Effects of AZD3480 on Cognition in Patients with Mild-to-Moderate Alzheimer's Disease: A Phase IIb Dose-Finding Study

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Abstract. AZD3480 is a selective agonist of the central $\alpha4\beta2$ and $\alpha2\beta2$ neuronal nicotinic cholinergic receptors (NNRs). Its effects on cognition were investigated in 567 patients with mild-to-moderate Alzheimer's disease (AD) (Mini Mental State Examination [MMSE] 12–26). Mean baseline MMSE was 21 (SD ± 3.7), with 61% of patients having mild disease (MMSE 21–26). Mean age was 74 (range 58–85) years. Patients were randomized to one of 5 treatment groups: AZD3480 5 mg, 20 mg or 35/100 mg, donepezil 10 mg (active comparator) or placebo, and treated once daily for 12 weeks. The primary outcome measure was change from baseline at Week 12 on the AD Assessment Scale-Cognitive Subscale (ADAS-Cog). Neither AZD3480 nor donepezil showed a statistically significant improvement versus placebo on ADAS-Cog. Improvements in a number of secondary outcome measures (MMSE, AD Cooperative Study-Clinical Global Impression of Change (ADCS-CGIC) and Disability Assessment for Dementia [DAD]) were observed for AZD3480 and for donepezil. A *post-hoc* analysis on ADAS-Cog, excluding patients with very mild AD (MMSE 25–26) indicated improvement versus placebo for AZD3480 20 mg (-1.4, 95% CI: -3.0; 0.2) and donepezil (-1.0, 95% CI: -2.3; 0.3). AZD3480 was well tolerated. The study did not meet proof of concept criteria, since neither AZD3480 nor donepezil were statistically significantly superior to placebo on ADAS-Cog and was considered to be inconclusive. Further studies are required to determine the therapeutic potential of stimulating $\alpha4\beta2$ receptors with NNRs in AD patients.

Keywords: Alzheimer's disease, AZD3480, cognitive impairment, NNR agonist, randomized controlled trial

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disease and the most common form of dementia in the elderly [1]. During the last two decades considerable research efforts have been directed towards identifying the cause of AD, with the ultimate aim of developing safe and effective pharmacological treatments. Currently approved anti-dementia drugs consist of the acetycholinesterase inhibitors (AChEIs) donepezil, galantamine, and rivastigmine; and the N-methyl-D-aspartate (NMDA) antagonist, memantine. They provide symptomatic treatment of AD but their efficacy is modest and the disease continues

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to progress [1–3]. Furthermore, the cholinesterase inhibitors affect peripheral as well as central acetylcholinesterase resulting in side effects in the gastrointestinal, cardiovascular, and nervous systems [1], which limit their use at higher doses.

Research into the molecular pharmacology of central nervous system (CNS) nicotinic receptors has highlighted therapeutic possibilities for the development of novel nicotinic agents. Evidence from both animal and human studies supports the involvement of neuronal nicotinic cholinergic receptors (NNRs) in systems serving cognition [4]. NNRs are widely distributed throughout the CNS including structures known to be important for cognition such as the cerebral cortex, hippocampus, and thalamus [5]. They are known to modulate cognition in animals and humans [6] and there is evidence that they are altered in the brains of patients with disease-related cognitive deficits [7, 8]. Among nicotinic receptor subtypes, the evidence is strongest for the involvement of the α4β2 and α7 subtypes in cognition [6]. The α4β2 subtype is only found in the CNS and is a prominent NNR subtype in the human brain [9]. Stimulation of this receptor regulates the release of several neurotransmitters known to be involved in cognition including glutamate, dopamine, acetylcholine, gamma-amino butyric acid (GABA), and noradrenaline [5, 10]. Activation also enhances hippocampal long-term potentiation, a synaptic strengthening associated with memory and learning [11, 12].

AZD3480 is a highly selective CNS-specific NNR agonist at the α4β2 NNR [13] and the co-localized α2β2 NNR [14]. It is devoid of activity at central α7 receptors and its very low activity on peripheral NNRs offers the potential for a low incidence of peripheral adverse events. AZD3480 has exhibited cognition-enhancing effects in non-clinical models of cognition [13]. It has also been shown to reverse fimbria fornix lesion-induced long-term potentiation deficits in rat hippocampal slice [14]. Moreover, AZD3480 has demonstrated an improvement on cognition in both healthy subjects and in phase II studies in patients with age-associated memory impairment (AAMI) and mild cognitive impairment (MCI) [15–20].

The objectives of this Phase IIb dose-finding study were to demonstrate that AZD3480 improves cognition in patients with mild-to-moderate AD relative to placebo and donepezil as an active comparator, to assess the dose relationship of three dose groups of AZD3480, and to assess safety and tolerability of AZD3480.

METHODS

Study design/procedures

This was a multicenter, double-blind, double-dummy, randomized, placebo-controlled, parallel-group, Phase IIb study (Study NCT00501111). The study was approved by local ethics committees in all centers and was undertaken in compliance with the Declaration of Helsinki and according to the principles of Good Clinical Practice (GCP).

Patients were enrolled into the study from 84 participating centers across Europe (Austria, 3; Belgium, 4; Bulgaria, 7; Czech Republic, 14; Germany, 13; Romania, 6; Russia, 10; Spain, 12; United Kingdom, 6; and Canada, 9). They were prospectively genotyped with respect to their metabolism of CYP2D6 substrates and analyzed with respect to concomitant medication (AZD3480 is metabolized predominantly by this enzyme) and then classified as either rapid or slow metabolizers (RMs or SMs). RMs were defined as patients with ≥1.5 functional CYP2D6 alleles who were not being treated concomitantly with any moderate or strong CYP2D6-inhibiting drug.

