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Summary of Alzheon Peer-Reviewed Scientific Publications

1. *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 AD Using Quantitative Systems Pharmacology Model*

Published June 2024 in *Drugs*

John A. Hey, PhD – Chief Scientific Officer of Alzheon, Jeremy Y. Yu, **Susan Abushakra, MD** – Chief Medical Officer of Alzheon, **Jean F. Schaefer**, **Aidan Power, MB, MRCPsych** – Chief Development Officer of Alzheon, **Patrick Kesslak**, **Martin Tolar MD, PhD** – Founder, President & CEO of Alzheon**.**

Actionable Insights: This analysis leveraged a comprehensive, validated well established Quantitative Systems Pharmacology (QSP) model of the amyloid cascade to evaluate the effects of treatment with ALZ-801/valiltramiprosate on amyloid fluid biomarkers, an oral inhibitor of beta-amyloid (Aβ) aggregation, in an open-label Phase 2 study of APOE4 carriers with early Alzheimer's disease. Utilizing longitudinal Alzheimer's fluid biomarker and RAVLT-total score data, the analysis showed ALZ-801 significantly arrested or reversed the decline in cerebrospinal fluid (CSF) $A\beta_{42}$ and plasma $A\beta_{42}/A\beta_{40}$ levels, respectively, an action consistent with an effective inhibition of amyloid aggregation in AD subjects, as well as reversing the decline in RAVLT-total scores over 104 weeks. The QSP model provides a robust framework for understanding and assessing the ALZ-801's pharmacodynamic effects using fluid biomarkers, and its potential disease-modifying capabilities in an investigational clinical trial setting.

Key Points: (A) The positive Phase 2 study achieved a significant reduction from baseline in plasma p-tau_{181} at all timepoints, reaching 31-43% over 52-104 weeks following treatment with ALZ-801/valiltramiprosate. In addition, a significant reduction in hippocampal volume atrophy was also observed, with a clear correlation between decreased atrophy and stable memory scores at the end of the study. (B) Utilizing published longitudinal trajectory CSF and plasma data, the QSP model indicated that ALZ-801 significantly increased the free non-aggregated fraction of amyloid in both CSF and plasma vs. the natural trajectory of AD progression: 92% slowing of the decrease in $A\beta_{42}$ in CSF and a 2.4% increase in plasma $A\beta_{42}/A\beta_{40}$ (vs. a 5.2% decrease in natural progression), supporting its mechanism of action in inhibiting amyloid aggregation. (C) 104-week treatment with ALZ-801 resulted in a 4.1% improvement in the RAVLT-total score vs. natural history trajectories (ADNI-1 and Mayo Normative Studies). (D) Conclusions: The significant reduction in plasma p-tau_{181} and increase

in non-aggregated, monomeric amyloid levels, coupled with the observed cognitive stabilization and decreased hippocampal atrophy, highlight ALZ-801's potential as a disease-modifying treatment. The QSP analysis added critical insights into the mechanism of action and its effects to arrest the progression of amyloid pathology and delineates how the upstream change in fluid amyloid biomarkers predict downstream benefit on cognitive outcomes. These results support further evaluation of treatment with ALZ-801/valiltramiprosate in a broader population of APOE4 carriers and in prevention trials in amyloid positive AD subjects.

2. *Effects of Oral ALZ-801 on Plasma Biomarkers, Brain Hippocampal Volume, and Cognition: Results of 2 Year Single Arm, Open Label, Phase 2 Trial in APOE4 Carriers with Early AD*

Published in June 2024 in *Drugs*

John A. Hey, PhD – Chief Scientific Officer of Alzheon, **Susan Abushakra, MD** – Chief Medical Officer of Alzheon**,** Kaj Blennow, Eric M. Reiman, Jakub Hort, Niels D. Prins, Katerina Sheardova, **Patrick Kesslak**, Larry Shen, Xinyi Zhu, **Adem Albayrak**, Jijo Paul, **Jean F. Schaefer, Aidan Power, MB, MRCPsych** – Chief Development Officer of Alzheon, **Martin Tolar MD, PhD** – Founder, President & CEO of Alzheon.

