates and covariate interactions were retained: Antidepressant Use, Vitamin E Use, Genotype, Cardiovascular Disease, Race, Type of ChEI Used, Total Intracranial Volume, Whole Brain Volume at Baseline, Baseline Hippocampus Volume \times Treatment, Age Group \times Treatment, Genotype \times Treatment, Race \times Treatment, Month \times Genotype, Month \times Cardiovascular Disease, Month \times Race, and Month \times Total Intracranial Volume. The HV adjusted model showed a significant treatment effect ($P = 0.011$). Least square estimates of the mean [95% CI] changes from Baseline were significantly different from zero for the placebo (-419.3 mm^3 [-642.1 to -196.4 mm^3]; $P < 0.001$) and 100 mg BID (-135.1 mm^3 [-249.5 to -20.7 mm^3]; $P = 0.021$) groups, while the change of 79.5 mm^3 (-182.5 to 341.5 mm^3) observed for the 150 mg BID group was not ($P = 0.550$). The decrease in HV were significantly less than placebo for both the 100 mg ($P = 0.035$) and 150 mg ($P = 0.009$) BID groups.

Safety

Table IV summarizes the most common adverse events (AE) and serious adverse events (SAE) of all

causalities. The proportion of patients experiencing at least one AE for the placebo, 100 mg BID and 150 mg BID groups was 92.1%, 95.2% and 94.8%, respectively. Nausea, syncope, vomiting, and weight decrease appeared to be dose-related. However, the incidence of these AEs in the 100 mg BID group was not statistically different from placebo. A total of 255 patients (24.2%) experienced at least one SAE with an incidence of 26.1%, 23.6% and 23.1%, for the placebo, 100 mg and 150 mg BID groups, respectively. Syncope and pneumonia were among SAEs that appeared to be dose-related. Thirty-two (3.0%) patients experienced an AE which resulted in death. Mortality rate was higher in the placebo group (4.0%) compared to the 100 mg (2.8%) and 150 mg (2.3%) BID groups. Gastrointestinal disorders were among the most frequent AEs that led to discontinuation, with a total of 38 (3.6%) patients. When examining the AE profile among the most frequently occurring AEs that led to discontinuation, nausea (Placebo: 1 [0.3%]; 100 mg BID: 3 [0.9%]; 150 mg BID: 10 [2.9%]) and vomiting (Placebo: 0 [0%]; 100 mg BID: 4 [1.1%]; 150 mg BID: 7 [2.2%]) appeared to be dose-dependent events. Laboratory test results were generally unremarkable, with no apparent clinical differences between groups.

Discussion

In this trial of tramiprosate in mild-to-moderate AD, the planned analyses did not show statistically significant between-group differences. Significant Site effects and the impact of variables not included in the planned analyses reduced model fit and validity. In addition, reduced power due to within- and between-patient variance, as well as the unexpected low deterioration in the placebo group, render these analyses inconclusive. Results of the

Table II. Changes in ADAS-cog Scores, CDR-SB Scores (ITT population) and hippocampus volume (MRI-ITT population)

Outcome/group	Baseline		Change from Baseline/Week																		
	n	Mean	SD	n	LS	95% CI															
ADAS-cog																					
Placebo BID	331	22.2	8.3	326	0.8	0.2-1.4	318	2.2	1.5-2.9	292	3.1	2.4-3.8	284	4.8	4.0-5.7	268	5.9	5.0-6.9	265	7.5	6.5-8.5
100 mg BID	325	22.1	8.6	322	1.1	0.6-1.7	300	1.2	0.7-1.8	282	2.7	2.1-3.3	279	3.8	3.2-4.5	262	5.3	4.5-6.0	258	6.8	5.9-7.7
150 mg BID	318	21.7	8.3	316	0.8	0.2-1.4	293	1.7	1.1-2.3	281	2.9	2.2-3.6	267	4.1	3.3-4.9	257	5.7	4.7-6.6	252	7.5	6.4-8.6
CDR-SB																					
Placebo BID	336	5.8	2.8	335	0.2	0.1-0.4	322	0.7	0.5-0.9	295	1.2	1.0-1.4	287	1.8	1.6-2.1	270	2.2	1.9-2.5	266	2.6	2.3-3.0
100 mg BID	327	5.7	2.6	326	0.4	0.2-0.5	301	0.7	0.5-0.9	282	1.1	0.9-1.3	275	1.6	1.4-1.9	260	1.9	1.7-2.2	258	2.4	2.1-2.8
150 mg BID	320	5.7	2.5	316	0.2	0.1-0.4	294	0.6	0.4-0.8	282	1.0	0.8-1.3	267	1.5	1.3-1.8	254	2.0	1.7-2.3	253	2.6	2.2-3.0
HV [mm ³]																					
Placebo BID	109	3294.4	723.4																109	-202.2	237.0
100 mg BID	103	3278.9	741.2																103	-210.6	237.5
150 mg BID	100	3405.9	702.6																100	-259.7	235.0

Higher scores on ADAS-cog and CDR-SB indicate greater impairment and LS mean changes from Baseline > 0 indicate deterioration. Hippocampus volume (HV) change < 0 indicates atrophy

adjusted models, which improved statistical fit and reduced the impact of Site effects, revealed a statistical trend toward a treatment effect on cognition and a treatment-related reduction in HV loss. Overall, tramiprosate was well tolerated.

