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ORIGINAL RESEARCH ARTICLE

Analysis of Cerebrospinal Fluid, Plasma β-Amyloid Biomarkers, and Cognition from a 2-Year Phase 2 Trial Evaluating Oral ALZ-801/Valiltramiprosate in APOE4 Carriers with Early Alzheimer's Disease Using Quantitative Systems Pharmacology Model

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Abstract

Introduction ALZ-801/valiltramiprosate is an oral, small-molecule inhibitor of beta-amyloid (Aβ) aggregation and oligomer formation in late-stage development as a disease-modifying therapy for early Alzheimer's disease (AD). The present investigation provides a quantitative systems pharmacology (QSP) analysis of amyloid fluid biomarkers and cognitive results from a 2-year ALZ-801 Phase 2 trial in APOE4 carriers with early AD.

Methods The single-arm, open-label phase 2 study evaluated effects of ALZ-801 265 mg two times daily (BID) on cerebrospinal fluid (CSF) and plasma amyloid fluid biomarkers over 104 weeks in APOE4 carriers with early AD [Mini-Mental State Examination (MMSE) ≥ 22]. Subjects with positive CSF biomarkers for amyloid ($A\beta_{42}/A\beta_{40}$) and tau pathology ($p\text{-}tau_{181}$) were enrolled, with serial CSF and plasma levels of $A\beta_{42}$ and $A\beta_{40}$ measured over 104 weeks. Longitudinal changes of CSF $A\beta_{42}$, plasma $A\beta_{42}/A\beta_{40}$ ratio, and cognitive Rey Auditory Verbal Learning Test (RAVLT) were compared with the established natural disease trajectories in AD using a QSP approach. The natural disease trajectory data for amyloid biomarkers and RAVLT were extracted from a QSP model and an Alzheimer's disease neuroimaging initiative population model, respectively. Analyses were stratified by disease severity and sex.

Results A total of 84 subjects were enrolled. Excluding one subject who withdrew at the early stage of the trial, data from 83 subjects were used for this analysis. The ALZ-801 treatment arrested the progressive decline in CSF $A\beta_{42}$ level and plasma $A\beta_{42}/A\beta_{40}$ ratio, and stabilized RAVLT over 104 weeks. Both sexes showed comparable responses to ALZ-801, whereas mild cognitive impairment (MCI) subjects (MMSE ≥ 27) exhibited a larger biomarker response compared with more advanced mild AD subjects (MMSE 22–26).

Conclusions In this genetically defined and biomarker-enriched early AD population, the QSP analysis demonstrated a positive therapeutic effect of oral ALZ-801 265 mg BID by arresting the natural decline of monomeric CSF and plasma amyloid biomarkers, consistent with the target engagement to prevent their aggregation into soluble neurotoxic oligomers and subsequently into insoluble fibrils and plaques over 104 weeks. Accompanying the amyloid biomarker changes, ALZ-801 also stabilized the natural trajectory decline of the RAVLT memory test, suggesting that the clinical benefits are consistent with its mechanism of action. This sequential effect arresting the disease progression on biomarkers and cognitive decline was more pronounced in the earlier symptomatic stages of AD. The QSP analysis provides fluid biomarker and clinical evidence for ALZ-801 as a first-in-class, oral small-molecule anti-Aβ oligomer agent with disease modification potential in AD.

Trial Registry https://clinicaltrials.gov/study/NCT04693520.

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Key Points

We report a quantitative systems pharmacology (QSP) analysis of fluid amyloid biomarkers and cognitive results from the ALZ-801 phase 2 trial in APOE4 carriers with early Alzheimer's disease (AD), relative to the AD trajectory data from literature.

ALZ-801 265 mg BID treatment for 2 years arrested the progressive decline in cerebrospinal fluid levels of monomeric $A\beta_{42}$, plasma $A\beta_{42}/A\beta_{40}$ ratio, and the cognitive outcome Rey Auditory Verbal Learning Test (RAVLT) in early AD.

The QSP analysis showed that effects on cerebrospinal fluid and plasma amyloid fluid biomarkers were more pronounced in mild cognitive impairment compared with mild AD subjects. Both sexes were responsive to ALZ-801 treatment.

Our analysis supports central nervous system target engagement, potential disease modification, and long-term clinical efficacy of ALZ-801 for patients with early AD.

1 Introduction

The central, causative, and early role of soluble aggregates of beta-amyloid (Aβ) called oligomers in the pathogenesis of Alzheimer's disease (AD) is implicated by a large and diverse set of genetic, biomarker, and genome-wide association studies in both familial and sporadic AD [1, 2]. The inhibition of formation of Aβ oligomers, particularly the highly aggregation prone $A\beta_{42}$ species, maintains amyloid in the physiologic, soluble monomeric form that can be cleared by multiple homeostatic brain clearance mechanisms, thereby blocking the neurotoxic effects of amyloid oligomers that initiate and drive AD pathogenesis [3, 4]. ALZ-801/valiltramiprosate is an orally bioavailable, small-molecule inhibitor of Aβ oligomer formation in late-stage clinical development as a disease-modifying treatment for early symptomatic AD. ALZ-801, administered twice-daily [bis in die (BID)], is being evaluated in the APOLLOE4 phase 3 trial in carriers of two copies of the ε4 allele of apolipoprotein E4 (APOE4), and in a phase 2 biomarker study in APOE4 carriers. After completion of the core 104-week period of the open-label phase 2 trial, a long-term extension (LTE) was added for an additional 104 weeks of treatment.

ALZ-801, (*S*)-3-(2-amino-3-methylbutanamido) propane-1-sulfonic acid, is a valine-conjugated prodrug of tramiprosate. Following oral dosing, ALZ-801 is rapidly and fully converted to tramiprosate plus valine; metabolism of tramiprosate in humans in turn generates the sole metabolite, 3-sulfopropanoic acid (3-SPA) [5]. Both tramiprosate and 3-SPA are pharmacologically active anti-Aβ oligomer agents, which mediate ALZ-801's central pharmacologic mechanism of action (MOA), and both readily penetrate the brain [5–7]. Furthermore, ALZ-801 exhibits substantially improved oral tolerability and pharmacokinetic consistency and, therefore, represents a novel prodrug approach to achieving an optimal delivery of tramiprosate into the brain [5].

The anti-Aβ aggregation properties of tramiprosate have been extensively documented [8–17]. We have recently elucidated its novel molecular MOA as an inhibitor of Aβ oligomer formation via a multiligand, enveloping, conformational modification action [6]. Furthermore, we found that 3-SPA exhibits similar inhibitory activities on Aβ oligomers [7]. Both tramiprosate and 3-SPA interact with and stabilize soluble Aβ monomers in a semicyclic conformational state that prevents aggregation and the resultant neurotoxic amyloid cascade implicated in pathogenesis of AD by blocking monomeric assembly. This molecular action prevents the formation of toxic oligomers [6, 7, 16]. In TgCRND8 mice, a transgenic experimental model of AD, chronic oral tramiprosate treatment results in a significant reduction (~30%) in insoluble amyloid plaques, as well as both soluble and insoluble $A\beta_{40}$ and $A\beta_{42}$ in the brain [11]. This anti-Aβ oligomer molecular MOA is consistent with the observed efficacy of tramiprosate in APOE4 carrier subgroups in phase 3 clinical trials [1, 2, 18–22]. Taken together, these data support the development of the tramiprosate prodrug, ALZ-801, as a potential disease-modifying oral AD treatment in APOE4 carriers with early AD.

Based on the anti-Aβ oligomer MOA of ALZ-801, we expect that its mode of action may exhibit unique effects on dynamic cerebrospinal fluid (CSF) and plasma fluid biomarkers compared to aducanumab and lecanemab, the anti-Aβ antibody agents that were approved in the USA for the treatment of AD. These antiamyloid antibodies show lack of specificity by targeting both soluble Aβ oligomers as well as insoluble amyloid plaques [23]. The 2-year ALZ-801 phase 2 biomarker study provides an opportunity to elucidate the effects of ALZ-801 on biomarkers of target engagement and on clinical outcomes in patients with early AD.

