Safety and efficacy of tau aggregation inhibitor therapy in mild or moderate Alzheimer's disease: a Phase 3 randomised controlled trial of leuco-methylthioninium bis(hydromethanesulfonate) (LMTM)

Serge Gauthier, Howard H. Feldman, Lon S. Schneider, Gordon K. Wilcock, Giovanni B. Frisoni, Jiri H. Hardlund, Peter Bentham, Karin A. Kook, Damon J. Wischik, Bjoern O. Schelter, Charles S. Davis, Roger T. Staff, Luc Bracoud, Kohkan Shamsi, John M.D. Storey, Charles R. Harrington, Claude M. Wischik

McGill Centre for Studies in Aging, Verdun, QC, Canada (Prof S Gauthier MD); **University of California, San Diego, CA, USA** (Prof H H Feldman MD); **University of Southern California, Los Angeles, CA, USA** (Prof L S Schneider MD); **Oxford University, Oxford, United Kingdom** (Prof G K Wilcock DM); **University of Geneva, Geneva, Switzerland** (Prof G B Frisoni); **TauRx Therapeutics Ltd, Aberdeen, UK** (J H Hardlund MD, D J Wischik PhD, P Bentham MBChB); **Salamandra LLC, Bethesda, MD, USA** (K A Kook PhD); **University of Aberdeen, Aberdeen, United Kingdom** (Prof B O Schelter PhD, Prof J M D Storey PhD, C R Harrington PhD, Prof C M Wischik MBBS); **CSD Biostatistics, Tucson, AZ, USA** (C S Davis PhD); **Aberdeen Royal Infirmary, NHS Grampian, Aberdeen, UK** (R T Staff PhD); **BioClinica, Lyon, France** (L Bracoud MSc); and **RadMD, New York, NY, USA** (K Shamsi MD)

Corresponding author:

Professor Claude M Wischik
TauRx Therapeutics Ltd, Aberdeen; School of Medicine, Medical Sciences and Nutrition, University of Aberdeen, United Kingdom. Email: cmw@taurx.com; Phone: +44 1224 438550

Word count (body of text) = 4031 words
Word count for abstract = 314 words

Includes 4 Tables and 2 Figures, with 4 Supplementary Tables

Abstract

Background

LMTM acts as a selective tau aggregation inhibitor (TAI) *in vitro* and in transgenic mouse models. It is a stabilised reduced form of the methylthioninium (MT) moiety previously found to have potential efficacy in Alzheimer's disease (AD).

Methods

This 15-month randomised controlled parallel arm trial in mild or moderate AD tested doses of 75 mg and 125 mg given twice daily (b.i.d.) compared with a control dose of 4 mg b.i.d. to maintain blinding with respect to urine/faecal discolouration (NCT01689246, EudraCT 2012-002866-11). 891 patients were randomised to either active dose or control in a 3:3:4 ratio, and stratified by severity, global region and AD-labelled co-medication status. Progression on the ADAS-cog and ADCS-ADL scales were co-primary outcomes, with reduction in brain lateral ventricular volume (LVV) as a key secondary outcome.

Findings

The prespecified primary analyses failed to demonstrate treatment benefit at either of the doses tested, but showed significant benefits for LMTM monotherapy relative to both controls and LMTM add-on therapy (ADAS-cog, p<0.0001; ADCS-ADL, p=0.0174). Prespecified analyses confirmed monotherapy treatment benefits for 150 mg/day (ADAS-cog -6.3 units, CI -8.9 – -3.6, p<0.0001; ADCS-ADL 6.5 units, CI 2.9 – 10.1, p=0.0013; LVV -2.7 cm^3, CI -4.0 – -1.4, p=0.0002) and 250 mg/day (ADAS-cog -5.8 units, CI -8.5 – -3.1, p<0.0001; ADCS-ADL 6.9 units, CI 3.3 – 10.6, p=0.0007; LVV -2.4 cm^3, CI -3.6 – -1.1, p=0.0012). The decline in patients taking LMTM as add-on therapy was indistinguishable from control. Gastrointestinal and urinary effects were the most common adverse events and causes for discontinuation, with non-clinically-significant dose-dependent reduction in haemoglobin the most common laboratory abnormality. Amyloid related imaging abnormalities were seen in fewer than 1% (8/885).

Interpretation

The results suggest that LMTM as monotherapy may be an efficacious and safe treatment for mild to moderate AD, but there is an unexplained attenuation of the effect when used as add-on to available approved treatments.

Funding

The study was financed by TauRx Therapeutics Ltd.

Research in context

Evidence before this study

Current approved treatments for AD offer symptomatic benefit without impacting on the underlying disease pathology. Disease modifying therapies have focussed for many years on the amyloid pathology without success so far. Pathological aggregation of tau protein to form the neurofibrillary tangles discovered by Alzheimer is highly correlated with clinical impairment in AD and begins 20 years before clinical symptoms appear. Targeting this process with tau aggregation inhibitor (TAI) therapy provides a rational approach both to treatment and prevention.

The publications identified in a PubMed search on 29 June 2016 were reviewed for randomised placebo-controlled studies in AD published since 1990, using the search terms “Alzheimer”, “trial”, and “tau” in any field. There are reports of two phase 2 studies in which progressive supranuclear palsy (PSP), a neurodegenerative disease also associated with prominent tau aggregation pathology, was treated with drugs aiming to inhibit tau phosphorylation. Tideglusib (NCT01049399; 12mo; 146 subjects) and davunetide (NCT01110720; 18mo; 313 subjects) both failed to show significant benefit in PSP. A phase 2 trial with methylthioninium chloride in mild to moderate AD (NCT00515333) has been the only trial of a TAI. Methylthioninium (MT) has TAI activity in vitro and in transgenic tau mouse models and demonstrated clinical benefit at 138 mg/day, but not at 218 mg/day, in a phase 2 trial in which the oxidised form of MT was dosed as monotherapy in mild or moderate AD.

Added value of this study

The present phase 3 study evaluates a larger study population over 15 months of treatment using a novel chemical entity to provide the MT moiety in a stable reduced form permitting higher doses to be absorbed in an efficacious form. Doses of 75 mg and 125 mg b.i.d. given as monotherapy demonstrated statistically significant efficacy on clinical and functional co-primary endpoints, as well as reduction in the rate of progression of brain atrophy, with a clinically acceptable safety profile. Doses that were effective as monotherapy failed to produce any benefit in patients taking LMTM as an add-on to approved symptomatic treatments.

Implications of all the available evidence

Findings confirming the present study in a soon to be completed 18-month trial in mild AD would support addition of TAI monotherapy to the treatment options currently available for mild or moderate AD.

Introduction

Current approved treatments for Alzheimer's disease, including the acetylcholinesterase inhibitors (AChEIs) and the N-methyl-D-aspartate receptor antagonist memantine, offer symptomatic benefit without impacting on the underlying disease pathology. Despite the urgent clinical need,[1-2] disease modifying therapies have been elusive thus far, with candidates targeting the amyloid aspect of Alzheimer's disease (AD) pathology proving unsuccessful across late stage clinical trials to date.[3]

Neurofibrillary tangles, the pathology discovered by Alois Alzheimer, are made up of paired helical filaments (PHFs), composed predominantly of a 12-kDa repeat-domain fragment of the microtubule-associated protein tau.[4-6] Numerous studies have confirmed a quantitative link for the spread of aggregated tau pathology with both the extent of clinical dementia and functional molecular imaging deficits in AD.[7-9] Since the process begins at least 20 years prior to any of the clinical manifestations,[10] targeting tau aggregation offers a rational approach to both treatment and prevention of AD.[9] Methylthioninium (MT), a diaminophenothiazine, acts as a tau aggregation inhibitor (TAI) *in vitro*,[12,13] dissolving PHFs isolated from human AD brain tissue *in vitro*,[13] and reducing tau pathology and associated behavioural deficits in transgenic mouse tau models at brain concentrations consistent with human oral dosing.[14,15]

Methylthioninium chloride (MTC, commonly known as methylene blue, the chloride salt of the oxidised form of MT (MT^+)), was tested clinically in a phase 2 study.[16] The minimum safe and effective dose was identified as 138 mg/day, but dose-dependent absorption limitations restricted utility at a higher dose of 218 mg/day. We have developed a stable reduced form of the MT moiety (leuco-methylthioninium dihydromesylate, LMTM) as a distinct novel chemical entity which retains TAI activity *in vitro* and *in vivo*,[13,15] has superior pharmaceutic properties in terms of solubility and pKa, and is not subject to the absorption limitations of the MT^+ form.[14]

We report here the results of a 15-month duration phase 3 randomised controlled double blind parallel group study in mild to moderate AD. The objective was to determine whether treatment with LMTM at doses of 75 mg and 125 mg given twice daily (b.i.d.) was safe and effective in modifying disease progression in AD. These doses were compared with a control dose of 4 mg b.i.d to maintain the blind with respect to urine/faecal discolouration. Patients were permitted to enter the trial whether or not they were taking currently approved AD medications, as it was considered infeasible for these drugs to be restricted given their extensive use. There were co-primary efficacy outcomes including the 11-item Alzheimer's Disease Assessment Scale – cognitive subscale (ADAS-cog) and the 23-item Alzheimer's Disease Cooperative Study Activities of Daily Living (ADCS-ADL). Magnetic resonance imaging (MRI) volumetry was selected as the key secondary outcome to evaluate a potential therapeutic effect on the rate of brain atrophy.