AD typically progresses from mild functional impairment to total dependence and death over a period of 5-20 years. A disease-modifying treatment initiated in the mild stage that slows progression by 25% would be expected to have a significant clinical impact. Consistent with this goal, and based on results from earlier trials, our study was powered to demonstrate a 2.5 point treatment effect on the ADAS-cog at 18 months. Although we had estimated an approximate 10-point deterioration in the placebo group, the 18-month non-adjusted and adjusted changes in ADAS-cog were only 7.4 points and 6.2 points, respectively. Accordingly, a 25% difference from placebo would be equivalent to an unadjusted change of 1.8 points and an adjusted change of 1.6 points. This trial was thus underpowered to detect a treatment effect, an issue of importance to other disease-modifying drug development programmes.

Statistical powering may also be affected by variance in longitudinal outcome measures that could obscure potential treatment signals and bias results toward the null hypothesis. In AD, many factors, such as concomitant medication type and dose, can affect outcome variability and their combined impact likely increases as trials become longer and involve larger cohorts. Simple ANCOVA models, such as those used in the planned analyses, may not have been sufficient to model unexplained variance arising from uncontrolled conditions. Indeed, the high unexplained variance found in the planned statistical models was the impetus for the development of more complex mixed-effects models that included multiple covariates and yielded improvement in modelling fit. This underscores the complexity of AD and the importance of careful consideration of statistical modelling

Table III. Change in ADAS-cog and CDR-SB (Adjusted Models) – ITT population

Visit / parameter	ADAS-Cog			CDR-SB		
	Placebo BID	100 mg BID	150 mg BID	Placebo BID	100 mg BID	150 mg BID
	(N = 341)	(N = 335)	(N = 329)	(N = 341)	(N = 335)	(N = 329)
Baseline/n	331	325	318	336	327	320
Mean (SD)	22.2 (8.3)	22.1 (8.6)	21.7 (8.3)	5.8 (2.8)	5.7 (2.6)	5.7 (2.5)
Week 13/n	297	295	293	302	299	295
LS mean change	-1.6	-1.2	-1.5	0.2	0.4	0.2
95% CI	-3.2;-0.0	-2.8-0.4	-3.0-0.1	-0.2-0.7	-0.1-0.8	-0.3-0.6
Difference (%) vs. placebo		0.4 (-25.0)	0.1 (-6.3)		0.2 (100.0)	0.0 (0.0)
P-value		0.302	0.761		0.314	0.656
Week 26/n	294	277	277	296	281	276
LS mean change	-0.2	-1.0	-0.7	0.8	0.7	0.6
95% CI	-1.8-1.4	-2.6-0.6	-2.3-0.9	0.3-1.2	0.3-1.2	0.2-1.1
Difference (%) vs. placebo		-0.8 (400.0)	-0.5 (250.0)		-0.1 (-12.5)	-0.2 (-25.0)
P-value		0.065	0.223		0.703	0.276
Week 39/n	265	264	264	268	266	265
LS mean change	1.0	0.7	0.9	1.2	1.2	1.0
95% CI	-0.7-2.7	-1.0-2.4	-0.8-2.6	0.7-1.7	0.7-1.7	0.5-1.5
Difference (%) vs. placebo		-0.3 (-30.0)	-0.1 (-10.0)		-0.0 (0.0)	-0.2 (-16.7)
P-value		0.525	0.845		0.721	0.329
Week 52/n	260	259	251	261	258	250
LS mean change	3.1	2.0	2.3	2.0	1.8	1.7
95% CI	1.4-4.8	0.4-3.7	0.6-4.0	1.5-2.5	1.3-2.3	1.2-2.2
Difference (%) vs. placebo		-1.1 (-35.5)	-0.8 (-25.8)		-0.2 (-10.0)	-0.3 (-15.0)
P-value		0.090	0.193		0.335	0.136
Week 65/n	246	243	236	247	244	237
LS mean change	4.5	3.8	4.2	2.5	2.3	2.3
95% CI	2.8-6.3	2.1-5.5	2.5-5.9	2.0-3.0	1.8-2.8	1.8-2.8
Difference (%) vs. placebo		-0.7 (-15.6)	-0.3 (-6.7)		-0.2 (-8.0)	-0.2 (-8.0)
P-value		0.217	0.535		0.280	0.378
Week 78/n	248	242	229	245	243	233
LS mean change	6.2	5.4	6.3	3.0	2.7	3.0
95% CI	4.5-7.9	3.6-7.1	4.6-8.0	2.5-3.5	2.2-3.2	2.5;3.5
Difference (%) vs. placebo		-0.8 (-12.9)	0.1 (1.6)		-0.3 (-10.0)	0.0 (0.0)
P-value		0.174	0.873		0.232	0.915