Many investigations have explored the natural history of AD trajectory and linked changes in fluid biomarkers to disease progression [24–33]. These studies significantly advanced the understanding of AD pathogenesis and contributed to improving the design of clinical trials. Data from longitudinal biomarker studies and the placebo arms of

clinical trials provide external comparators that can be used for evaluation of drug effects on underlying AD pathology.

Quantitative systems pharmacology (QSP) is a valuable tool that can integrate the complex, dynamic information of molecular pathways and biological processes from multiple tissue compartments which, when coupled with other known clinical risk factors, may help improve the prediction of disease progression [34, 35]. QSP analysis has been increasingly deployed as a discerning tool to define AD trajectories, as well as to characterize effects of pharmacologic interventions on biomarkers as an indicator of target engagement and to predict clinical benefits [36–45]. Of these, the recent QSP study by Geerts et al. [45] provides an in-depth model for understanding of the pathophysiology of AD progression based on the biomarker changes in both central nervous system (CNS) and plasma. This QSP model offers an age-dependent framework for prediction of the levels of CSF and plasma AD biomarkers and for analysis of biomarker and clinical efficacy data from drug trials.

Besides amyloid biomarkers, the natural disease trajectory for some cognitive measures has been described in the AD population, including the Rey Auditory Verbal Learning Test (RAVLT), a cognitive outcome included in the ALZ-801 phase 2 trial [30, 31, 39, 46, 47]. This test measures hippocampal-dependent learning and memory (i.e., immediate and delayed memory), which are impacted early in the disease in APOE4 carriers [48–50]. The normative RAVLT data in the general population has also been defined [51].

In this study, we aim to provide an analysis of CSF and plasma amyloid biomarker data from the ALZ-801 phase 2 trial, leveraging the available QSP data in literature. We also compared the cognitive results from the ALZ-801 phase 2 trial and the population trajectory of RAVLT in the context of both AD and normal aging.

2 Methods

2.1 Protocol Approval and Informed Consent

The study was conducted at seven sites in the Czech Republic and the Netherlands, according to the principles of the Declaration of Helsinki. The study was approved by the institutional review boards of all study centers, upon approval of the clinical trial applications by the respective health and regulatory authorities. The study protocol and informed consents were also approved by the Independent Ethics Committee at each study center, and all patients and/or their legal representatives and study partners provided written informed consent. The trial was conducted in accordance with the International Council for Harmonization (ICH) Guidelines for Good Clinical Practice (GCP) and applicable local regulatory requirements.

2.2 ALZ-801 Phase 2 Trial Design and Endpoints

The single-arm, open-label phase 2 biomarker study (ALZ-801-201 ADBM) was designed to evaluate the longitudinal effects of 265 mg BID of ALZ-801 on CSF and plasma amyloid and tau biomarkers of AD pathology over 104 weeks in APOE4 carriers (APOE4/4 homozygotes and APOE3/4 heterozygotes) with early AD [52]. The study included subjects with mild cognitive impairment (MCI) with the Mini-Mental State Examination (MMSE) score $\geq$ 27 and mild AD with the MMSE score of 22-26; these two groups are collectively called early AD. A total of 84 subjects including 31 homozygotes and 53 heterozygotes were enrolled at seven study sites, three in the Netherlands and four in the Czech Republic. Excluding one subject who dropped out during the early stage of the trial, data from 83 subjects were used for this analysis. All eligible subjects had positive biomarkers of amyloid and tau pathology in CSF (i.e., high CSF p-tau$_{181}$ and low Aβ_{42}/Aβ_{40} levels). After a 2-week titration, during which a single 265 mg oral tablet of ALZ-801 was taken in the evening, subjects received 265 mg tablet twice-daily with the morning and evening meals. The demographic and baseline clinical characteristics of study subjects are presented in Table 1.

The primary efficacy outcome of the ALZ-801 phase 2 trial was the change from baseline in plasma p-tau$_{181}$ over 104 weeks, and the secondary outcomes were the changes of plasma Aβ_{42}/Aβ_{40} ratio over 104 weeks; the changes of free CSF Aβ_{42} and Aβ_{40} were exploratory. The cognitive endpoints included RAVLT, as well as other cognitive and disease-staging measures, including the functional Clinical Dementia Rating—Global scale (CDR-G). Magnetic resonance imaging (MRI) was conducted for brain volumetric measures, with the hippocampal volume being the primary imaging outcome. Specific MRI sequences were

Table 1 Demographics and baseline profiles of enrolled subjects (N = 84)

Characteristics	Mean (SD) or %
Age (years)	68.9 (5.4)
Female	52%
APOE genotype	4/4: 37% 3/4: 63%
MMSE	25.7 (2.6)
MCI	70%
Mild AD	30%
CDR-Global	0.58 (0.18)
Cognitive measures	
RAVLT—total	30.1 (13.0)

RAVLT—total, sum of RAVLT—immediate + RAVLT—delayed

also obtained to monitor the occurrence of amyloid-related imaging abnormalities (ARIA).

The plasma biomarkers and cognitive outcomes were obtained at baseline and at 13, 26, 52, 78, and 104 weeks. CSF biomarkers and MRIs were obtained at baseline, 52 and 104 weeks. Levels of CSF $A\beta_{42}$ were measured by the Lumipulse Fujirebio assay, and both plasma $A\beta_{42}$ and $A\beta_{40}$ were determined by the Euroimmun ELISA assay. All biomarker analyses were performed at the Neurochemistry Laboratory, University of Gothenburg (Molndal, Sweden), blinded to subject's demographic information.

2.3 QSP Trajectory Models for CSF and Plasma Amyloid Biomarker Changes in AD

The longitudinal trajectory data for CSF $A\beta_{42}$ and plasma $A\beta_{42}/A\beta_{40}$ ratio from Geerts et al. [45] were included for comparative QSP analyses. This comprehensive, mechanistic, and validated QSP model integrates multiple molecular processes of the amyloid cascade, including the synthesis of $A\beta_{42}$ and $A\beta_{40}$ from the amyloid precursor protein, the enzymatic degradation of Aβ monomers by insulin-degrading enzyme or neprilysin, the aggregation of monomers into oligomers, protofibrils and plaques (forward reactions, i.e., formation), the splitting off of monomers from higher order oligomers (backward reactions, i.e., breakdown), and the clearance of oligomers, protofibrils and plaques by microglia (illustrated in Fig. 1).

Importantly, the impact of APOE4 genotype on Aβ clearance [33, 53, 54] was considered in the QSP model. The model was calibrated with clinical data from multiple observational studies and major phase 2 and phase 3 trials of antiamyloid agents, including solanezumab, crenezumab, bapineuzumab, gantenerumab, aducanumab, and lecanemab [45]. Parameters were adjusted so that the levels of soluble and insoluble oligomers, protofibrils, and plaques agreed with the ranges measured from postmortem AD brains [55–57]. Also, levels of free CSF $A\beta_{42}$ and $A\beta_{40}$ were aligned with observed values in longitudinal clinical studies, and calculation of plasma $A\beta_{42}$ and $A\beta_{40}$ monomers included first-order liver synthesis rates that were adjusted to generate concentrations and treatment-induced changes as observed in clinical studies. When combined with a physiologically based pharmacokinetic (PBPK) model, the binding of monoclonal antibodies to soluble Aβ species, together with increased microglial clearance, was able to reproduce and project the observed changes in amyloid imaging and fluid biomarkers [45].