The study consisted of a 2-week run-in period (no treatment), a 12-week treatment period and a 2-week follow-up period after administration of the last dose (Fig. 1). Patients were randomized to one of five treatment regimens: AZD3480 5 mg, AZD3480 20 mg, AZD3480 35/100 mg (i.e., 35 mg for SMs [to limit exposure caused by the large variability] and 100 mg for RMs), donepezil 5 mg, then 10 mg, or placebo. Randomization was stratified according to country, and to severity of dementia as measured by Mini Mental State Examination (MMSE 21–26 [mild] versus MMSE 12–20 [moderate]). Randomized treatments were administered once daily for 12 weeks in a double-dummy fashion with active treatment of AZD3480 administered in the morning and donepezil in the evening. All RM patients assigned to the 35 mg dose group of AZD3480 had their dose increased to 100 mg after 4 weeks' treatment and continued on this for the remainder of the study. Donepezil doses were chosen according to current label (5 mg once daily in the evening, which was then increased in all patients to 10 mg after 4 weeks' treatment and continued for the remainder of the study).

Patient eligibility

Key inclusion criteria included: male or female, aged 60 to 85 years, with a clinical diagnosis of
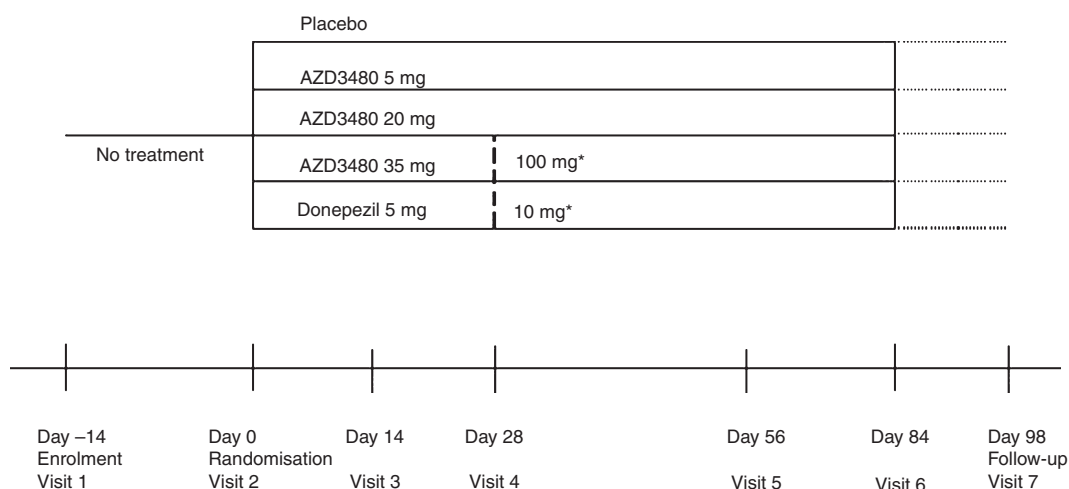
Fig. 1. Study design and sequence of treatment periods. *Dose increased only for rapid metabolisers.

probable AD according to National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria and with an MMSE score of between 12 and 26 (mild-to-moderate AD); computerized tomography (CT) or magnetic resonance imaging (MRI) scan within the last 2 years (performed after onset of dementia) consistent with the diagnosis of AD; a specified caregiver (at home, or in a community dwelling) capable of accompanying the patient on all clinic visits and visiting the patient ≥3 times weekly for the study duration.

Key exclusion criteria included: use of AChEIs or memantine for treatment of AD within 8 weeks prior to enrolment and throughout the duration of the study (except investigational drug after randomization); use of smoking cessation therapy within 4 weeks of enrolment, or during the study; initiation or dose change of Ginkgo Biloba or other therapies with anticholinergic effect within 4 weeks of enrolment, or during the study; dementia other than AD, significant neurological disease, major depressive disorder or other major psychiatric disorder.

Study endpoints/outcome measures

The primary outcome variable was the change in cognitive function from baseline to Week 12, as measured by the 11-item ADAS-Cog (Alzheimer's Disease Assessment Scale-Cognitive Subscale). Secondary outcome measures included change from baseline on global function as measured by Alzheimer's Disease Cooperative Study-Clinical Global Impression of Change (ADCS-CGIC), cognitive function measured by MMSE and on the computerized cognitive drug research (CDR) test battery, caregiver-reported outcomes and evaluation of the safety and tolerability of AZD3480.

Efficacy assessments

Appropriately trained and certified raters administered all tests/interviews relating to the assessment of cognitive functions.

The ADAS Cog includes components for memory, language, and praxis and was designed specifically for evaluating the severity of major dysfunctions in cognitive behavior characteristic of patients with AD [21]. Tests were administered at baseline, at Week 4 and at Week 12 (or at the end of treatment). A summary score, computed across all dimensions of the 11-item ADAS-Cog was used as the primary outcome variable in the statistical analysis. The maximum score is 70 points, a lower score indicating better cognitive function.

Cognitive function was also assessed using MMSE, a commonly used screening tool for cognitive performance [22]. Tests were administered at enrolment and at the end of treatment (Week 12).

ADCS-CGIC, a standardized rating method, provides a semi-structured format allowing clinicians to gather the necessary information from both AD patients and caregivers to form a clinical global impression of change [23]. Change from baseline was assessed at the end of treatment (Week 12). The ADCS-CGIC rater was blinded to all other assessments during the study.