Higher scores on ADAS-cog and CDR-SB indicate greater impairment and LS mean changes from Baseline > 0 indicate deterioration. The following covariates and covariate interactions were retained in the adjusted ANCOVA Models. ADAS-cog: Race, Type of ChEI Used, Age (quartiles), Disease Severity, Vitamin E dose, Antidepressant Use, Genotype, Visit X Disease Severity, Visit X Age (quartiles), and Visit X Memantine Dose. CDR-SB: Cardiovascular Disease, Type of ChEI Used, Disease Severity, Memantine Use, Years of Education (quartiles), Antidepressant Use, Genotype, Vitamin E Use, and Visit X Disease Severity

aimed at improving statistical fit and validity of results.

Results of the HV analysis illustrate the importance of statistical modelling. Our planned analysis showed a trend toward greater HV loss in

the 150 mg BID tramiprosate group, a finding consistent with the AN-1792 amyloid vaccine trial, which showed a non-significantly greater HV loss in the antibody responders [26]. While the underlying neurological basis of the HV loss in the

Table IV. Most common adverse events (> 5%) and serious adverse events (> 1%) in any treatment group, all causalities, safety population

Preferred term (PT)	Placebo BID (N = 353) n (%)	100 mg BID (N = 352) n (%)	150 mg BID (N = 347) n (%)
Adverse events			
Fall	51 (14.4)	58 (16.5)	45 (13.0)
Depression	43 (12.2)	29 (8.2)	33 (9.5)
Nausea	42 (11.9)	58 (16.5)	75 (21.6)
Diarrhoea	41 (11.6)	41 (11.6)	49 (14.1)
Dizziness	39 (11.0)	50 (14.2)	38 (11.0)
Urinary tract infection	39 (11.0)	38 (10.8)	32 (9.2)
Upper respiratory tract infection	34 (9.6)	27 (7.7)	32 (9.2)
Agitation	31 (8.8)	34 (9.7)	26 (7.5)
Headache	29 (8.2)	25 (7.1)	28 (8.1)
Vomiting	28 (7.9)	33 (9.4)	47 (13.5)
Fatigue	25 (7.1)	26 (7.4)	23 (6.6)
Back Pain	25 (7.1)	23 (6.5)	23 (6.6)
Weight decrease	24 (6.8)	38 (10.8)	52 (15.0)
Insomnia	23 (6.5)	18 (5.1)	16 (4.6)
Cough	22 (6.2)	16 (4.5)	16 (4.6)
Nasopharyngitis	21 (5.9)	22 (6.3)	18 (5.2)
Anxiety	20 (5.7)	21 (6.0)	17 (4.9)
Syncope fainting	13 (3.7)	19 (5.4)	25 (7.2)
Serious adverse events			
Syncope	9 (2.5)	10 (2.9)	13 (3.8)
Congestive cardiac failure	7 (2.0)	4 (1.1)	1 (0.3)
Fall	4 (1.1)	5 (1.4)	1 (0.3)
Myocardial infarction	4 (1.1)	2 (0.6)	0 (0.0)
Pneumonia	2 (0.6)	4 (1.1)	8 (2.3)

MedDRA (Version 9.0) was used. PT presented in decreasing order of incidence in the placebo group. Patients are counted once per PT using the most severe level for that event. For some PT, incidence may be derived from more than one System Organ Class

vaccine trial remains unclear, inclusion of covariates selected on the basis of clinical relevance and statistical significance in our trial reversed the direction of the between-group difference, with no change in HV in the 150 mg BID group and a lower volume change relative to placebo in the 100 mg BID group. Interestingly, the adjusted model identified a significant interaction between treatment and several covariates, specifically, age, hippocampus volume at baseline and APOE genotype. These variables have previously been found to exert an impact on HV in a number of longitudinal imaging studies [27-29]. Our study suggests that these variables may modulate the effects of an anti-amyloid intervention on HV changes over the course of the trial. Confirmation of the impact of the above covariates and their interaction with treatment in other data sets would

corroborate our findings and allow the development of more complex imaging analysis plans in future trials.

In our study, we did not find close agreement between cognitive/clinical and HV changes. Psychometric tests and vMRI may not measure precisely the same disease processes, and changes in biological and cognitive measures may not occur over the same time period [17, 30]. Nevertheless, volumetric MRI and cognitive measures are complementary and may be implemented in AD trials in order to document potential treatment effects on the underlying disease and to provide regulatory support for disease modification claims.

This trial is among the first published Phase III trials of anti-amyloid compounds for the treatment of AD. Planned analyses did not demonstrate statistically significant between-group differences,

but were confounded by weakness of the statistical models. Post-hoc adjusted analyses showed a treatment effect in reducing HV loss and a trend toward reduced cognitive decline. These results must be interpreted in consideration of the limitations of the outcome measures, the heterogeneity of the disease and the impact of demographic, genetic and clinical variables on patient outcome.

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