2.4 Comparison of Changes in CSF and Plasma Amyloid Biomarkers following ALZ-801 Treatment versus QSP Trajectory Model of AD

To analyze the effect of ALZ-801 treatment on key amyloid biomarkers that define the natural course of AD progression, we compared ALZ-801 phase 2 fluid biomarker results with the published trajectory curves of CSF $A\beta_{42}$

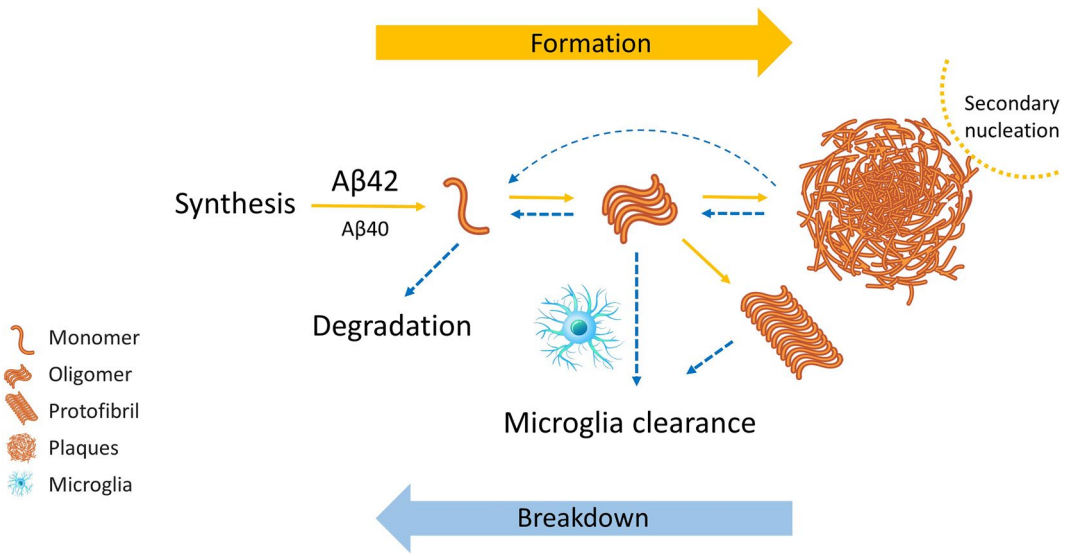

Fig. 1 Schematic illustration of quantitative systems pharmacology (QSP) model underlying natural history trajectories of amyloid biomarkers. The QSP model considers multiple molecular processes including synthesis of both $A\beta_{40}$ and $A\beta_{42}$, enzymatic degradation of Aβ monomers, formation of higher order oligomers by nucleation, monomers splitting off from the higher order oligomers, formation of plaques from oligomers, formation of protofibrils in an off-pathway structure, breakdown of protofibrils into two pieces, secondary nucleation on the plaque surface, and microglia-dependent clearance of oligomers, protofibrils, and, to a lesser extent, of plaques [45]. *Aβ* beta-amyloid

and plasma $A\beta_{42}/A\beta_{40}$ ratio as described by Geerts et al. [45]. We extracted the generic time-course data of the APOE4 carriers from Geerts et al. [45] and then modeled the mean ALZ-801 phase 2 biomarker data onto the natural history curves based on patient age. Analyses were also stratified by disease severity and sex. Since the biomarker assay methodologies and dynamic ranges differ across studies, we converted ALZ-801 phase 2 biomarker data to concentration unit scales based on the relative percentage change.

2.5 Comparison of Changes in Cognitive Outcomes following ALZ-801 Treatment versus QSP Trajectory Model of AD

The mean RAVLT data were modeled onto the natural history trajectory curve derived from the Alzheimer's Disease Neuroimaging Initiative (ADNI) noninterventional study [30], by matching the clinical test score values from AD subjects. Analyses were also stratified by disease severity and sex. The ADNI dataset includes a mixture of both APOE4 carrier and noncarrier AD subjects and, therefore, represents a conservative estimate of disease progression compared with the ALZ-801 phase 2 trial population, as the rate of disease progression is accelerated in a gene-dose manner by the increasing number of APOE4 alleles [33, 53, 54]. Additionally, the natural history data from the Mayo Normative Studies were included as a reference, which defines the age dependent RAVLT changes of the normal aging process [51].

3 Results

3.1 Summary of ALZ-801 Phase 2 Trial Results

As previously summarized [52], levels of plasma p-tau_{181} (the primary study endpoint) were significantly reduced at all time-points reaching 31–43% over 52–104 weeks ($p = 0.045$). The results for plasma $A\beta_{42}/A\beta_{40}$ ratio and for CSF $A\beta_{42}$ are presented in Table 2. At 104 weeks, the hippocampal volume imaging showed 3.7% atrophy in the group treated with ALZ-801 versus 5.0% atrophy in the matched ADNI external control (i.e., ~25% less than the matched APOE4-carrier AD subjects from the ADNI group; $p = 0.0014$). RAVLT—total (immediate and delayed memory) and digit symbol substitution test (DSST) scores improved at 26 weeks compared with baseline, and RAVLT remained stable at 104 weeks. The cognitive effects on RAVLT correlated significantly with the reduction of hippocampal atrophy ($r = 0.44$, $p = 0.0002$) [52]. The CSF $A\beta_{42}$, plasma $A\beta_{42}/A\beta_{40}$ ratio, and RAVLT data were used for the QSP analysis as below.

3.2 QSP Analysis of CSF $A\beta_{42}$ Levels

Figure 2 shows a comparison of the CSF $A\beta_{42}$ level changes over 2 years of treatment with ALZ-801 versus the natural disease history trajectory. The natural AD trajectory data of the APOE4 carriers was derived from Geerts et al. [45]. Specifically, during the ages of 65–80 years, the QSP analysis shows a rapid increase in the brain amyloid plaque load indicated by the standardized uptake value ratio (SUVR) of insoluble amyloid determined by the positron emission tomography (PET) imaging (right *y* axis) and a concomitant steep decrease of free CSF $A\beta_{42}$ concentrations (left *y* axis)

Table 2 Levels of CSF $A\beta_{42}$ and plasma $A\beta_{42}/A\beta_{40}$ in APOE4 carrier early AD subjects treated with ALZ-801 for 2 years

Visits	All subjects ($n = 83$)	MCI ($n = 36$)	Mild AD ($n = 47$)	Males ($n = 40$)	Females ($n = 43$)
CSF $A\beta_{42}$ (pg/mL)					
Baseline	466.0 (18.4)	444.3 (22.3)	483.0 (27.7)	475.0 (31.5)	457.5 (20.1)
Week 52	426.0 (20.3)	423.7 (24.5)	427.9 (30.9)	456.8 (33.2)	394.5 (22.3)
Week 104	456.4 (23.2)	465.0 (28.0)	449.5 (35.7)	478.7 (36.7)	430.5 (26.4)
Plasma $A\beta_{42}/A\beta_{40}$ ratio					
Baseline	0.197 (0.005)	0.203 (0.010)	0.192 (0.006)	0.195 (0.005)	0.198 (0.009)
Week 26	0.211 (0.011)	0.228 (0.022)	0.198 (0.008)	0.202 (0.009)	0.221 (0.020)
Week 52	0.215 (0.010)	0.230 (0.021)	0.204 (0.009)	0.210 (0.010)	0.220 (0.019)
Week 78	0.225 (0.021)	0.252 (0.0412)	0.202 (0.015)	0.197 (0.010)	0.255 (0.042)
Week 104	0.206 (0.011)	0.223 (0.021)	0.192 (0.010)	0.200 (0.011)	0.214 (0.021)

Data are expressed as means (standard error of the mean)

Aβ, beta-amyloid; AD, Alzheimer's disease; CSF, cerebrospinal fluid; MCI, mild cognitive impairment