Caregivers completed the following instruments at baseline and at Week 12 (end of treatment): Disability Assessment for Dementia (DAD [24, 25]), Zarit Burden Interview (ZBI [26–29]), Cohen-Mansfield Agitation Inventory (CMAI-long form with expanded definitions [30]), and Activity and Affect Indicators of Quality of Life (AAIQOL [31]).

The CDR computerized test battery was used in this study in parallel with the established measures of cognition to compare its sensitivity in detecting changes in cognition over more established methods. The version used (CDR Ltd, UK) was specifically developed for use with patients suffering from dementia and has been validated extensively [32–34]. The test battery included 11 tests from which composite factors scores were calculated for power of attention, continuity of attention, quality of working memory, quality of episodic memory and speed of memory.

Safety

Safety was assessed by monitoring adverse events (AEs), blood pressure, pulse rate, and clinical laboratory variables. AEs were coded according to the Medical Dictionary for Regulatory Activities (MedDRA), and were collected from the first administration of study drug until the patient's final visit in the study. Serious AEs were collected from enrolment to the follow-up visit.

Statistical analyses

Several assumptions and criteria were used for calculating the proof of concept (PoC) analysis sample size. AZD3480 and donepezil superiority over placebo in ADAS-Cog after 12 weeks treatment by moderate and mild AD respectively was assumed to be: AZD3480, 4 and 3 points; donepezil, 3 and 2 points. The standard deviation was assumed to be 6 points. If any AZD3480 dose group in any of the two sub-populations (moderate and mild AD) were statistically significantly superior versus placebo (one-sided $p < 0.1$) and numerically better than donepezil as assessed by mean change from baseline at Week 12 on the 11-item ADAS-Cog the PoC were to be considered as proven. Acceptable probabilities for erroneous conclusions used in calculations were: 10% for declaring PoC for a placebo-like drug; 6% for not declaring PoC for an efficacious drug; and 10% that donepezil would not be statistically significantly superior to placebo. Assuming a drop-out rate of 10% after randomiza-

tion these assumptions and criteria resulted in a sample size of 525 (336 mild AD and 189 moderate AD).

The full efficacy analysis population included all randomized patients, classified by treatment group, who received at least one dose of study treatment and, for any efficacy variable, had both a valid baseline assessment and at least one valid assessment after randomization. If more than 7 days had elapsed since the last dose of study treatment, an efficacy assessment was regarded as invalid. The last observation carried forward (LOCF) method was used for missing Week 12 data. For individual efficacy variables, the number of patients included in an analysis was dictated by the availability of data for that particular variable.

In the statistical analyses, patients treated with AZD3480 35 mg and 100 mg were treated as one group (high-exposure group), while those treated with 5 mg and 20 mg were treated as separate treatment groups.

The change from baseline to the end of the randomized treatment period (Week 12) was analyzed with a linear model using analysis of variance (ANOVA) or covariance (ANCOVA). The results were reported as p-values, estimates and confidence intervals.

For the PoC efficacy analyses (analysis of ADAS-Cog by AD severity), treatment, country and baseline ADAS-Cog total score were included as explanatory variables in the model. Country was treated as a random effect.

For the combined efficacy analysis of ADAS-Cog (i.e., mild and moderate AD sub-groups combined), the model included treatment, baseline ADAS-Cog total score, country, and severity group as explanatory variables. Country was treated as a random effect while severity group was treated as a fixed effect.

Secondary efficacy variables were analyzed using ANCOVA models, following the same conventions as the PoC analysis and the combined analysis of ADAS-Cog. This article focuses on results from analyses combining the mild and moderate subpopulations.

A *post-hoc* analysis was undertaken excluding patients with very mild AD (baseline MMSE 25–26). This was based on expert opinion received during the course of the trial. This analysis included 452 patients.

RESULTS

A total of 659 patients with mild-to-moderate AD (MMSE 12–26) were enrolled into the study and 567 received randomized treatment. The majority of

patients who were enrolled into the study but subsequently not randomized was as a result of abnormal laboratory data, ECG or disallowed medication use (exclusion criteria of the study). Of the randomized patients, 506 completed the study, with similar rates of completion across the treatment groups (Fig. 2).

Overall, 61 (10.8%) patients discontinued treatment: 20 (8.2%) AZD3480-treated patients, 19 (11.6%) placebo-treated patients, and 22 (13.8%) donepezil-treated patients. There was no apparent dose-related pattern of discontinuations among the AZD3480 patients. The most common primary reasons for withdrawal from treatment were AEs (23 patients, 4.0%) and voluntary discontinuation (23 patients, 4.0%).

Baseline demographic variables and disease characteristics are summarized for all patients in Table 1. The majority of patients were white (99%), and 63% were female. The mean age was 74 years and the mean BMI 25.7 kg/m^2. Forty-four percent of patients were categorized as RMs. Per the design, the majority of patients (61%) had a baseline MMSE score of between 21 and 26, categorizing them as having mild AD; 16% of patients had very mild AD (MMSE 25–26). The

mean MMSE score at baseline was 21 (SD $\pm$ 3.7). Most patients had been diagnosed with AD within the previous year and 87% were treatment naïve.

Baseline demographics and disease characteristics were generally well-balanced across individual treatment groups.

Efficacy

Of 567 patients who received treatment, 558 were included in the full analysis set according to criteria described in the Statistical analyses section. Data from these patients, where available, were included in the individual analyses of each efficacy variable.