Methods

Patients

Patients were recruited at 115 sites across 16 countries in EU, North America, Asia and Russia between 29 January 2013 and 26 June 2014, and last patient visit was on 30 November 2015.

Inclusion criteria. Patients aged <90 years with a diagnosis of mild to moderate probable AD according to National Institute of Aging (NIA) and Alzheimer's Association (AA) criteria were included with Mini-Mental State Examination (MMSE) score of 14–26 inclusive and with a Clinical Dementia Rating (CDR) total score of 1 or 2. Concomitant use of AChEIs and/or memantine at a stable dose for

at least 18 weeks prior to screening was permitted. Concomitant use of serotonergic antidepressant, antipsychotic (except clozapine or olanzapine) and sedative medications was permitted at stable doses where clinically feasible. Drugs with methaemoglobinaemia warnings or cautions were excluded. Each patient had one or more adult informants participate with them in this trial.

Exclusion criteria. Patients were excluded from the study if they had a significant central nervous system cause for dementia other than AD. Because MT^+ in high doses can induce methaemoglobinaemia, patients at risk were excluded. A more detailed list of inclusion/exclusion criteria is provided in the protocol in Supplementary Materials.

Changes to protocol or Statistical Analysis Plan (SAP) after trial commencement. All amendments are listed in the Protocol provided in Supplementary Materials. In summary, a protocol amendment in August 2013 increased the study duration from 12 to 15 months in light of the placebo decline rates reported in external studies[17,18] which were lower than our initial estimates. The target recruitment was also adjusted to include two-thirds moderate patients to better reflect the expected distribution of tau pathology[9] across both AD studies being conducted. RUD-lite and collection of cerebrospinal (CSF) fluid markers were added as exploratory endpoints. A further amendment in June 2015 changed from the co-primary endpoint from ADCS-CGIC to ADCS-ADL in light of data from external studies[17,18] making relevant placebo decline estimates possible and to conform with recommendations received from the European Medicines Agency. Other amendments entailed primarily clarifications arising from site and/or monitor queries. Substitution of LVV for WBV as the key secondary outcome and addition of TPV were based on advice from the Scientific Advisory Board (SAB) prior to finalisation of the SAP and were not reflected in a protocol amendment.

Randomisation and masking

Patients were randomised at baseline to LMTM 75 mg b.i.d. or 125 mg b.i.d. (expressed as MT base equivalent) or control in a 3:3:4 ratio using an Interactive Web Response System (IWRS) managed by BioClinica. The randomisation was stratified according to geographical region (3 levels: North America, Europe, rest of world), use of AD-labelled co-medications (2 levels, using or not using), severity (2 levels, mild MMSE 20 – 26 and moderate MMSE 14 – 19 inclusive) and site PET capability (2 levels, yes/no).

A total of 600 blocks of length 10 with 3:3:4 treatment allocations were generated by BioClinica using a Java 1.6 api class random number generator that uses a 48-bit seed based on the time the list is generated. The subject randomisation file consisted of the trial randomisation number, treatment group code/description and block number. This file was provided to the manufacturer of the Investigational Medicinal Product (IMP) and a drug kit number list was generated and subsequently uploaded into the IWRS. The randomisation file and IMP kit list were unavailable to personnel involved in study conduct and analysis, but was available to the unblinded statistician providing analyses exclusively for the Data Safety Monitoring Board (DSMB).

Study participants, their informant, and all assessors remained blinded to treatment assignment throughout the study, and safety assessors were not permitted to be involved in the primary efficacy assessments. As LMTM is associated with both urinary[19] and faecal discolouration, the low dose of 4 mg b.i.d. was selected as the control based on repeat dose phase 1 studies, being the minimum that would allow the blind to be maintained and well below the 69 mg/day dose of MTC that was

previously reported to lack clinical efficacy.[16] Clinical study drug supplies were identical in appearance for all three treatment arms.

Ethical conduct of the study

All patients provided written informed consent prior to enrolling in the study; legal representatives provided consent on behalf of patients with reduced decision-making capacity. Informants for the participants also provided consent for involvement. The study was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonisation Guidelines for Good Clinical Practice, and approval of the study protocol and all related documents was obtained from the appropriate Independent Ethics Committees and Institutional Review Boards for all study sites. An independent Data and Safety Monitoring Board was established for oversight of accruing safety information. The trial is registered at www.clinicaltrials.gov (NCT01689246) and the European Union Clinical Trials Registry (2012-002866-11).

Outcome measures: clinical and imaging assessments

ADAS-cog and ADCS-ADL assessments were performed at baseline and every 13 weeks thereafter with the final on-treatment visit at Week 65. These were repeated at the final off-treatment safety visit at Week 69.

Secondary efficacy measures included Clinical Global Impression of Change (ADCS-CGIC, administered by an independent rater at the same visits as the co-primary endpoints) and MMSE (administered on screening and at Weeks 26, 52, 65 and 69). Cranial MRI scans were performed at baseline/screening and every 13 weeks using a standardized protocol at prequalified sites. MRI data were collected centrally by an imaging corelab (Bioclinica) and reviewed centrally by RadMD for eligibility and safety (Amyloid Related Imaging Abnormalities, or ARIA monitoring). Volumetric data were used to measure change in lateral ventricular volume (LVV) as the key secondary outcome measure. Temporo-parietal volume (TPV), whole brain volume (WBV) and hippocampal volume (HV, estimated as the mean of left and right) were included as exploratory endpoints, as was ^{18}F-fluorodeoxyglucose positron emission tomography (FDG-PET) performed during screening and at Weeks 39 and 65 in a subset of patients in sites with this imaging capability, and determination of change in CSF total tau, phospho-tau and amyloid-β_{1-42} between baseline and Week 65 in a subsample of those consenting to lumbar puncture.

Patients were monitored throughout for adverse events (AEs) and clinical laboratory testing, physical and neurological examinations and 12-lead electrocardiograms were performed at all clinic visits (screening, baseline and Weeks 2, 6, 13, 26, 39, 52, 65 and 69). Patients were also assessed at all visits for suicidal ideation and intent using the Columbia-Suicide Severity Rating Scale (C-SSRS),[20] and were systematically monitored for potential serotonin syndrome using a rating scale derived from 4 published diagnostic criteria[21] due to a theoretical potential for serotonin syndrome.[22]

Statistical methods

Sample size. Enrolment of 833 patients was targeted (with 891 patients actually recruited) in order to obtain data on approximately 500 patients completing the study, assuming a 30–40% drop-out rate. This sample size was estimated to provide at least 90% power for detecting a treatment differences of 2.40 units on the ADAS-cog scale and 3.80 units on the ADCS-ADL scale at a two-sided alpha of 0.05 after correction for multiple comparisons, under the assumption that both doses have

an effect size corresponding to a 50% reduction in the expected rate of decline assumed to be 4·76 ± 8·85 (mean ± sd) units and -7·52 ± 14·06 units respectively over 15 months.