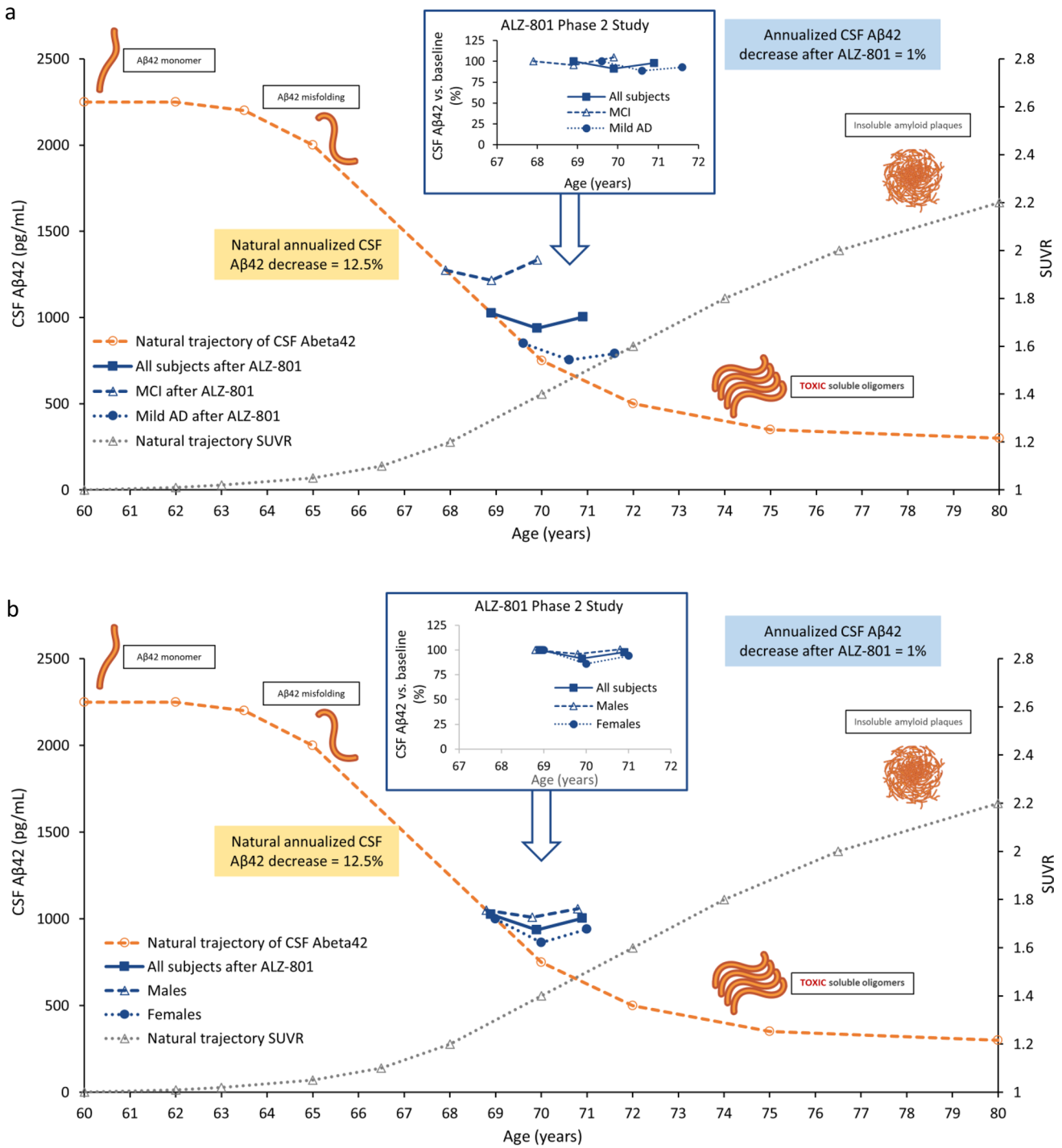

Fig. 2 Comparison of CSF $A\beta_{42}$ changes in early AD subjects treated with ALZ-801 for 2 years versus natural disease trajectory. The natural trajectories of CSF $A\beta_{42}$ (left *y* axis) and SUVR (right *y* axis) are shown from years 60–80, which represent the most dramatic period of worsening of disease pathology and fluid biomarker changes. The mean phase 2 CSF $A\beta_{42}$ changes are superimposed based on the age; the biomarker data are converted to the same unit based on the relative percentage change. **a** Analysis by disease severity (n = 36 for MCI and n = 47 for mild AD). **b** Analysis by sex (n = 40 for males and n = 43 for females). CSF $A\beta_{42}$ and SUVR trajectory data extracted from [45]. *Aβ* beta-amyloid, *AD* Alzheimer's disease, *CSF* cerebrospinal fluid, *SUVR* standardized uptake value ratio

in the natural progressive course of AD, reflecting an acceleration of the underlying pathogenesis.

The mean CSF $A\beta_{42}$ data from the ALZ-801 phase 2 trial was superimposed with the natural disease trajectory, shown as all subjects together, as well as separately by disease severity and sex. The age for the APOE4 carrier subjects was 68.9 ± 5.4 years [mean ± standard deviation (SD)], which aligns with the steepest and most linear phase of CSF $A\beta_{42}$ decline in the natural disease trajectory. Overall, ALZ-801 treatment in early AD subjects arrested the rapid decline of the CSF $A\beta_{42}$ free fraction. Although not directly modeled in our analysis, this stabilizing effect on CSF $A\beta_{42}$ is expected to reduce its aggregation into neurotoxic soluble amyloid oligomers and ultimately the formation of insoluble plaque deposits as indicated by the SUVR in Fig. 2. After 2 years of ALZ-801 treatment, the average natural rate of CSF $A\beta_{42}$ decline was slowed by 92% (annualized rate of CSF $A\beta_{42}$ decrease of 1% versus 12.5% with and without ALZ-801 therapy, respectively).

When analyzed by disease severity at the study entry, the MCI subjects in the phase 2 study had a younger age on average (67.9 ± 4.9, $n = 36$) than the mild AD subjects (69.6 ± 5.6, $n = 47$), and they showed an annualized rate of CSF $A\beta_{42}$ increase of 2.3% versus 3.5% decrease in the mild AD subjects (Fig. 2a). This is consistent with results from clinical trials of antiamyloid agents, which show that patients with AD with milder disease are more responsive to treatments than those with more advanced and severe disease.

Figure 2b shows a comparison of the CSF $A\beta_{42}$ level changes after ALZ-801 treatment based on sex. Subjects of both sexes were responsive to ALZ-801 treatment. The male subjects showed a reversal of CSF $A\beta_{42}$ decline (with an annualized increase of 0.4%), while the female subjects showed a reduced annualized CSF $A\beta_{42}$ decrease (− 3.0%) versus the natural trajectory (− 12.5%). A composite comparison of the 2-year changes of CSF $A\beta_{42}$ with versus without ALZ-801 265 mg BID therapy by disease severity and sex is shown in Fig. 3.

3.3 QSP Analysis of Plasma $A\beta_{42}/A\beta_{40}$ Ratio

We compared plasma $A\beta_{42}/A\beta_{40}$ ratio changes following ALZ-801 treatment over a period of 2 years to the natural history of AD in APOE4 carriers (Fig. 4). Similar to CSF $A\beta_{42}$ as above, there is a rapid and dramatic decrease of plasma $A\beta_{42}/A\beta_{40}$ ratio in the natural course of AD progression from ages 65–80 as shown in data from Geerts et al. [45], which also temporally coincides with amyloid plaque disposition in the brain (Fig. 2). The ALZ-801 phase 2 plasma $A\beta_{42}/A\beta_{40}$ ratio data were superimposed on the natural AD trajectory curve, shown as all subjects combined, as well as separately by disease severity and sex. The mean age of the subjects aligns with the steepest, linear phase of plasma $A\beta_{42}/A\beta_{40}$ decline of the natural disease trajectory. In summary, oral ALZ-801 treatment over 2 years arrested and reversed the plasma $A\beta_{42}/A\beta_{40}$ ratio decline in early AD subjects, with an annualized plasma $A\beta_{42}/A\beta_{40}$ increase of 2.4% versus a decrease of 5.2% without ALZ-801 therapy. When analyzed by disease severity at study entry (Fig. 4a), the MCI subjects in the phase 2 study showed an annualized plasma $A\beta_{42}/A\beta_{40}$ increase of 4.9% versus a smaller annualized increase of 0.1% in the mild AD subjects.