11-item ADAS-Cog

The combined analysis of change from baseline in ADAS-Cog total score at Week 12 (LOCF) is shown in Table 2. Negative differences indicate an improvement. LS mean changes from baseline at Week 12 were not statistically significantly superior to placebo for any of the AZD3480 dose groups. This was also the case
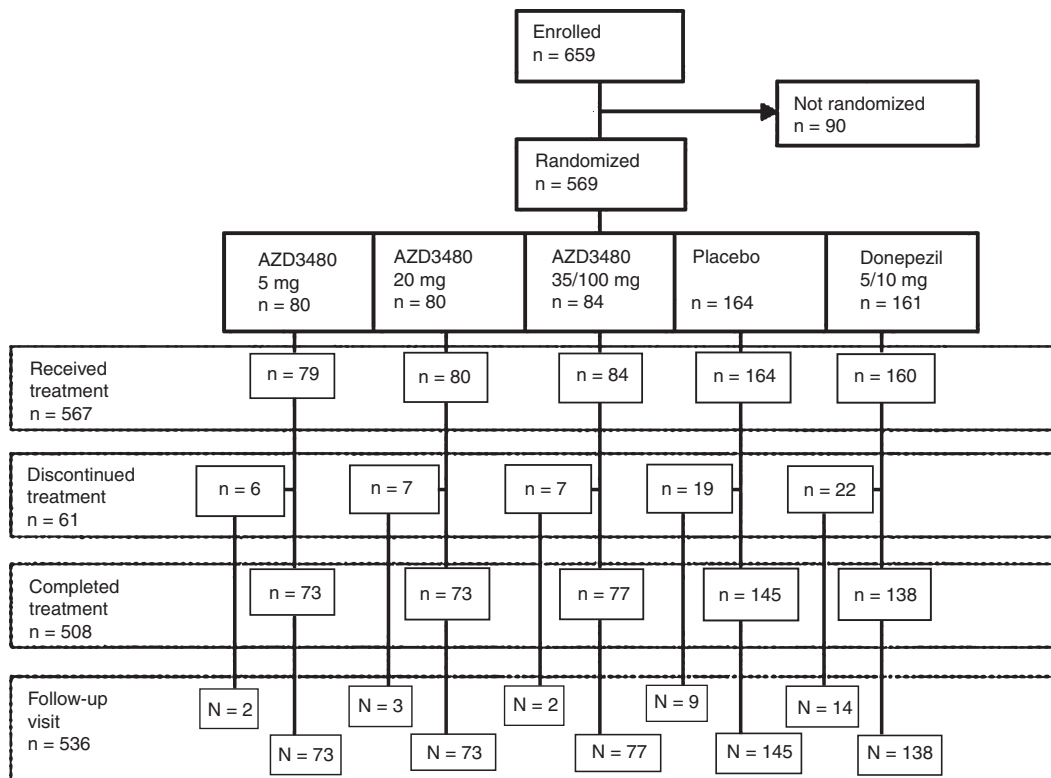

Fig. 2. Patient disposition.

Table 1
Baseline patient demographics

		AZD3480			Placebo	Donepezil	Total
		5 mg (n = 77)	20 mg (n = 78)	35/100 mg (n = 82)	(n = 163)	5/10 mg (n = 158)	(n = 558)
Age	Mean (SD)	74.0 (6.01)	73.8 (6.51)	72.7 (6.24)	73.5 (6.42)	73.9 (6.48)	73.6 (6.36)
Gender	Male	23 (29.9)	28 (35.9)	27 (32.9)	73 (44.8)	54 (34.2)	205 (36.7)
	Female	54 (70.1)	50 (64.1)	55 (67.1)	90 (55.2)	104 (65.8)	353 (63.3)
Race	White	77 (100.0)	77 (98.7)	81 (98.8)	162 (99.4)	158 (100.0)	555 (99.5)
	Black	0	1 (1.3)	1 (1.2)	0	0	2 (0.4)
	Asian	0	0	0	1 (0.6)	0	1 (0.2)
CYP2D6 metabolic	Unknown*	0	1 (1.3)	1 (1.2)	4 (2.5)	7 (4.4)	13 (2.3)
capacity n (%)	Rapid	28 (36.4)	29 (37.2)	39 (47.6)	76 (46.6)	71 (44.9)	243 (43.5)
	Slow	49 (63.6)	48 (61.5)	42 (51.2)	83 (50.9)	80 (50.6)	302 (54.1)
BMI (kg/m^2)	Mean (SD)	26.31 (4.45)	25.04 (3.34)	25.09 (3.77)	26.09 (3.83)	25.7 (3.29)	25.7 (3.72)
MMSE severity	Mild 21–26	46 (59.7)	46 (59.0)	50 (61.0)	99 (60.7)	97 (61.4)	338 (60.6)
category n (%)	Moderate 12–20	31 (40.3)	32 (41.0)	32 (39.0)	64 (39.3)	61 (38.6)	220 (39.4)
Years since AD diagnosis	Mean (SD)	0.81 (1.246)	0.74 (1.074)	0.70 (1.085)	0.83 (1.481)	0.85 (1.194)	0.80 (1.259)
Previously treated AD[#]	No	63 (81.8)	71 (91.0)	70 (85.4)	145 (89.0)	137 (86.7)	486 (87.1)
n (%)	Yes	14 (18.2)	7 (9.0)	12 (14.6)	18 (11.0)	21 (13.3)	72 (12.9)

* If CYP2D6 genotype was unavailable by the time of Visit 4, the patient was treated as a slow metabolizer for the purpose of drug administration only.