Analysis plan: The last version of the SAP was finalised on 9 February 2016 prior to database lock on 10 February 2016 and unblinding on 11 February 2016. The primary efficacy analyses of change from baseline in ADAS-cog and ADCS-ADL scores to week 65 (week 52 if the withdrawal rate exceeded 40%) were conducted in the modified intent-to-treat (mITT) population (all randomised patients who took at least one dose of study treatment and had both a baseline and at least one post-baseline efficacy assessment). The primary analysis was specified as a mixed model repeated-measures (MMRM) analysis with an unstructured covariance matrix and no imputation for missing data. The model included visit (5 levels corresponding to assessments at weeks 13, 26, 39, 52 and 65), treatment (3 levels corresponding to control, 75 mg b.i.d. and 125 mg b.i.d.), treatment-by-visit interaction, the stratification variables as additive terms, and baseline ADAS-cog or ADCS-ADL as a covariate. A similar exploratory analysis was specified in the SAP with the covariate for taking or not taking AD-labelled medications as an interaction term with treatment and as an interaction term with visit in the model. The same methodology was used for all secondary analyses. Westfall's method for multiple comparison correction was used in each step to ensure control of the familywise error with alpha 0·05.[23]

Role of the funding source

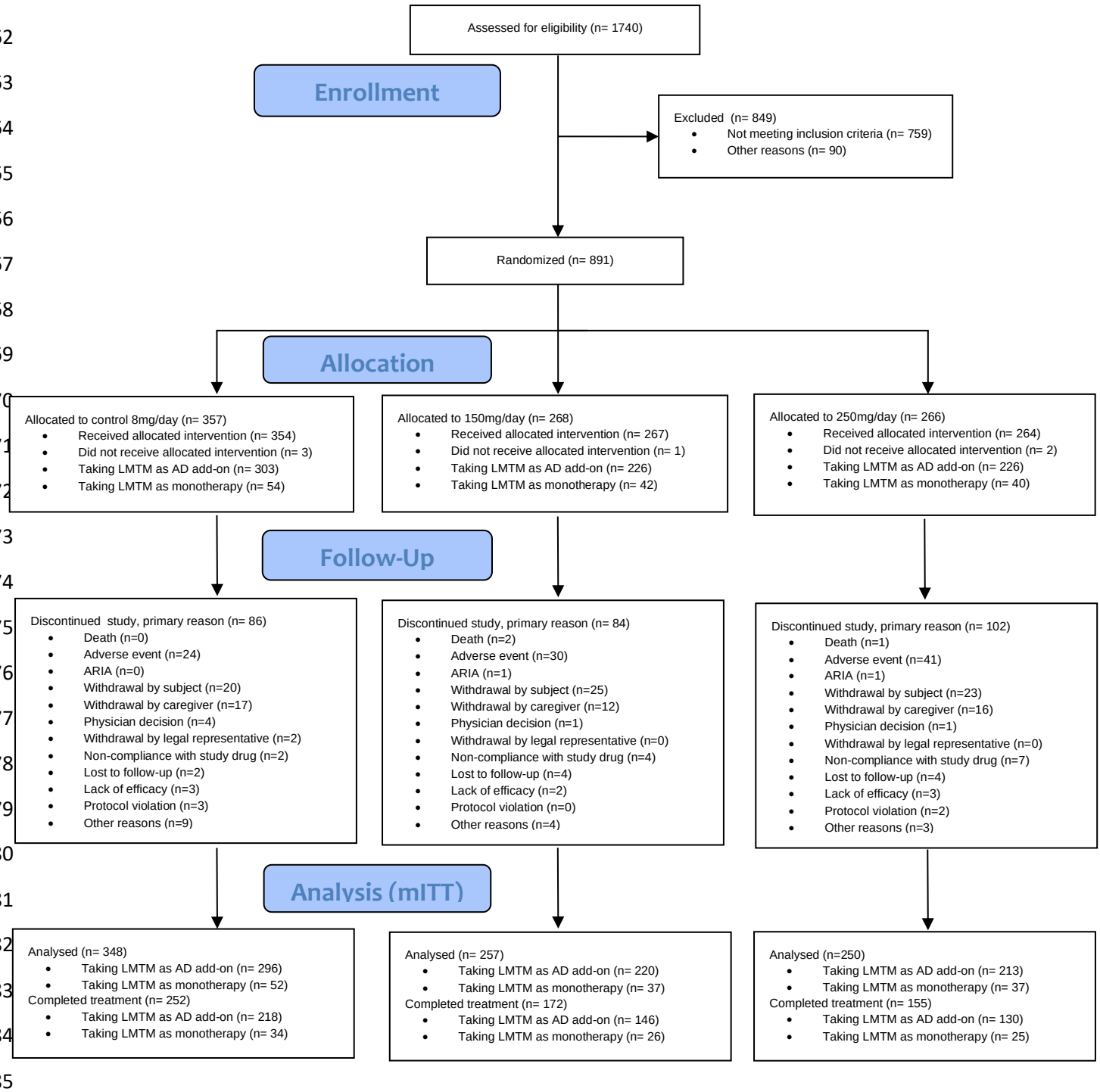
The study was financed entirely by TauRx Therapeutics Ltd. TauRx took the lead in study design and conduct, data interpretation, and report preparation. The decision to submit the paper was taken jointly by SAB members (SG, HHF, LSS, GKW, GFB, and CMW).

Results

Patients

The patient disposition and trial design is shown in Figure 1. Of 891 patients randomised, 885 received at least one dose of study drug and comprised the safety and mITT populations. The baseline demographics and clinical characteristics of the safety population are shown in Table 1. Although 7 patients had a CDR score of 0.5 they were not required to discontinue if already randomised. There were 618 patients completing the study to 65 weeks (with 579 remaining on treatment), for an overall study withdrawal rate of 31%. MRI scans from all scheduled visits were available from 880 patients pre-treatment and 554 at 65 weeks. FDG-PET data were available from 101 patients at 65 weeks, of whom 6 were not taking AD-labelled treatments. Lumbar puncture data were available from 38 patients at baseline, of whom 5 were not taking AD treatments.

Figure 1. Screening and randomised populations.



286 **Table 1.** Patient baseline demographics and clinical characteristics (safety population)

Characteristic	Control LMTM 4 mg b.i.d.	LMTM 75 mg b.i.d.	LMTM 125 mg b.i.d.	Total
	n=354	n=267	n=264	n=885
Age (years)				
Mean (SD)	70·7 (8·5)	71·0 (9·3)	70·1 (9·3)	70·6 (9·0)
Median (min; max)	72·0 (40; 89)	72·0 (39; 88)	71·0 (32; 89)	72·0 (32; 89)
Sex				
Male, n (%)	134 (38)	93 (35)	113 (43)	340 (38)
Female, n (%)	220 (62)	174 (65)	151 (57)	545 (62)
Race				
American Indian or Alaska Native, n (%)	2 (0·6)	3 (1·1)	2 (0·8)	7 (0·8)
Asian, n (%)	41 (11·6)	32 (12·0)	30 (11·4)	103 (11·6)
Black or African American, n (%)	3 (0·8)	3 (1·1)	4 (1·5)	10 (1·1)
White, n (%)	307 (86·7)	226 (84·6)	225 (85·2)	758 (85·6)
Other, n (%)	1 (0·3)	0	2 (0·8)	3 (0·3)
Multiple Race, n (%)	0	3 (1·1)	1 (0·4)	4 (0·5)
Years since diagnosis				
Mean (SD)	2·8 (2·4)	2·9 (2·3)	2·8 (2·2)	2·8 (2·3)
Dementia severity				
CDR 0·5, n (%)	4 (1·1)	1 (0·4)	2 (0·8)	7 (0·8)
CDR 1, n (%)	261 (73·7)	209 (78·3)	192 (72·7)	662 (74·8)
CDR 2, n (%)	89 (25·1)	57 (21·3)	70 (26·5)	216 (24·4)
MMSE				
Mean (SD)	18·6 (3·45)	18·8 (3·44)	18·5 (3·40)	18·6 (3·43)
Median (min; max)	18·0 (14; 26)	19·0 (14; 26)	18·0 (14; 26)	18·0 (14; 26)
MMSE severity				
MMSE ≥20, n (%)	134 (38)	105 (39)	98 (37)	337 (38)
MMSE <20, n (%)	220 (62)	162 (61)	166 (63)	548 (62)
ADAS-Cog:				
Mean (SD)	27·2 (10·1)	26·5 (9·4)	26·7 (9·7)	26·9 (9·8)
Median (min; max)	26·3 (7; 57)	26·3 (8; 54)	26·3 (8; 56)	26·3 (7; 57)
ADCS-ADL:				
Mean (SD)	55·9 (12·7)	58·0 (11·1)	57·5 (12·7)	57·0 (12·3)
Median (min; max)	58·0 (17; 78)	58·5 (16; 78)	60·0 (13; 78)	59·0 (13; 78)
Whole brain volume (cm^3)				
Mean (SD)	927 (108)	922 (115)	939(101)	929 (108)
Median (min; max)	917 (681; 1,233)	922 (602; 1,207)	934 (682; 1,264)	925 (602; 1,264)
Lateral ventricular volume (cm^3)				
Mean (SD)	52 (23)	52 (26)	51 (23)	52 (24)
Median (min; max)	49 (15; 154)	44 (12; 160)	47 (15; 138)	47 (12; 160)
Hippocampal volume (mm^3)				
Mean (SD)	2·3 (0·6)	2·7 (0·6)	2·9 (0·6)	2·8 (0·6)
Median (min; max)	2·7 (1·4; 4·5)	2·7 (1·4; 4·4)	2·8 (1·5; 5·0)	2·7 (1·4; 5·0)
AD-approved co-medications				
AChEI only, n (%)	183 (52)	151 (57)	150 (57)	484 (55)
Memantine only, n (%)	32 (9)	16 (6)	15 (6)	63 (7)
AChEI and memantine, n (%)	93 (26)	60 (23)	61 (23)	214 (24)
CSF biomarkers (ng/L)				
Total tau, mean (SD) [n]	143·9 (68·4) [19]	156·4 (72·5) [15]	113·2 (54·7) [5]	144·8 (68·2) [39]
Phospho-tau, mean (SD) [n]	59·2 (25·3) [20]	61·2 (20·3) [15]	58·1 (12·8) [5]	59·8 (21·9) [40]
Aβ1-42, mean (SD) [n]	264·7 (96·6) [20]	276·0 (85·9) [15]	235·8 (62·1) [5]	265·3 (88·0) [40]
***APOE* genotype**				
ε4 allele present, n (%)	144 (47.5)	91 (41.9)	114 (52.5)	349 (47.4)
ε4 allele absent, n (%)	159 (52.5)	126 (58.1)	103 (47.5)	388 (52.6)