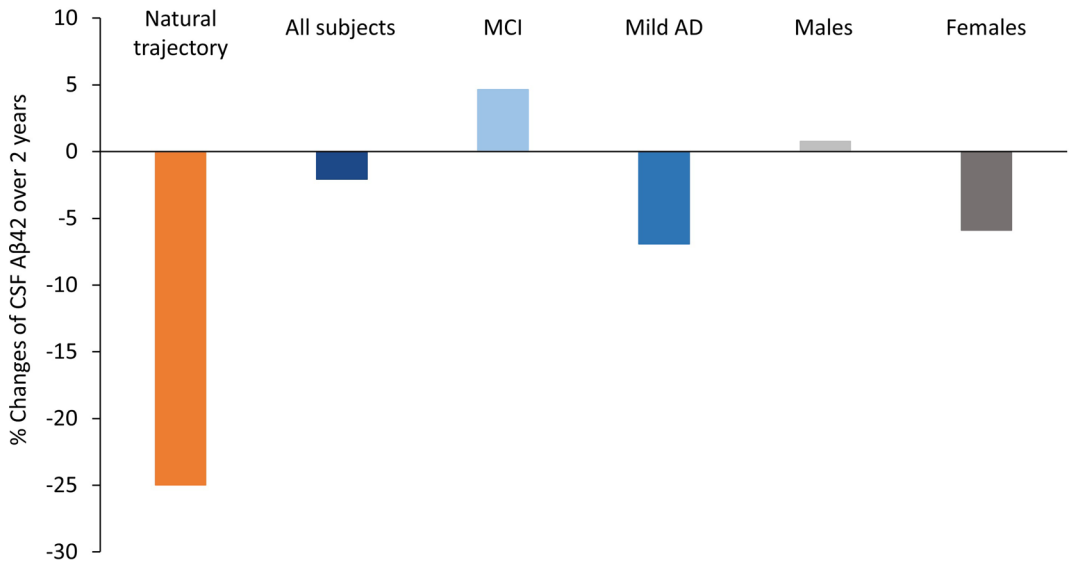

Fig. 3 Comparison CSF $A\beta_{42}$ changes in early AD subjects followed for 2 years with versus without ALZ-801 therapy by disease severity and sex. The mean percentage changes were calculated based on the levels at week 104 relative to the baseline from Fig. 2. *Aβ* beta-amyloid, *AD* Alzheimer's disease, *CSF* cerebrospinal fluid, *MCI* mild cognitive impairment

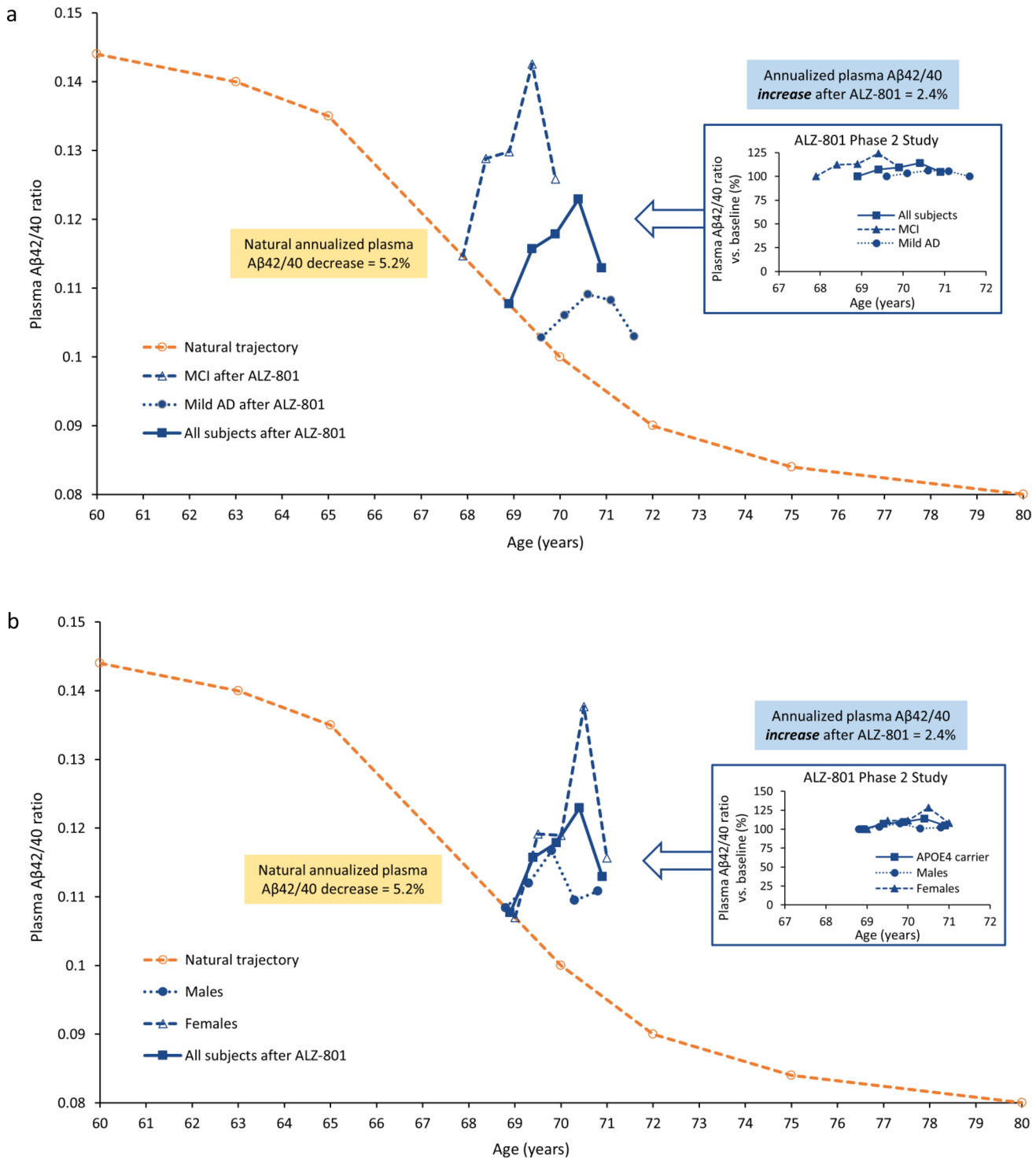

Fig. 4 Comparison of plasma $A\beta_{42}/A\beta_{40}$ ratio changes in early AD subjects treated with ALZ-801 for 2 years versus natural disease trajectory. The natural disease trajectory of plasma $A\beta_{42}/A\beta_{40}$ ratio is shown from years 60–80, which represents the most dramatic period of disease pathology and fluid biomarker changes. The mean phase 2 plasma $A\beta_{42}/A\beta_{40}$ ratio changes are superimposed based on the age; the biomarker data was converted to the same unit based on the relative percentage change. **A** Analysis by disease severity ($n = 36$ for MCI and $n = 47$ for mild AD). **B** Analysis by sex ($n = 40$ for males and $n = 43$ for females). Plasma $A\beta_{42}/A\beta_{40}$ trajectory data extracted from [45]. *Aβ* beta-amyloid, *AD* Alzheimer's disease, *MCI* mild cognitive impairment

Figure 4b shows a comparison of the plasma $A\beta_{42}/A\beta_{40}$ ratio changes after ALZ-801 treatment by sex. Subjects of both sexes showed a positive response to ALZ-801 treatment. The female subjects showed an annualized plasma $A\beta_{42}/A\beta_{40}$ increase of 4.0% versus an annualized increase of 1.1% in the male subjects. A composite comparison of the 2-year changes of plasma $A\beta_{42}/A\beta_{40}$ ratio with versus without ALZ-801 therapy by disease severity and sex is shown in Fig. 5.