[#] Previously treated with acetycholinesterase inhibitors or memantine.

Table 2
ADAS-Cog 11-item total score: Change from baseline versus placebo at Week 12 (LOCF)

Treatment	n	Mean baseline value (SE)	Change from baseline		Difference versus Placebo		
			LS Mean (SE)	95% CI	LS Mean (SE)	95% CI	One-sided p-value
AZD3480 5 mg	77	22.4 (1.02)	0.1 (0.60)	−1.1, 1.23	0.4 (0.73)	−1.0, 1.81	0.701
AZD3480 20 mg	77	22.5 (1.08)	−1.0 (0.60)	−2.2, 0.13	−0.7 (0.73)	−2.1, 0.73	0.166
AZD3480 35/100 mg	81	22.7 (1.12)	0.8 (0.58)	−0.4, 1.90	1.1 (0.72)	−0.3, 2.49	0.936
Placebo	157	24.0 (0.92)	−0.3 (0.42)	−1.2, 0.50	na	na	na
Donepezil 5/10 mg	153	23.8 (0.83)	−1.1 (0.43)	−1.9, −0.21	−0.7 (0.59)	−1.9, 0.44	0.111

ADAS-Cog, Alzheimer's Disease Assessment Scale-Cognitive Subscale; LOCF, last observation carried forward; na, not applicable. Negative differences indicate an improvement.

when the mild and moderate AD sub-populations were analyzed separately.

The effect of treatment with donepezil was numerically better than placebo (LS mean difference to placebo of −0.7 [SE 0.59]), but was not statistically significant.

The observed cases and LOCF analyses of change from baseline to Week 12 over time are shown in Fig. 3. Patients treated with AZD3480 20 mg and those treated with donepezil produced a similar mean improvement from baseline at Week 4 that was still present at Week 12. Patients treated with AZD3480 5 mg and 35/100 mg showed no mean improvement at either time point. Patients treated with placebo showed a mean improvement at Week 4 that was similar to those treated with AZD3480 20 mg and those treated with donepezil. The mean score then showed a decline back towards baseline but without reaching the baseline score.

Sensitivity analyses were performed to identify if any center had very differing results, which might have affected the overall outcome: the results were very variable and no such centers could be identified.

In subgroup analyses by gender, both positive and negative differences from placebo were somewhat larger in females compared with males. However, this may have been driven by severity category imbalances as the female group was more severe on ADAS-Cog. When added as an explanatory factor in the analysis model of total cohort, gender did not explain the variability in ADAS-Cog change in any statistically significant way.

A *post-hoc* analysis on ADAS-Cog excluding patients with very mild AD at baseline (MMSE 25–26) resulted in slightly increased estimates of the effect size: −1.4, 95% CI: (−3.0; 0.2) for AZD3480 20 mg, and −1.0, 95% CI: (−2.3; 0.3) for donepezil (Table 3).
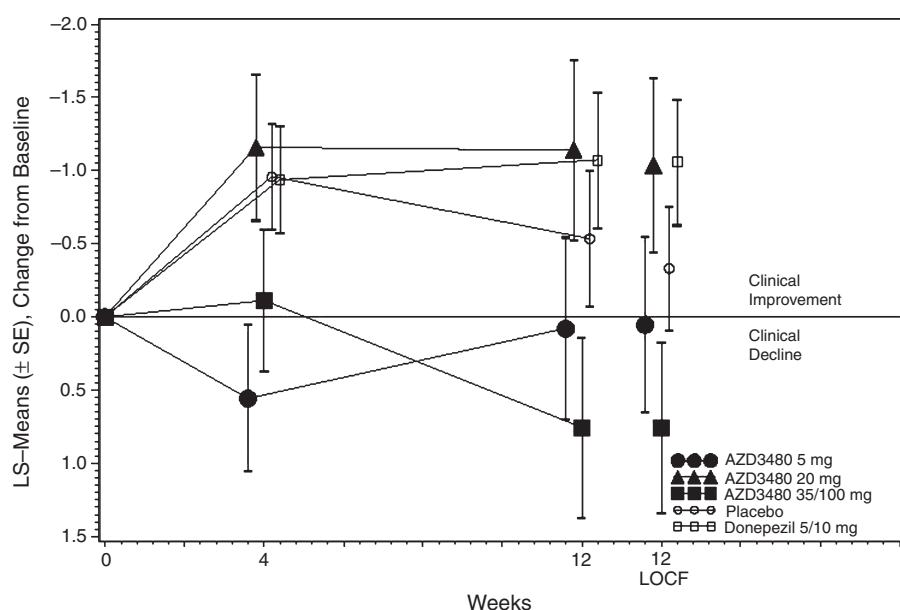
Fig. 3. ADAS-Cog 11-item total score: change from baseline to Week 12: Observed cases and LOCF. ADAS-Cog, Alzheimer's Disease Assessment Scale-Cognitive Subscale; LOCF, last observation carried forward.