287

Efficacy analyses of primary and secondary outcomes

Table 2 reports baseline values, change from baseline in the control arm and treatment effects shown as differences with respect to the control arm for the primary and secondary outcomes. None of the treatment effects was significant in the primary or secondary analyses. This is shown in Figure 2 (A1, B1, C1, D1, E1). Table 2 also shows the main effects for the covariates included in the primary analysis model. Patients taking LMTM as monotherapy experienced a lower rate of overall clinical decline than patients in the control arm or patients taking the test doses of LMTM as add-on to existing AD treatments. This difference remained statistically significant after correction for multiple comparisons. Mild patients also had a lower overall rate of progression. There was no effect of geographic region.

Table 2. Efficacy analyses for primary and secondary outcomes using primary analysis with the stratification covariates as additive terms in the model. Treatment effects are shown as differences with respect to control change from baseline at 65 weeks. Estimates for the covariates severity and usage of AD-labelled treatments are shown. Population weights are used for all covariates in the mixed model repeated measures analysis, except for the AD treatment term where the contrast was set to "taking approved AD treatments". The effect for geographic regions is not shown as it was not significant. All p values have been adjusted for multiple comparisons using the Westfall procedure.

				Treatment effects		Covariate effects	
		Baseline	Control (4 mg b.i.d.) change from baseline	75 mg b.i.d.	125 mg b.i.d.	Severity (mild)	Taking LMTM as monotherapy
			n = 348	n = 257	n = 250		
ADAS-cog	Mean	27·15	6·32	-0·02	-0·43	-1·03	-2·30
	95% CI	26·09, 28·21	5·31, 7·34	-1·60, 1·56	-2·06, 1·20	-1·57, -0·49	-3·35, -1·25
	p value			0·9834	0·9323	0·0009	< 0·0001
ADCS-ADL	Mean	55·91	-8·22	-0·93	-0·34	1·62	2·00
	95% CI	54·58, 57·24	-9·63, -6·82	-3·12, 1·26	-2·61, 1·93	1·02, 2·23	0·65, 3·35
	p value			0·8659	0·9479	< 0·0001	0·0174
LVV (cm^3)	Mean	52·40	7·18	-0·60	-0·58	-0·12	-0·13
	95% CI	49·93, 54·87	6·63, 7·74	-1·47, 0·27	-1·46, 0·31	-0·25, 0·01	-0·42, -0·16
	p value			0·6049	0·6049	0·3490	0·6158
CGIC	Mean		-1·03	-0·06	0·01	0·16	0·42
	95% CI		-1·16, -0·90	-0·27, 0·14	-0·21, 0·22	0·09, 0·23	0·27, 0·57
	p value			0·7866	0·9504	< 0·0001	< 0·0001
MMSE	Mean	18·60	-3·73	0·06	0·50	0·03	1·95
	95% CI	18·24, 18·96	-4·23, -3·23	-0·71, 0·84	-0·29, 1·30	-0·51, 0·56	1·24, 2·66
	p value			0·9997	0·6888	0·9997	< 0·0001

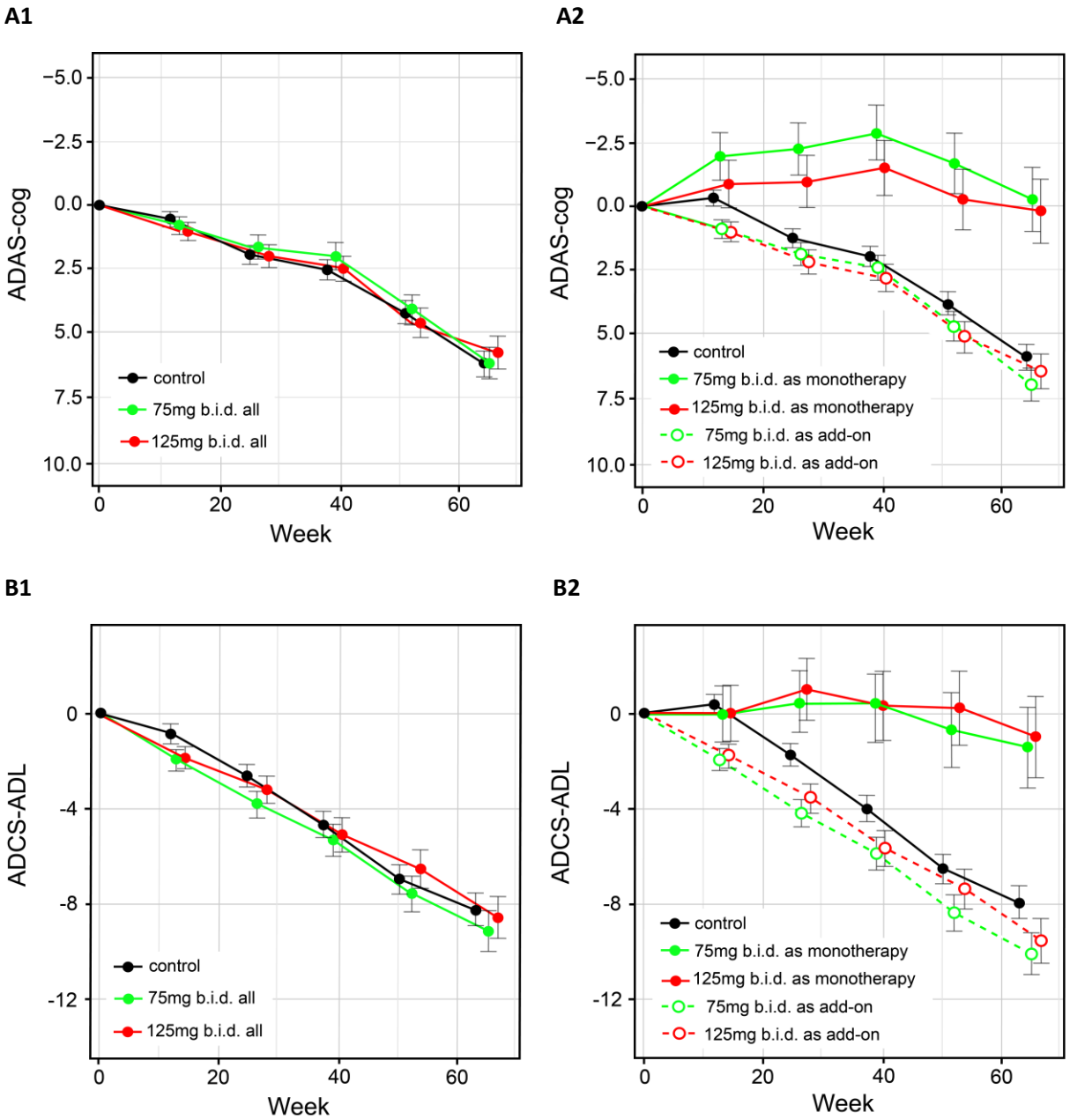
Efficacy analyses of primary and secondary outcomes with AD co-medication status as an interaction term in the analysis model

Since taking LMTM as monotherapy showed significant benefit in the primary analysis model, a further analysis pre-specified in the SAP was undertaken which included it as an interaction term with LMTM treatment and as an interaction term with visit in the model. As can be seen in Table 3 and Figure 2, in patients taking LMTM as monotherapy the differences with respect to control as randomised were significant after correction for multiple comparisons on all treatment outcomes. In patients taking the same doses of LMTM as add-on to approved AD treatments the decline was indistinguishable from controls.