3.4 Analysis of RAVLT Results

RAVLT, a cognitive endpoint of ALZ-801 phase 2 trial, was included in this analysis together with the amyloid biomarkers. In Fig. 6, we aligned the natural history trajectories of RAVLT—total score, which is a summation of immediate and delayed recall scores, from both a normal population of the Mayo Normative Studies [51] and a representative AD population of the ADNI study [30]. The normal aging trajectory data were extracted and calculated from the average data in combined sexes with an average of 12 years of education from the Mayo Normative Studies [51]. The RAVLT-total results from the ALZ-801 Phase 2 study were modeled onto the ADNI trajectory curve based on the raw score value. Since the ADNI cohort included a mixture of APOE4 carriers and noncarriers, it may serve as a conservative reference for this analysis. Overall, RAVLT score declines gradually over the lifespan after adulthood is reached, following a polynomial equation ($y = -0.00562x^2 + 0.2236x + 59.555$, where y is RAVLT—total score, and x is age). The RAVLT trajectory of AD progression showed a sigmoid curve [30], whereas other studies reported quadratic shaped trajectory curves (i.e., without the lower plateau phase) [39, 46, 47]. Interestingly, RAVLT appears to be sensitive to detect a change in the rate of decline in AD subjects beginning at 55 years of age. There is a rapid, linear decline from 60 to 75 years of age, covering the age and RAVLT score range of the ALZ-801 phase 2 study subjects, which parallels the changes in CSF and plasma Aβ biomarkers as described above.

In summary, the 2-year treatment with ALZ-801 arrested the steep decline of RAVLT—total score (Fig. 6a) in early AD subjects, with an annualized increase of 1.4% versus a decrease of 2.7% (Δ +4.1% improvement favoring ALZ-801) with and without ALZ-801 therapy, respectively. A comparison of the 2-year changes in RAVLT—total score by disease severity and sex is shown in Fig. 6b. There was a cognitive benefit regardless of the status of disease severity or sex, although there appeared to be larger treatment efficacy in MCI than in mild AD subjects.

4 Discussion

The well-defined QSP model data derived from the integrative molecular processes of the amyloid cascade and multiple late-stage antiamyloid antibody clinical trial datasets by Geerts et al. [45] were used to conduct a comparative analysis of the amyloid biomarker and cognitive results from the 2-year phase 2 trial evaluating oral ALZ-801/valiltramiprosate treatment in early AD subjects [52]. In their seminal

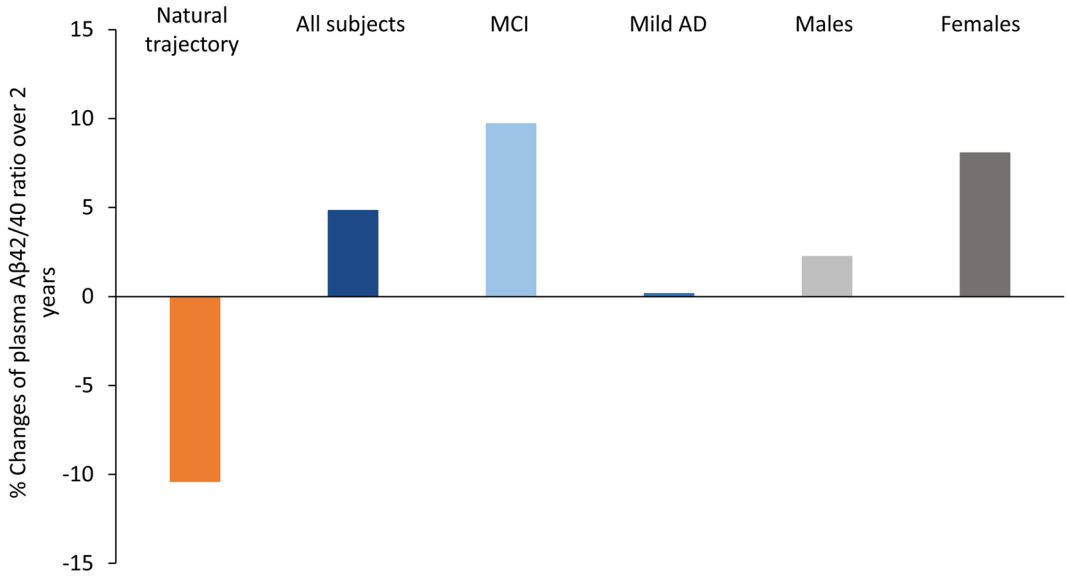

Fig. 5 Comparison of plasma $A\beta_{42}/A\beta_{40}$ changes in early AD subjects followed for 2 years with versus without ALZ-801 therapy by disease severity and sex. The mean percentage changes were calculated based on the levels at week 104 relative to the baseline from Fig. 4. *Aβ* beta-amyloid, *AD* Alzheimer's disease, *MCI* mild cognitive impairment