Table 3
ADAS-Cog 11-item total score: sub-analysis excluding very mild patients (MMSE 25–26). Change from baseline versus placebo at Week 12 (LOCF)

Treatment	n	Mean baseline value (SE)	Change from baseline		Difference versus Placebo		
			LS Mean (SE)	95% CI	LS Mean (SE)	95% CI	One-sided p-value
AZD3480 5 mg	66	23.3 (9.1)	0.1 (0.7)	−1.1, 1.4	0.3 (0.8)	−1.2, 1.9	0.662
AZD3480 20 mg	64	24.3 (9.2)	−1.6 (0.7)	−2.9, −0.3	−1.4 (0.8)	−3.0, 0.2	0.040
AZD3480 35/100 mg	61	25.4 (10.0)	0.7 (0.7)	−0.7, 2.0	0.8 (0.8)	−0.8, 2.5	0.848
Placebo	131	25.2 (11.5)	−0.2 (0.5)	−1.1, 0.7	na	na	na
Donepezil 5/10 mg	130	25.4 (10.0)	−1.2 (0.5)	−2.1, −0.3	−1.0 (0.7)	−2.3, 0.3	0.065

ADAS-Cog, Alzheimer's Disease Assessment Scale – Cognitive Subscale; LOCF, last observation carried forward; na, not applicable. Negative differences indicate an improvement.

MMSE

The combined (mild and moderate subgroups) analysis of change in MMSE from baseline at Week 12 (LOCF) is shown in Table 4. Positive scores indicate an improvement. Treatment with AZD3480 20 mg and 5 mg showed an improvement relative to placebo, as did donepezil treatment (unadjusted p-values 0.009, 0.091 and 0.001, respectively). AZD3480 35/100 mg treatment showed no improvement relative to placebo.

ADCS-CGIC

The combined analysis of change in ADCS-CGIC scores from baseline at Week 12 (LOCF) is shown in Table 5. Negative differences indicate an improvement. At Week 12, treatment with AZD3480 20 mg showed an improvement versus placebo (unadjusted p-value <0.001), as did AZD3480 35/100 mg and donepezil treatment (unadjusted p-values 0.070 and 0.036, respectively). The observed percentage of patients rated on the ADCS-CGIC scale as showing improvement (rating = 1 to 3) was greater than placebo for all 3 AZD3480 dose groups (AZD3480 range: 35.6% to 46.7%; placebo 27.7%) and was largest for the 20 mg dose (Fig. 4). The corresponding figure for donepezil was 33.3%. The frequency of decline for patients treated with 20 mg AZD3480 was less than placebo whereas the other dose groups were similar to placebo.

CDR

Treatment with AZD3480 did not show any improvement in CDR composite scores in the combined analysis. The variability in composite scores was very high.

Table 4
MMSE total score: Change from baseline versus placebo at Week 12 (LOCF)

Treatment	n	Mean baseline value (SE)	Change from baseline		Difference versus Placebo		
			LS Mean (SE)	95% CI	LS Mean (SE)	95% CI	One-sided *p*-value
AZD3480 5 mg	74	20.8 (0.40)	0.4 (0.35)	−0.3, 1.08	0.5 (0.38)	−0.2, 1.27	0.091
AZD3480 20 mg	75	20.8 (0.41)	0.8 (0.35)	0.1, 1.47	0.9 (0.38)	0.2, 1.67	0.009
AZD3480 35/100 mg	77	21.1 (0.42)	−0.1 (0.35)	−0.8, 0.59	0.0 (0.38)	−0.7, 0.79	0.454
Placebo	145	20.7 (0.31)	−0.1 (0.28)	−0.7, 0.42	na	na	na
Donepezil 5/10 mg	138	20.6 (0.31)	0.9 (0.28)	0.4, 1.47	1.0 (0.32)	0.4, 1.68	0.001

MMSE, Mini Mental State Examination; LOCF, last observation carried forward; na, not applicable. Positive scores indicate an improvement.

Table 5
ADCS-CGIC score: Change from baseline at Week 12 (LOCF)

Treatment	n	Rating of change from baseline		Difference versus placebo		
		LS Mean (SE)	95% CI	LS Mean (SE)	95% CI	One-sided *p*-value
AZD3480 5 mg	73	3.9 (0.12)	3.7, 4.18	−0.1 (0.14)	−0.4, 0.18	0.239
AZD3480 20 mg	75	3.6 (0.12)	3.3, 3.79	−0.5 (0.14)	−0.8, −0.21	<0.001
AZD3480 35/100 mg	78	3.8 (0.12)	3.6, 4.07	−0.2 (0.14)	−0.5, 0.07	0.070
Placebo	148	4.0 (0.09)	3.9, 4.22	na	na	na
Donepezil 5/10 mg	141	3.8 (0.10)	3.6, 4.02	−0.2 (0.12)	−0.4, 0.02	0.036