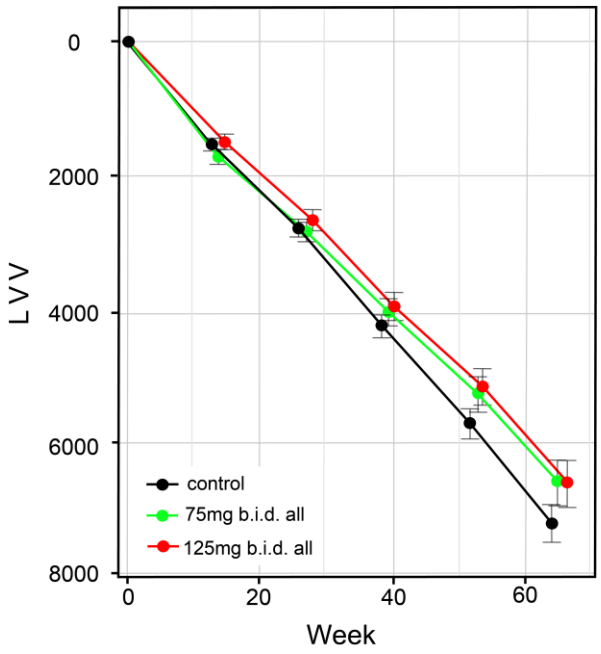
Table 3. Efficacy analyses for primary and secondary outcomes using prespecified analysis with the covariate for LMTM as monotherapy or add-on as an interaction term with treatment and an interaction term with visit in the model. Baseline values are shown according to add-on treatment status. Treatment effects are shown as differences with respect to change from baseline in the control arm as randomised at 65 weeks. All p values have been adjusted for multiple comparisons using the Westfall procedure.

			Baseline and treatment effect for LMTM as add-on therapy			Baseline and treatment effect for LMTM as monotherapy		
		Control (4 mg b.i.d.) change from baseline	**Baseline**	**75 mg b.i.d.**	**125 mg b.i.d.**	**Baseline**	**75 mg b.i.d.**	**125 mg b.i.d.**
		n = 348		n = 220	n = 213		n = 37	n = 37
ADAS-cog	Mean	5·98	26·75	1·02	0·50	26·18	-6·25	-5·79
	95% CI	4·99, 6·98	26·05, 27·45	-0·58, 2·61	-1·15, 2·14	24·42, 27·94	-8·92, -3·59	-8·47,-3·11
	p value			0·3622	0·5555		< 0·0001	< 0·0001
ADCS-ADL	Mean	-7·92	57·73	-2·16	-1·62	54·73	6·48	6·93
	95% CI	-9·29, -6·55	56·86, 58·60	-4·37, 0·05	-3·91, 0·68	52·39, 57·07	2·87, 10·09	3·29, 10·57
	p value			0·1027	0·1674		0·0013	0·0007
LVV (cm^3)	Mean	7·19	52·75	-0·27	-0·31	45·72	-2·71	-2·35
	95% CI	6·64, 7·73	50·99, 54·51	-1·14, 0·60	-1·19, 0·58	41·03, 50·41	-4·00, -1·42	-3·64, -1·05
	p value			0·7334	0·7334		0·0002	0·0011
CGIC	Mean	-0·97		-0·22	-0·10		0·90	0·59
	95% CI	-1·10, -0·84		-0·43, -0·01	-0·312, 0·12		0·54, 1·26	0·23, 0·95
	p value			0·0738	0·3891		< 0·0001	0·0037
MMSE	Mean	-3·47	18·58	-0·25	0·11	19·30	1·92	2·82
	95% CI	-3·95, -2·98	18·33, 18·83	-1·04, 0,·55	-0·71, 0·93	18·69, 19·91	0·46, 3·39	1·36, 4·27
	p value			0·7756	0·7885		0·0287	0·0006

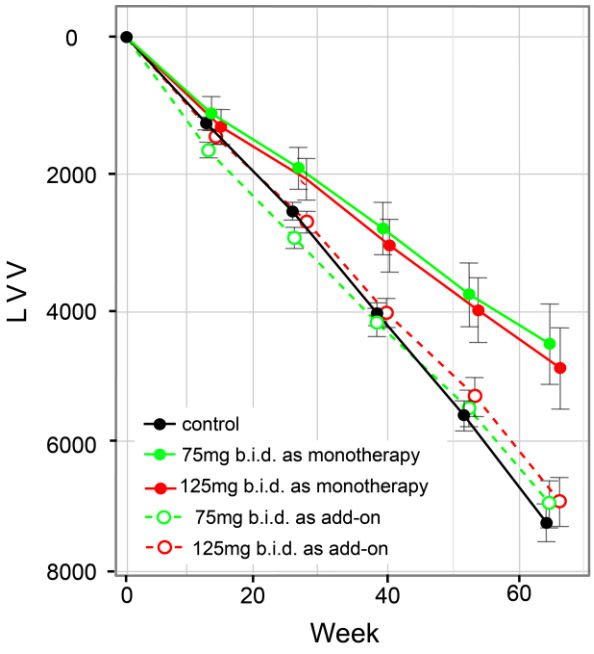
Figure 2. Least squares estimates of mean change from baseline in ADAS-cog (A), ADCS-ADL (B), LVV (C), ADCS-CGIC (D, treated as numerical value) and MMSE (E) using either primary analysis model with AD co-medication status as an additive term in the model (A1, B1, C1, D1, E1), or prespecified repeat of primary analysis with AD-co-medication status as an interaction term in the model showing effect of LMTM treatment as either monotherapy or as add-on to existing AD treatments (A2, B2, C2, D2, E2). In both analysis pairs, the control arm is as randomised. Numbers of subjects analysed in each of the study arms are shown in Tables 2 and 3, and numbers completing treatment are shown in Figure 1.



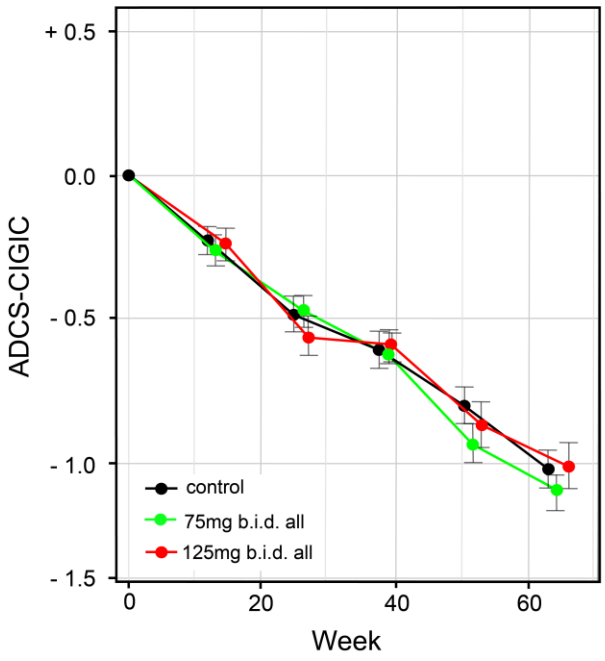
C1


0
2000
4000
6000
8000
L V V
0
20
40
60
Week
control
75mg b.i.d. all
125mg b.i.d. all