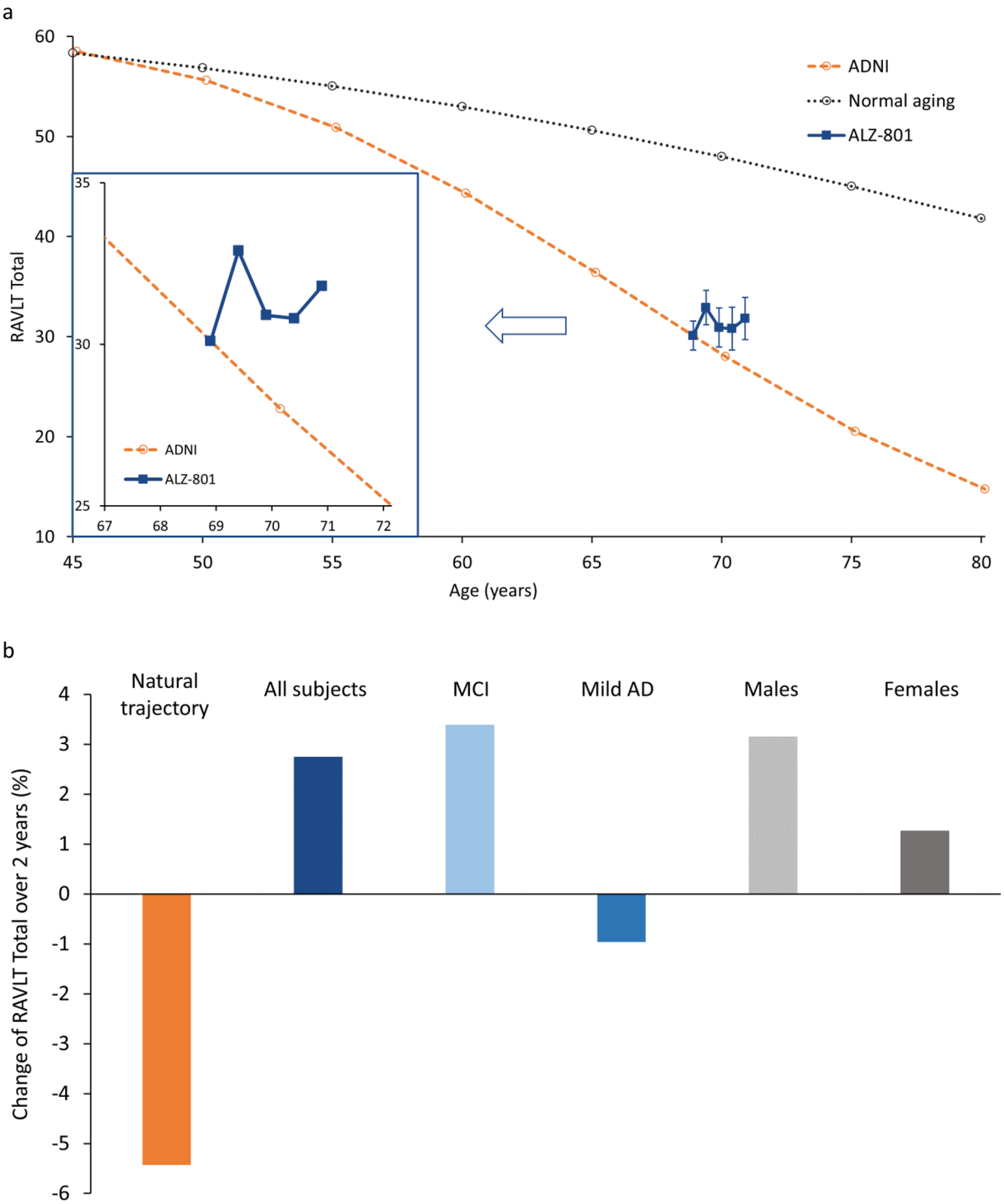

Fig. 6 Comparison of RAVLT changes in early AD subjects treated with ALZ-801 for 2 years versus natural disease trajectory. **a** RAVLT—total score changes after ALZ-801 therapy versus the longitudinal natural history trajectory of AD of the ADNI study and normal aging. The ALZ-801 Phase 2 RAVLT—total data are superimposed based on the actual score values. Error bars indicate standard error of the mean. **b** Mean RAVLT—total score changes with versus without ALZ-801 therapy by disease severity and sex. AD and normal aging RAVLT trajectory data derived and extracted from ADNI and Mayo Normative Studies, respectively [30, 51]. *Aβ* beta-amyloid, *AD* Alzheimer's disease, *ADNI* Alzheimer's Disease Neuroimaging Initiative, *MCI* mild cognitive impairment, *RAVLT* Rey Auditory Verbal Learning Test

work, Geerts et al. [45] described a dynamic natural course of longitudinal amyloid biomarker changes over a lifetime in both APOE4 carrier and noncarrier patients with AD, which is instrumental not only in understanding disease pathogenesis but also in aiding the evaluation of drug efficacy based on fluid biomarkers in interventional trials. Their analysis confirmed that the disease progression in APOE4 carriers has an earlier onset than in noncarriers by approximately 3–4 years.

We posit that QSP analysis could be a predictive endpoint in early stages of disease including MCI or early AD, particularly in subjects with MMSE ≥ 24, who generally exhibit minimal decline in standard functional outcomes (e.g., CDR-SB) prior to more rapid clinical progression in later AD stages, which are characterized by accelerated rates of decline. In addition, QSP analysis can be an important endpoint in drug trials in presymptomatic patients with AD assessing slowing of conversion to symptomatic AD by providing a quantifiable objective endpoint such as plasma $A\beta_{42}/A\beta_{40}$ ratio to support drug efficacy of antiamyloid therapies. Such biomarker surrogate outcomes are useful to demonstrate drug effects in prevention trials of preclinical AD, because standard functional outcome endpoints including CDR-SB are less sensitive at early AD stages. In these cases, inclusion of QSP with a biomarker as a key endpoint could improve study power and allow for smaller and/or shorter drug trials in the higher-risk APOE4 carrier populations. Inclusion of QSP analysis would represent a novel approach to developing AD drugs that can prevent symptomatic AD.

The outputs of specific interest from this QSP model include free CSF $A\beta_{42}$ monomer level, plasma $A\beta_{42}/A\beta_{40}$ ratio, and brain amyloid SUVR, which quantifies insoluble brain amyloid deposits such as fibrils and plaques. It is noted that free monomeric CSF $A\beta_{42}$ in patients with AD rises slowly and gradually over time until approximately 65 years of age followed by a sharp and steep decline that, in turn, temporally leads into the last symptomatic phase of the disease. This rapid decrease of CSF $A\beta_{42}$ concentrations coincides with an equally steep, mirror-image increase of deposited brain amyloid plaque load within a short duration, reflecting an acceleration of the underlying pathogenesis.

Importantly, it is well known that evolution from free CSF $A\beta_{42}$ monomers to small soluble amyloid aggregates known as Aβ oligomers implicated in AD pathogenesis precedes the formation of insoluble, deposited plaques [1, 2]. Highly neurotoxic, soluble Aβ oligomers have been shown to initiate and drive AD progression in a large number of mechanistic and clinical trials with antiamyloid agents [3–7, 58, 59]. Similarly, plasma free $A\beta_{42}/A\beta_{40}$ ratio mirrors the changes in CSF and follows the same temporally linked steep decline. This modeled natural disease history is consistent with that reported by Pfizer and InSysBio [36] and agrees with overall clinical AD trajectories. The description of the APOE genotype effect offers additional insights to its contribution to the rate of disease progression, considering that after aging APOE4 is the strongest risk factor for late-onset AD [33, 53, 54]. The recent study by Fortea et al. [33] shows a clear APOE4 gene-dose effect on CSF $A\beta_{42}$ reductions (i.e., leftward shift in patients with APOE4/4 > APOE3/4 > APOE3/3), suggesting that AD of APOE4/4 homozygous patients represents a distinct semidominant genetic form of the disease, with near-full penetrance of AD pathology at 65 years of age.

Clinically, CSF $A\beta_{42}$ is a defined biomarker for amyloid deposition in AD, which has been previously shown to be inversely correlated with the deposition of amyloid plaques in the brains of patients with AD [27, 60]. Unlike CSF $A\beta_{40}$, which stays stable, CSF levels of $A\beta_{42}$ are significantly reduced during AD pathogenesis [61, 62]. This phenomenon is thought to result from the deposition of soluble $A\beta_{42}$ into insoluble amyloid plaques, thereby preventing transit of soluble $A\beta_{42}$ from the brain into CSF (i.e., plaques acting as a sink), due to its highly amyloidogenic proclivity of $A\beta_{42}$ species. Similar to CSF $A\beta_{42}$, plasma $A\beta_{42}/A\beta_{40}$ ratio is also a reliable marker for brain amyloid load, being inversely related to amyloid plaques in AD [27, 28].

Our analysis shows that the natural rate of decline in both CSF $A\beta_{42}$ and plasma $A\beta_{42}/A\beta_{40}$ ratio in AD was either arrested or reversed following treatment with oral ALZ-801 265 mg BID in APOE4 carrier subjects. These findings support the MOA, target engagement and clinical efficacy of ALZ-801 treatment, resulting in mitigation of the otherwise rapid disease progression during the early symptomatic phase of AD. These effects are consistent with the MOA of ALZ-801 as a potent inhibitor of Aβ oligomer formation in brain [6, 7, 16].

We found that subjects with both MCI and Mild AD showed a substantial biomarker effect on both CSF $A\beta_{42}$ and plasma $A\beta_{42}/A\beta_{40}$ ratio versus the natural disease trajectory. In addition, the MCI subgroup displayed a trend of improved efficacy in biomarkers versus mild AD, suggesting a greater clinical benefit based on disease severity. This is consistent with the well-established finding that patients with AD at the earliest stages of symptomatic disease severity (i.e., MCI) exhibit the greatest disease-modifying benefit, likely related to the fact that these subjects are also at the earliest stages of a rapidly advancing progression of pathology. Consequently, patients with more advanced brain pathology display a smaller benefit, reflective of the more severe, irreversible injury and neuronal loss characteristic of the later stages of the disease. Importantly, early AD subjects of both sexes were responsive to ALZ-801 treatment. In contrast, lecanemab results from the CLARITY phase 3 study appeared to show a greater clinical benefit in males versus females [63].

It is noteworthy that the changes in CSF $A\beta_{42}$ levels follow a biphasic pattern after ALZ-801 treatment, i.e., an initial reduction followed by a later increase (Fig. 2). Based on its MOA, we posit that ALZ-801 treatment impacts three dynamic processes: (1) prevention of oligomer formation favoring retention in the monomeric fraction, which subsequently leads to (2) enhanced Aβ clearance from the brain by either enzymatic degradation or efflux to glymphatics and plasma (the initial fast action of ALZ-801 treatment), followed by (3) long-term reduction in amyloid deposition, which may take years to translate into a lower insoluble amyloid plaque load. Therefore, the initial decrease of CSF $A\beta_{42}$ reflects the target engagement [3–7].