ADCS-CGIC, Alzheimer's disease Cooperative Study-Clinical Global Impression of Change; LOCF, last observation carried forward; na, not applicable. Rating scale: 1–3 = improvement, 4 = no change, 5–7 = decline. Negative differences versus placebo indicate an improvement.
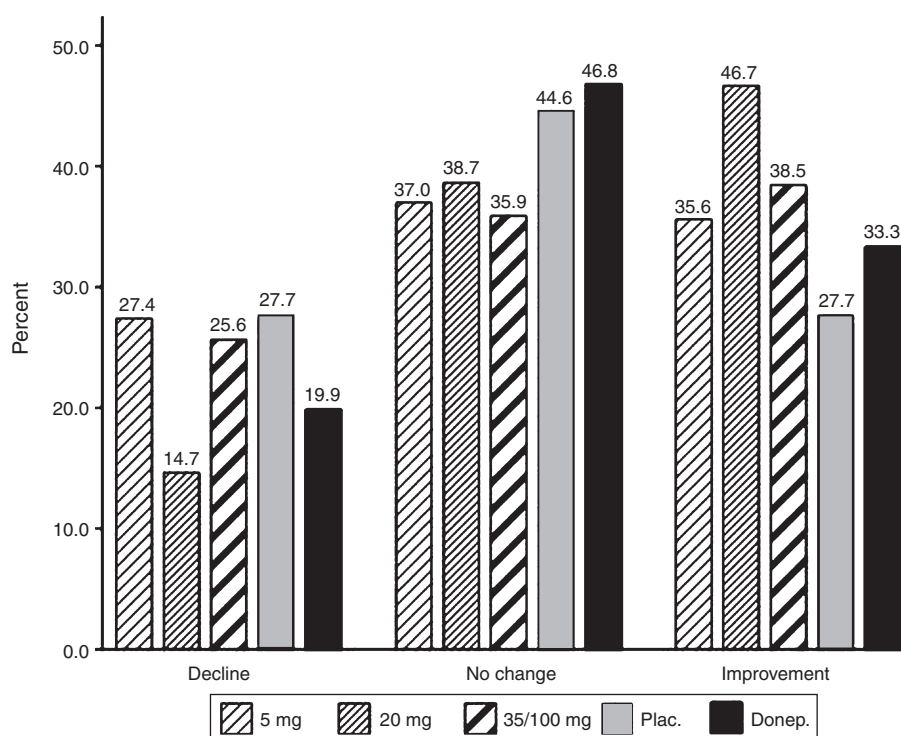

Fig. 4. ADCS-CGIC score: change from baseline at Week 12 (LOCF). ADCS-CGIC, Alzheimer's disease Cooperative Study-Clinical Global Impression of Change; LOCF, last observation carried forward.

Caregiver-reported outcomes

The combined analysis of change from baseline in DAD total score at Week 12 (LOCF) is shown in Table 6. Positive scores indicate an improvement relative to placebo. Treatment with AZD3480 35/100 mg showed an improvement relative to placebo (unadjusted *p*-value <0.1), with an LS mean difference of 2.9 (SE 2.15; 95% CI −1.3, 7.11). Treatment

Table 6
DAD total score: Change from baseline versus placebo at Week 12 (LOCF)

Treatment	n	Mean baseline value (SE)	Change from baseline		Difference versus Placebo		
			LS Mean (SE)	95% CI	LS Mean (SE)	95% CI	One-sided p-value
AZD3480 5 mg	61	76.6 (2.37)	0.2 (1.84)	−3.4, 3.80	2.8 (2.20)	−1.5, 7.13	0.102
AZD3480 20 mg	58	77.4 (2.58)	−1.2 (1.89)	−4.9, 2.51	1.4 (2.24)	−3.0, 5.82	0.264
AZD3480 35/100 mg	66	73.4 (3.14)	0.3 (1.77)	−3.2, 3.76	2.9 (2.15)	−1.3, 7.11	0.090
Placebo	124	74.8 (2.17)	−2.6 (1.32)	−5.2, −0.02	na	na	na
Donepezil 5/10 mg	109	71.0 (2.41)	−0.8 (1.42)	−3.6, 2.00	1.8 (1.85)	−1.8, 5.46	0.164

DAD, Disability Assessment for Dementia; LOCF, last observation carried forward; na, not applicable. Positive scores indicate an improvement.

with AZD3480 5 mg showed a numerical improvement similar to that seen with 35/100 mg (unadjusted p-value = 0.102). Treatment with AZD3480 did not show improvement compared with placebo on the CMAI, AAIQOL, or ZBI.

Safety

The safety population included all patients who received at least one dose of study treatment. In general, few patients reported an AE for any particular preferred term. The most common AEs (occurring in ≥3 patients among AZD3480 treatment groups or in ≥2 patients in any single treatment group) reported overall were headache, nasopharyngitis, diarrhea, dizziness, and fatigue (Table 7). Incidences in the AZD3480 groups were generally similar to placebo, although those treated with 35/100 mg had slightly higher rates than placebo for dizziness (4.8% versus 1.2%) and fatigue (4.8% versus 0.6%). Patients treated

with donepezil had slightly higher rates of diarrhea (5.0% versus 1.8%) and vomiting (4.4% versus 0.6%) compared with placebo. Most AEs were considered by the investigator to be of mild or moderate intensity. No nicotine withdrawal symptoms were identified.

DISCUSSION

The primary outcome measure of change from baseline on ADAS-Cog 11-item total score was not statistically significant relative to placebo for either AZD3480 or donepezil. Therefore, donepezil could not establish assay sensitivity and it was not possible to draw any firm conclusions regarding the efficacy of AZD3480 on the ADAS-Cog. A contributory factor could be the large number of centers (84) and countries (10) involved in the trial, which may have increased variability. The observed mean effect of donepezil on ADAS-Cog relative to placebo was certainly lower than predicted and this suggests that the study may have