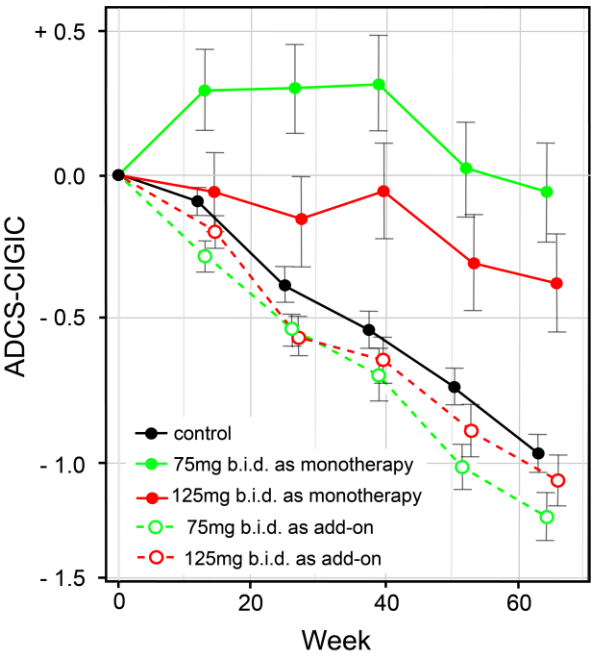
C2


0
2000
4000
6000
8000
L V V
0
20
40
60
Week
control
75mg b.i.d. as monotherapy
125mg b.i.d. as monotherapy
75mg b.i.d. as add-on
125mg b.i.d. as add-on

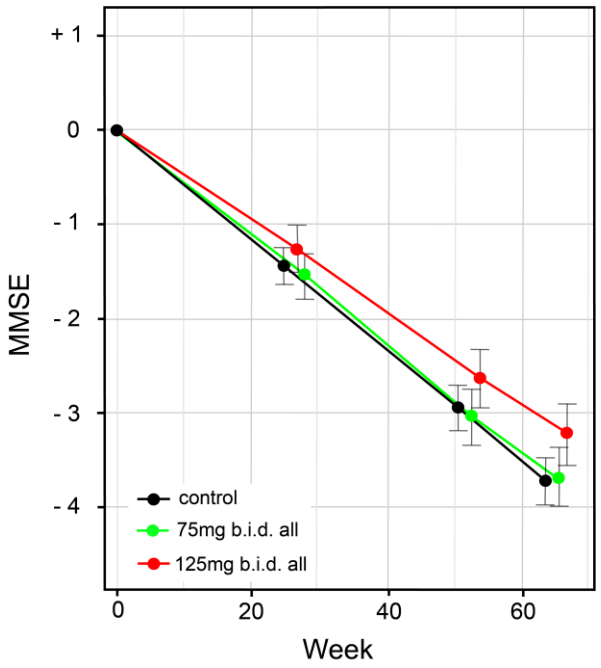
D1


+ 0.5
0.0
- 0.5
- 1.0
- 1.5
ADCS-CIGIC
0
20
40
60
Week
control
75mg b.i.d. all
125mg b.i.d. all

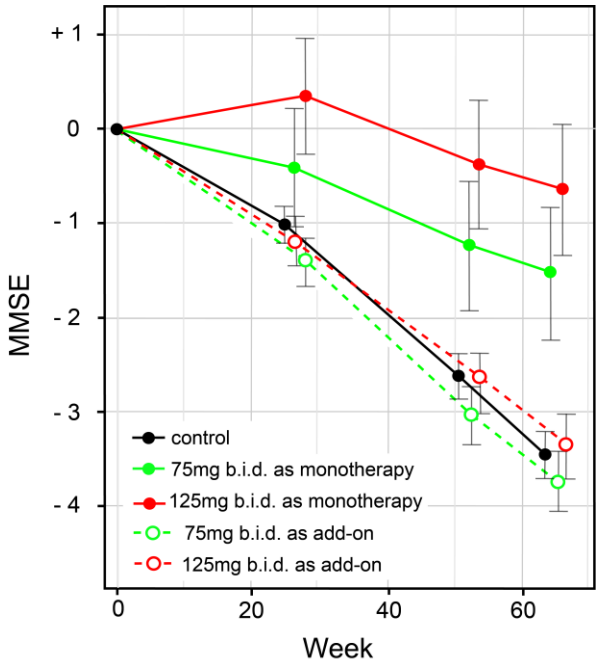
D2


+ 0.5
0.0
- 0.5
- 1.0
- 1.5
ADCS-CIGIC
0
20
40
60
Week
control
75mg b.i.d. as monotherapy
125mg b.i.d. as monotherapy
75mg b.i.d. as add-on
125mg b.i.d. as add-on

346 **E1**



E2



347

348

349

350

Additional analyses

Mild and moderate subjects. The same analyses were repeated for mild and moderate patients as separate subgroups (prespecified in the SAP). As can be seen from Supplementary Table 1, efficacy on all outcomes was again restricted to patients taking LMTM as monotherapy, with treatment benefits being more consistent in mild than in moderate patients.

Comparison at baseline of patients taking or not-taking AD-labelled medications. The baseline characteristics of patients taking LMTM as monotherapy or add-on were compared in *post hoc* analyses (Supplementary Table 2). No difference was found in age or sex distribution. There was no difference in baseline ADAS-cog or MMSE. Mild (but not moderate) patients not taking these medications were marginally worse on the ADCS-ADL scale, had a slightly larger HV and smaller LVV on baseline MRI, with no difference in WBV, TPV or in extent of vascular pathology burden as indicated by Fazekas score at baseline.[24] No differences were found for baseline bilirubin or creatinine clearance which might suggest differences in metabolism or excretion of LMTM. There were no differences in *APOE4* frequency. Mild (but not moderate) patients taking LMTM as monotherapy were significantly over-represented in sites located predominantly in Russia, Eastern Europe (Poland and Croatia) and Malaysia. There was a trend for moderate (but not mild) patients to have left education at an earlier age. Pooled mild/moderate analyses showed the same results.

Analyses of TPV, HV and WBV are shown in Supplementary Table 3. For patients taking the 75 mg b.i.d. and 125 mg b.i.d. doses as monotherapy. TPV and WBV benefits were restricted to patients taking LMTM as monotherapy and were seen in both mild and moderate patients. Benefit on rate of hippocampal atrophy was seen only in mild patients at the highest dose. FDG-PET data were not analysed further as there were only 6 patients in centres with this capability receiving LMTM as monotherapy.

Similarly, the small number of patients precluded further analysis of CSF data. RUD-lite, although included as an exploratory outcome, will be analysed in conjunction with the recently completed study in mild AD.

380

381 **Safety outcomes**

382 The gastrointestinal and urinary tracts were the body systems most commonly affected by adverse
383 events (AEs), and related AEs were also the most common reasons for discontinuing high dose
384 LMTM (9%, 48/531) compared with 2% (6/354) in the control arm. The incidence of targeted
385 gastrointestinal AEs was two-fold higher in patients receiving LMTM as add-on therapy (241/761,
386 32%) compared with those receiving LMTM alone (22/124, 18%). The treatment emergent AEs
387 occurring in ≥5% on high dose LMTM and greater than in the control arm are shown in Table 4.

388

389 **Table 4.** Most common treatment emergent adverse events occurring in ≥5% on 75 mg b.i.d. or 125
390 mg b.i.d. LMTM and greater than in control arm.