Free CSF $A\beta_{42}$ is a reliable indicator of brain plaque load [27, 60], and its gradual increase reflects decreased brain amyloid deposition and a recovery to homeostasis in the longer term. Therefore, the biphasic CSF $A\beta_{42}$ pattern reflects the composite changes of these dynamic processes. This working model may also explain the rapid increase of plasma $A\beta_{42}/A\beta_{40}$ following ALZ-801 treatment, which suggests Aβ efflux from the CNS. This hypothesis is consistent with the previously observed dose-dependent CSF $A\beta_{42}$ reduction after 3 months of treatment with tramiprosate, the active molecule in ALZ-801, in a phase 2 study [9], the dose-dependent increase of CSF $A\beta_{42}$ at 78 weeks in the aducanumab phase 3 trials [23], as well as the dose- and time-dependent CSF $A\beta_{42}$ and plasma $A\beta_{42}/A\beta_{40}$ ratio increases at 1–1.5 years in the lecanemab phase 2 trial [45]. Our observations suggest that ALZ-801 as a small molecule agent may have unique, differential effects on amyloid fluid biomarker dynamics as compared to the anti-amyloid antibody treatments.

A potential limitation of the QSP working hypothesis is that it does not fully explain the apparent qualitative difference in the response of CSF $A\beta_{42}$ versus the single time point drop in plasma $A\beta_{42}/A\beta_{40}$ ratio at 104 weeks (Fig. 4). As this occurred at the last time point of the 2-year study, it is unclear whether this reflects a transient change or the beginning of a more meaningful trend. Plasma Aβ, of which the CNS contribution is known to be a small fraction, is known to have highly complex dynamics that can be influenced by multiple factors such as the synthesis from the liver, the efflux from the brain, the aggregation cascade, and multiple clearance and elimination processes. The ongoing ALZ-801 Phase 2 trial extension for an additional 2 years may offer further insights. Nevertheless, since we calculated annualized rates of change based on the biomarker results at 104 weeks versus baseline, if the observed reduction of plasma $A\beta_{42}/A\beta_{40}$ ratio at this sampling time point reflects only a transient change, which suggest an underestimation of the extent of biomarker effects of ALZ-801 treatment.

RAVLT is a sensitive and validated cognitive instrument that measures episodic, immediate, and delayed hippocampus-dependent memory that has been employed for evaluation and discrimination of normal aging subjects from MCI and AD subjects, and for early AD diagnosis [49, 50]. The decline of RAVLT score correlates with brain atrophy measured by MRI volume changes, particularly in the medial temporal lobe, hippocampus, and amygdala, structures that play an important role in AD pathogenesis [49, 50]. Several groups have modeled the natural history trajectory of RAVLT in patients with AD using the public database of the ADNI study [30, 31, 39, 46, 47]. Of these, the RAVLT trajectory reported by Hadjichrysanthou et al. [30] showed a sigmoid curve, whereas in some other studies quadratic shaped trajectory curves (i.e., without the lower plateau phase and potentially faster decline) have also been reported [39, 46, 47]. We used the sigmoid trajectory data [30] as a conservative reference in this analysis.

We found that RAVLT test is a sensitive instrument that can detect a deviation in AD from the normal aging trajectory as early as ~55 years of age. In AD, there is a rapid, linear decline from approximately 60 to 75 years, encompassing the age and RAVLT score range of the ALZ-801 Phase 2 trial subjects; this temporally parallels the changes in CSF and plasma Aβ biomarkers. In agreement with both the CSF and plasma Aβ biomarker findings, we showed that the AD-related cognitive decline of the RAVLT trajectory was also arrested, with a reversal trend after 2 years of ALZ-801 treatment. The treatment effect was observed in all subject groups regardless of disease severity or sex, although MCI subjects showed a larger response. This clinical benefit and apparent disease modification finding is of particular significance, considering that RAVLT is a sensitive measure of hippocampal-dependent episodic memory that is preferentially affected in APOE4 carriers at the early AD stages [48–50]. The RAVLT benefits were also significantly correlated with the hippocampal volume effects of ALZ-801 [52]. Taken together, these cognitive results align with and corroborate the Aβ biomarker data. Of note, the ADNI comparator arm included a mixture of APOE4 carriers and noncarriers while the ALZ-801 phase 2 study is all in APOE4 carriers. Therefore, it is likely that the current analysis of ALZ-801 biomarker effects is conservative, considering that disease progression and amyloid accumulation in APOE4 carriers are more aggressive and accelerated [33, 45, 53, 54].

There are a few limitations in this study. First, our analysis is based on the data derived from the previously reported QSP and population biomarker models, which may not be exhaustive, although the model considered several disease molecular mechanisms and clinical parameters. For example, there could be additional disease biology and processes (e.g., impact on Aβ efflux from brain to plasma) that are not directly accounted for. Furthermore, co-medications in the ADNI cohort could influence the

relationship between biomarkers and cognitive outcome, which is difficult to model. Secondly, the present comparison of our phase 2 biomarker results shows that ALZ-801 arrests or reverses the natural disease trajectory in an open-label trial. While this should not be weighed similarly to an interventional trial where two exactly matched groups are studied, our analysis supports the clinical benefit versus the well-calibrated QSP model as well as the well-established, large ADNI clinical database. Taken together, the present analysis strongly supports a beneficial treatment effect of ALZ-801 to arrest or slow AD progression.

5 Conclusions

The QSP analysis demonstrates a positive therapeutic effect of oral ALZ-801/valiltramiprosate on the natural trajectory of biomarkers and on memory test in a genetically defined and biomarker-enriched early AD population. These analyses provide a mechanistic fluid biomarker basis for the MOA and degree of disease modification (i.e., clinical benefit) of ALZ-801 linked to the stage of the disease. We posit that a similar biomarker-based analysis can be used as a sensitive and predictive clinical marker of conversion from preclinical or presymptomatic to symptomatic AD based on changes of free CSF $A\beta_{42}$ and/or plasma $A\beta_{42}/A\beta_{40}$ ratio. The use of such a surrogate biomarker would allow more efficient prevention trials in the high-risk APOE4 carriers.

By leveraging publicly sourced QSP data, we evaluated the CSF $A\beta_{42}$ levels and plasma $A\beta_{42}/A\beta_{40}$ ratio results from the ALZ-801 phase 2 biomarker study and compared them with the dynamic natural AD trajectory in APOE4 carrier subjects. The effects of ALZ-801 treatment on CSF and plasma $A\beta_{42}$ levels support its CNS target engagement and promising clinical efficacy, and provide rationale for evaluating ALZ-801 in APOE4 carriers in a phase 3 pivotal trial in early AD.

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Declarations

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Conflict of interest J.A.H., S.A., A.P., J.F.S., P.K., and M.T. are employees of Alzheon, Inc. J.Y.Y. serves as a consultant to Alzheon, Inc. All authors own Alzheon stocks and/or stock options.

Ethics approval The protocol was approved by the assigned ethics committees in the Netherlands and in Czech Republic and by the institutional ethics committees in the Czech Republic.

Consent to participate All subjects and their partners, caregivers or legal representatives signed informed consent before participation in the study.

Consent for publication Not applicable.

Availability of data and material ADNI datasets are publicly available at the website: http/ADNI.LONI.USC.edu. The ALZ-801 data is proprietary to Alzheon Inc.

Code availability Not applicable.

Author contributions J.A.H. wrote the article in collaboration with J.Y.Y. and J.F.S.; S.A., J.A.H., A.P., P.K., and M.T. designed and conducted the ALZ-801-201 ADBM study and the statistical analysis plan. All authors have read and approved the final submitted manuscript and agree to be accountable for the work.

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