Table 7
Most common AEs* to end of treatment

Preferred term	AZD3480			Placebo	Donepezil	Total
	5 mg (n = 79) n (%)	20 mg (n = 80) n (%)	35/100 mg (n = 84) n (%)	(n = 164) n (%)	5/10 mg (n = 160) n (%)	(n = 567) n (%)
Patients with any AE	21 (26.6)	23 (28.8)	34 (40.5)	60 (36.6)	60 (37.5)	198 (34.9)
Headache	3 (3.8)	2 (2.5)	4 (4.8)	9 (5.5)	5 (3.1)	23 (4.1)
Nasopharyngitis	1 (1.3)	2 (2.5)	2 (2.4)	9 (5.5)	3 (1.9)	17 (3.0)
Diarrhea	1 (1.3)	2 (2.5)	1 (1.2)	3 (1.8)	8 (5.0)	15 (2.6)
Dizziness	2 (2.5)	2 (2.5)	4 (4.8)	2 (1.2)	5 (3.1)	15 (2.6)
Fatigue	1 (1.3)	1 (1.3)	4 (4.8)	1 (0.6)	4 (2.5)	11 (1.9)
Insomnia	0	0	2 (2.4)	3 (1.8)	6 (3.8)	11 (1.9)
Nausea	0	0	2 (2.4)	2 (1.2)	6 (3.8)	10 (1.8)
Vomiting	1 (1.3)	0	1 (1.2)	1 (0.6)	7 (4.4)	10 (1.8)
Anxiety	0	1 (1.3)	1 (1.2)	4 (2.4)	2 (1.3)	8 (1.4)
Arthralgia	3 (3.8)	0	2 (2.4)	1 (0.6)	0	6 (1.1)
Back Pain	1 (1.3)	0	3 (3.6)	0	2 (1.3)	6 (1.1)
Nightmare	0	1 (1.3)	1 (1.2)	0	4 (2.5)	6 (1.1)
Influenza	1 (1.3)	0	2 (2.4)	0	2 (1.3)	5 (0.9)
Edema Peripheral	1 (1.3)	0	2 (2.4)	1 (0.6)	1 (0.6)	5 (0.9)
Urinary Tract Infection	0	1 (1.3)	1 (1.2)	1 (0.6)	2 (1.3)	5 (0.9)
Blood Pressure Increased	1 (1.3)	0	1 (1.2)	0	2 (1.3)	4 (0.7)
Urinary Incontinence	0	2 (2.5)	0	0	1 (0.6)	3 (0.5)

*Defined as ≥3 patients in AZD3480 treatment groups or ≥2 patients in any single treatment group.

been underpowered. It is possible that a longer duration of treatment e.g., 24 weeks may have resulted in assay sensitivity with donepezil. However, the power in our study was based on the mean −2.7 points reported in the registration trial over 12 weeks 10 mg/day donepezil treatment [35], and the weighted mean difference of −2.98 (LOCF, 12 week studies) or −2.45 (12 week completers) reported in the Cochrane meta-analysis for 10 mg/day donepezil [36]. Other recent studies of investigational drugs which have included donepezil as a control have also reported relatively small effects on ADAS-Cog (−0.8 and −1.2 respectively) compared with what had been reported in earlier studies, even when the treatment period was 24 weeks [37, 38]. One important difference between recent studies and studies conducted with donepezil in the 1990s is the availability today of a number of approved drugs for the symptomatic treatment of AD which may affect the type of patients selected for placebo-controlled clinical trials. However, 87% of patients in this study had not been previously treated with acetyl-cholinesterase inhibitors or memantine. It is likely that a proportion were comparable to patients studied in the 1990s since some were recruited from countries where access to acetylcholinesterase inhibitors and memantine is difficult. The small effect of donepezil in the present study may in part be because the improvement in the placebo group had not declined back to baseline by 12 weeks. It may also be due to the relatively mild severity of patients (mean MMSE 21) compared with those that were included in earlier studies (mean MMSE 19) [35, 39].

A *post-hoc* analysis excluding very mild patients (MMSE 25–26) did increase the effect of donepezil and the effect of the AZD3480 20 mg dose. This may be due to improved diagnostic certainty, especially given the mean time since diagnosis was relatively short (0.8 years). It may also be due to the increased sensitivity of the ADAS-Cog as the severity of the cohort increases [40].

The CDR test battery did not offer any advantages over ADAS-Cog in this study in detecting an effect of AZD3480. However, the variability in composite scores was very high which could have masked any potential gain. This may indicate difficulties in applying this measure to a large multicentre multinational trial compared with a small number of highly practiced centers. Improvements in several secondary outcome measures (MMSE, ADCS-CGIC, DAD) were observed for AZD3480, especially for the 20 mg dose. It is possible that these scales are capturing an effect such as an improvement in function or behavior that

is not seen on the more specific assessments of cognition like the ADAS-Cog. This suggests that stimulation of α4β2 nicotinic receptors might be having some effects on outcomes relevant to AD although the dose response relationship is puzzling. Further studies will be required to test this. Interestingly, a recent study with AZD3480 in adults with attention-deficit hyperactivity disorder (ADHD) showed improvements with a 50 mg dose in clinical symptoms measured on the Conners' Adult ADHD Rating Scale-Investigator Rating (CAARS-INV) and on a core cognitive deficit for ADHD indicating effects on cognitive function [41]. However, the relevance of these findings to AD is not known.

The overall safety and tolerability profile for the AZD3480 dose groups was similar to placebo and with fewer gastrointestinal-related adverse events (diarrhea, nausea, and vomiting) than observed with donepezil. The pattern of AEs for donepezil was as expected. No nicotine withdrawal symptoms were identified during the 2-week follow-up period after end of treatment with AZD3480.

In conclusion, the study did not meet its proof of concept criteria. However, as the effect on the primary outcome measure ADAS-Cog was not statistically significant versus placebo for either AZD3480 or the active control donepezil, the study was considered to be inconclusive. Nevertheless, an improvement in several secondary outcome measures was observed with AZD3480 and donepezil, and AZD3480 was well tolerated. There were no signs of any nicotine withdrawal symptoms. Further studies are required to determine the therapeutic potential in AD of stimulating of the α4β2 receptor with neuronal nicotinic agonists.

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