MedDRA System Organ Class / Preferred term	Control	High dose LMTM	
	4 mg b.i.d. (n = 354)	75 mg b.i.d. (n = 267)	125 mg b.i.d. (n = 264)
At least one TEAE	296 (83·6%)	224 (83·9%)	229 (86·7%)
Blood and lymphatic system disorders	17 (4·8%)	29 (10·9%)	25 (9·5%)
Anemia	10 (2·8%)	22 (8·2%)	15 (5·7%)
Gastrointestinal disorders	87 (24·6%)	105 (39·3%)	111 (42·0%)
Diarrhea	33 (9·3%)	63 (23·6%)	67 (25·4%)
Nausea	14 (4·0%)	22 (8·2%)	19 (7·2%)
Vomiting	2 (0·6%)	25 (9·4%)	18 (6·8%)
Infections and infestations	88 (24·9%)	83 (31·1%)	76 (28·8%)
Urinary tract infection	29 (8·2%)	29 (10·9%)	26 (9·8%)
Investigations	80 (22·6%)	87 (32·6%)	80 (30·3%)
Blood folate decreased	21 (5·9%)	18 (6·7%)	19 (7·2%)
Renal and urinary disorders	29 (8·2%)	61 (22·8%)	65 (24·6%)
Dysuria	3 (0·8%)	7 (2·6%)	27 (10·2%)
Pollakiuria	6 (1·7%)	15 (5·6%)	18 (6·8%)
Urinary incontinence	9 (2·5%)	18 (6·7%)	12 (4·5%)
Respiratory, thoracic and mediastinal disorders	28 (7·9%)	32 (12·0%)	22 (8·3%)
Cough	12 (3·4%)	14 (5·2%)	11 (4·2%)

Adverse events of special interest included haemolytic anaemia, serotonin syndrome and ARIA. There was no case of clinically significant haemolytic anaemia. The incidence of MedDRA terms for anaemia-related events was 22% (115/531) in patients receiving high dose LMTM, compared to 16% (58/354) in controls. Dose-related mean decreases in haemoglobin were maximal at 6 weeks (-0.66 and -1.08 g/dL for the 75 mg and 125 mg b.i.d. arms respectively), with no change in the control arm (-0·01 g/dL). Although 22% (196/885) of patients entered the study taking a selective serotonin reuptake inhibitor (SSRI), only two had transient symptoms consistent with serotonergic excess. The temporal course and presentation were not consistent with serotonin syndrome in either case. In total, 8/885 (<1%) patients developed ARIA (6 ARIA-H and 2 ARIA-E) during the study, with no dose relationship. There was no indication of increase in suicidality at higher doses relative to control.

With respect to other significant events, 9 patients who participated in the study died, 3 in each treatment arm; none was judged by the investigator as related to treatment. The most common reasons were progression of AD (1 randomised to LMTM 125 mg b.i.d. and 2 to control) or cancer (1 in each treatment group); 1 subject randomised to LMTM 75 mg b.i.d. had a myocardial infarction and there was no etiology in the remaining 2 patients. By protocol, ARIA, serotonin toxicity, and suicidality, discussed above, were to be reported as serious adverse events (SAEs). An additional 96 patients had one or more other non-fatal SAEs, in a frequency that was evenly balanced between the 3 treatment arms. The overall number of SAEs and incidence by body system most commonly affected is presented in Supplementary Table 4, and were judged by the investigator as possibly related to treatment in only 14% (20/139) of the cases, the most common being convulsion (all 4 occurring in the control arm).

Discussion

The study results failed to demonstrate a treatment benefit on either of the co-primary outcomes at either 75 mg b.i.d. or 125 mg b.i.d. doses in the prespecified analysis. However, the primary analysis model showed that patients taking LMTM as monotherapy had significantly lower decline than control patients or those taking LMTM as an add-on to existing AD treatments. Given the significant interaction of LMTM treatment with AD co-medication status, an analysis prespecified in the SAP with this covariate as an interaction term in the model was undertaken as the first supporting analysis. This confirmed a significant treatment benefit on both cognition and activities of daily living for patients taking LMTM as monotherapy at both of the doses tested compared with controls as randomised, and also confirmed that there was an unexplained attenuating effect of existing AD treatments. The higher dose of 125 mg b.i.d. resulted in similar efficacy to that seen at the 75 mg b.i.d. dose. The same pattern of monotherapy efficacy was found for the secondary clinical outcomes (ADCS-CGIC and MMSE) and reduction in LVV, and all remained statistically significant after correction for multiple comparisons. The reduction in LVV was confirmed by corresponding increases in TPV and WBV. This is the first report of a treatment intervention in AD showing concordance between reduction in rate of clinical decline and reduction in rate of progression of brain atrophy.

The rates of decline seen in the control arm are consistent with those reported in recent studies or randomised controlled trials.[23,24] The same was found to be true for the rate of progression of brain atrophy in the mild AD group measured by change of LVV in comparison with data available from the ADNI program.[25,26] Further analyses of the potential effect of the 4 mg b.i.d. dose taken alone in patients randomised to the control arm in this and the recently completed study in mild AD are in progress. The similarity in the decline seen in the control arm as randomised relative to recent studies supports the face validity of the present trial as being representative of currently available trial populations in mild or moderate AD.

The overall safety of LMTM as monotherapy is consistent with prior experience with MTC.[16] Adverse events affecting the gastrointestinal and urinary tracts were the most common and, similarly, were the most common reason for discontinuing high dose LMTM. Reporting of reductions in red cell indices was greater in patients receiving higher doses of LMTM, consistent with effects previously described for MTC.[14] Although 22% (196/885) of patients were taking SSRIs, only two had transient symptoms meeting any of the criteria for serotonin toxicity, although neither was taking an SSRI (or any other serotonergic drug). None of the 9 deaths that occurred during the study was judged as being related to treatment. Eight patients developed ARIA during the study and there was no dose relationship. This frequency is consistent with the placebo rates reported in recent trials.[17-18]

The reason for the loss of benefit when LMTM is combined with symptomatic AD treatments remains to be explained. To date, an interference with TAI activity *in vitro* has been ruled out ([13] and unpublished data), as has an effect of oral LMTM on cholinergic efficacy of donepezil in the scopolamine mouse model (unpublished data). Likewise absorption effects have been ruled out in preliminary analyses of plasma data from a subset of patients (unpublished data). Avenues currently being explored include further blood analyses, the potential effect of cholinergic pathology on cognition and brain atrophy[27] in tau transgenic mouse models, the interaction between cholinesterase and amyloid pathology[28] and whether induction of transporters by chronic

administration of AD symptomatic treatments[29,30] might lower the concentration of MT at the site of action. Although the cognitive efficacy seen in the present study is similar to that reported in the earlier MTC monotherapy study,[16] the failure as yet to provide an explanation for the unexpected pharmacological interaction we have documented remains an important of weakness of the present report.

A further limitation is that this trial was not designed to test the efficacy of LMTM as monotherapy versus add-on to existing symptomatic treatments. The findings are therefore open to the criticism that the groups taking or not taking AD-labelled treatments in addition to LMTM may not have been comparable. We have excluded a number of obvious confounding factors, including age, sex, clinical severity at baseline, extent of coexisting vascular pathology and biological factors that could potentially affect metabolism or excretion of MT. The over-representation of patients from countries with more limited access to AD symptomatic treatments and younger age at completion of education point to socio-economic factors determining treatment access, rather than patient-specific confounding factors.

The relatively small number of patients taking LMTM as monotherapy raises the possibility that the benefit seen in this group is a chance finding. However, the treatment effect was seen in two different arms of the trial and was of such a magnitude as to remain statistically significant in the primary analysis of the whole population after correction for multiple comparisons, and remained so in the first prespecified supporting analysis similarly corrected. The primary efficacy analysis of the similarly designed and recently completed independent study in mild AD (NCT01689233, EudraCT 2012-002847-28) was modified prior to unblinding in the light of the results reported here to take account of the previously unsuspected effect of AD comedication status. Preliminary efficacy results, which will be reported in due course, show statistically significant benefit for LMTM on the same co-primary and secondary outcomes as the present study in a larger monotherapy population. Notwithstanding the limitations of the present study, its results argue in support of LMTM monotherapy being an efficacious and safe treatment for mild to moderate AD with potentially larger effect size than currently available treatments.

Contributors

JH, PB, KAK, DJW, BS, CSD, RTS, LB, KS, JMDS, CRH, and CW were all involved in study design and data interpretation. SG, HHF, LSS, GKW, GBF, JH, PB, KAK, DJW, BS, CSD, RTS, LB, KS, JMDS, CRH, and CW were all involved in the data analysis. All authors critically revised the report, commented on drafts of the manuscript and approved the final report.

Conflicts of interest

SG has received clinical trial support from Lilly and Roche in DIAN-TU, TauRx Therapeutics Ltd (TauRx), and Lundbeck; has been a DSMB member of ADCS, ATRI, API, and Eisai; has been a scientific advisor to Affiris, Boehringer-Ingelheim, Lilly, Roche, Servier, Sanofi, Schwabe, Takeda, and TauRx. HHF has received clinical trial support from TauRx, Lilly, and Roche; he has served as DSMB member for Eisai and DMC member for Genentech/Banner Health; he has served as member of scientific advisory board for TauRx, and Tau Consortium and has been consultant to Arena and Merck Pharmaceuticals. LSS has received grant and research support from Baxter, Genentech, Johnson & Johnson, Eli Lilly, Lundbeck, Novartis, Pfizer, Roche, TauRx, and NIH. Within 3 years of the beginning of the work he has served as a consultant for, and received consulting fees from, Abbvie, AC Immune, Allon, AstraZeneca, Baxter, Biogen Idec, Biotie, Bristol-Myers Squibb, Cerespir, Chiesi, Cognition, Elan, Eli Lilly, Forum (EnVivo), GlaxoSmithKline, Johnson & Johnson,

Lundbeck, MedAvante, Merck, Novartis, Piramal, Pfizer, Roche, Servier, Takeda, TauRx, Toyama (FujiFilm), and Zinfandel. GW has been a scientific advisor to Cytox Ltd, GSK Research and Development Ltd, Nutricia Limited, Red and Yellow Memory Services Ltd, Roche Products Ltd, Shire Pharmaceutical Development, and TauRx. GBF has served in advisory boards for Lilly, BMS, Bayer, Lundbeck, Elan, Astra Zeneca, Pfizer, TauRx, Wyeth, GE, and Baxter; he is a member of the editorial board of Lancet Neurology; he has received grants from Wyeth Int.l, Lilly Int.l, Lundbeck Italia, GE Int.l, Avid/Lilly, Roche, Piramal, and the Alzheimer's Association; he has received lecture fees when speaking at the invitation of Lundbeck, Piramal, and GE. JH, JMDS, CRH, and CMW are officers of, and hold beneficial interests inTauRx. PB, KAK, DJW, BS, CSD, RTS, LB and KS are paid consultants to TauRx. JMDS, CRH, and CMW are inventors on patents relating to LMTM and tau aggregation inhibitors that are owned by WisTa Laboratories Ltd, an affiliate of TauRx.

Acknowledgements

The study was sponsored by TauRx Therapeutics Ltd, Singapore. We gratefully acknowledge study investigators and the generosity of study participants.

References

1. Winblad B, Amouyel P, Andrieu S, et al. Defeating Alzheimer's disease and other dementias: a priority for European science and society. *Lancet Neurol* 2016; **15**: 455-532.
2. Alzheimer's Disease International. World Alzheimer Report 2015: The global impact of dementia, an analysis of prevalence, incidence, cost and trends. *World Alzheimer Report* 2015.
3. Mullane K, Williams M. Alzheimer's therapeutics: Continued clinical failures question the validity of the amyloid hypothesis-but what lies beyond? Biochem Pharmacol 2013; 85: 289-305.
4. Wischik CM, Crowther RA, Stewart M, Roth M. Subunit structure of paired helical filaments in Alzheimer's disease. *J Cell Biol* 1985; **100**: 1905-12.
5. Wischik CM, Novak M, Edwards PC, Klug A, Tichelaar W, Crowther RA. Structural characterization of the core of the paired helical filament of Alzheimer disease. *Proc Natl Acad Sci USA* 1988; **85**: 4884-8.
6. Wischik CM, Novak M, Thøgersen HC, et al. Isolation of a fragment of tau derived from the core of the paired helical filament of Alzheimer's disease. *Proc Natl Acad Sci USA* 1988; **85**: 4506-10.
7. Brier MR, Gordon B, Friedrichsen K, et al. Tau and Aβ imaging, CSF measures, and cognition in Alzheimer's disease. *Science Transl Med* 2016; **8**: 338ra66.
8. Maruyama M, Shimada H, Suhara T, et al. Imaging of tau pathology in a tauopathy mouse model and in Alzheimer patients compared to normal controls. *Neuron* 2013; **79**: 1094-108.
9. Wischik CM, Harrington CR, Storey JMD. Tau-aggregation inhibitor therapy for Alzheimer's disease. *Biochem Pharmacol* 2014; **88**: 529-39.
10. Braak H, Del Tredici K. The pathological process underlying Alzheimer's disease in individuals under thirty. *Acta Neuropathol* 2011; **121**: 171-81.
11. Lai RYK, Harrington CR, Wischik CM. Absence of a role for phosphorylation in the tau pathology of Alzheimer's disease. *Biomolecules* 2016; **6**: 19.

12. Wischik CM, Edwards PC, Lai RYK, Roth M, Harrington CR. Selective inhibition of Alzheimer disease-like tau aggregation by phenothiazines. *Proc Natl Acad Sci USA* 1996; **93**: 11213-8.

13. Harrington CR, Storey JMD, Clunas S, et al. Cellular models of aggregation-dependent template-directed proteolysis to characterize tau aggregation inhibitors for treatment of Alzheimer's disease. *J Biol Chem* 2015; **290**: 10862-75.

14. Baddeley T, C., McCaffrey J, Storey JMD, et al. Complex disposition of methylthioninium redox forms determines efficacy in tau aggregation inhibitor therapy for Alzheimer's disease. *J Pharmacol Exptl Therapeutics* 2015; **352**: 110-8.

15. Melis V, Magbagbeolu M, Rickard JE, et al. Effects of oxidized and reduced forms of methylthioninium in two transgenic mouse tauopathy models. *Behav Pharmacol* 2015; **26**: 353-68.

16. Wischik CM, Staff RT, Wischik DJ, et al. Tau aggregation inhibitor therapy: an exploratory phase 2 study in mild or moderate Alzheimer's disease. *J Alzheimer's Dis* 2015; **44**: 705-20.

17. Salloway S, Sperling R, Fox NC, et al. Two phase 3 trials of bapineuzumab in mild-to-moderate Alzheimer's disease. *New Eng J Med* 2014; **370**: 322-33.

18. Doody RS, Thomas RG, Farlow M, et al. Phase 3 trials of solanezumab for mild-to-moderate Alzheimer's disease. *New Eng J Med* 2014; **370**: 311-21.

19. DiSanto AR, Wagner JG. Pharmacokinetics of highly ionized drugs. II. Methylene blue - absorption, metabolism, and excretion in man and dog after oral administration. *J Pharmaceut Sci* 1972; **61**: 1086-90.

20. Posner K, Brown GK, Stanley B, et al. The Columbia–Suicide Severity Rating Scale: Initial Validity and Internal Consistency Findings From Three Multisite Studies With Adolescents and Adults. *Am J Psychiatry* 2011; **168**: 1266-77.

21. Alusik S, Kalatova D, Paluch Z. Serotonin syndrome. *Neuroendocrinol Lett* 2014; **35**: 265-73.

22. Ramsay RR, Dunford C, Gillman PK. Methylene blue and serotonin toxicity: inhibition of monoamine oxidase A (MAO A) confirms a theoretical prediction. *Br J Pharmacol* 2007; **152**: 946-51.

23. Westfall PH. Multiple testing of general contrasts using logical constraints and correlations. *J Am Stat Assoc* 1997; **92**: 299-306.

24. Murray AD, Staff RT, Shenkin SD, Deary IJ, Starr JM, Whalley LJ. Brain white matter hyperintensities: relative importance of vascular risk factors in nondemented elderly people. *Radiology* 2005; **237**: 251-7.

25. Frisoni GB, Fox NC, Jack CR, Scheltens P, Thompson PM. The clinical use of structural MRI in Alzheimer disease. *Nature Reviews Neurology* 2010; **6**: 67-77.

26. Nestor SM, Rupsingh R, Borrie M, et al. Ventricular enlargement as a possible measure of Alzheimer's disease progression validated using the Alzheimer's disease neuroimaging initiative database. *Brain* 2008; **131**: 2443-54.

27. Risacher SL, McDonald BC, Tallman EF, et al. Association between anticholinergic medication use and cognition, brain metabolism, and brain atrophy in cognitively normal older adults. *JAMA Neurol* 2016; **73**: 721-32.

28. Inestrosa NC, Alvarez A, Pérez CA, et al. Acetylcholinesterase accelerates assembly of amyloid-β-peptides into Alzheimer's fibrils: possible role of the peripheral site of the enzyme. *Neuron* 1996; **16**: 881-91.

589 29. Mohamed LA, Keller JN, Kaddoumi A. Role of P-glycoprotein in mediating rivastigmine effect
590 on amyloid-β brain load and related pathology in Alzheimer's disease mouse model. *Biochim*
591 *Biophys Acta* 2016; **1862**: 778-87.

592 30. Mohamed LA, Qosa H, Kaddoumi A. Age-related decline in brain and hepatic clearance of
593 amyloid-beta is rectified by the cholinesterase inhibitors donepezil and rivastigmine in rats.
594 *ACS Chem Neurosci* 2015; **6**: 725-36.

595