Actionable Insights: In APOE4 carriers with early AD, open-label treatment with ALZ-801/valiltramiprosate at a dose 265 mg twice daily by mouth significantly reduced plasma p-tau$_{181}$ levels, starting at 13 weeks and sustained at every visit through 104 weeks. Compared with an external control (ADNI-1 matched cohort), ALZ-801 treatment resulted in stabilization of memory test scores over 104 weeks which significantly correlated with the observed reduction in hippocampal atrophy. ALZ-801 also displayed a favorable safety profile, with no instances of vasogenic brain edema.

Key Points: (A) This Phase 2 study included 84 participants, 52% female, mean age of 69.0 years, 37% homozygotes, and mean MMSE score of 25.7 with 43% considered to have mild cognitive impairment (MCI). (B) The primary outcome was achieved with a significant 31% reduction from baseline in plasma p-tau$_{181}$ at 104 weeks. (C) The key secondary outcome of hippocampal volume atrophy, compared to an external control, also demonstrated a statistically significant reduction at 104 weeks. (D) Memory scores remained stable over 104 weeks and correlated significantly with the observed reduction in hippocampal volume atrophy. (E) Safety assessments revealed common adverse events such as COVID-19 infection and mild nausea, with no drug-related serious adverse events despite enrolling APOE4 carriers, who are at higher risk for ARIA-E events due to higher CAA burden. (F) Conclusions: Treatment with ALZ-801 resulted in a significant reduction in plasma p-tau$_{181}$ and hippocampal

volume atrophy, clear demonstrations of target engagement. Together with the observed stabilization of cognitive function and safety profile, these data strongly support the potential of ALZ-801/valiltramiprosate as a disease-modifying treatment for early AD in the high-risk APOE4 carrier population.

3. *The Single Toxin Origin of Alzheimer's Disease and Other Neurodegenerative Disorders Enables Targeted Approach to Treatment and Prevention*

Published in February 2024 in *International Journal of Molecular Sciences*

Martin Tolar MD, PhD – Founder, President & CEO of Alzheon, **John A. Hey, PhD** – Chief Scientific Officer of Alzheon, **Aidan Power, MB, MRCPsych** – Chief Development Officer of Alzheon, **Susan Abushakra, MD** – Chief Medical Officer of Alzheon.

Actionable Insights: Novel biomarkers and disease-modifying treatments have advanced our understanding of Alzheimer's pathogenesis and provide support for our thesis that the aggregation of misfold ed native proteins initiates and drives the pathogenic cascade that leads to Alzheimer's disease and other age-related neurodegenerative disorders. The destruction of brain structures by oligomer assemblies results in characteristic clinical syndromes described in Alzheimer's disease and Parkinson's disease, amyotrophic lateral sclerosis, progressive supranuclear palsy, and many other neurological diseases. These well-conserved pathways of brain neurodegeneration offer multiple opportunities for potential disease-modifying interventions to slow the progression or even prevent the onset of clinical symptoms if administered early in the pathogenic process. ALZ-801 may provide advantages as an oral agent that efficiently crosses the blood brain barrier, selectively interacts with amyloid monomers to inhibit their misfolding, and blocks the formation of neurotoxic soluble amyloid oligomers in a concentration-dependent manner, without affecting insoluble amyloid plaques or fibrils.

Key Points: (A) Soluble beta amyloid aggregates called oligomers or protofibrils initiate and drive pathogenesis of Alzheimer's disease. (B) All other biochemical effects and neurodegenerative changes observed in Alzheimer's brain are a direct response to or downstream effect of initial toxic insult by amyloid oligomers. (C) Other neurodegenerative disorders follow a similar path in which normal brain proteins become trapped in aging brain due to impaired clearance and then misfold and aggregate into neurotoxic species that exhibit prion-like behavior. (D) Inhibition of amyloid misfolding and aggregation by ALZ-801/valiltramiprosate could slow disease progression in Alzheimer's patients.

4. *Neurotoxic Soluble Amyloid Oligomers Drive Alzheimer's Pathogenesis and Represent a Clinically Validated Target for Slowing Disease Progression*

Published in June 2021 in *International Journal of Molecular Sciences*

Martin Tolar MD, PhD – Founder, President & CEO of Alzheon, **John A. Hey, PhD** – Chief Scientific Officer of Alzheon, **Aidan Power, MB, MRCPsych** – Chief Development Officer of Alzheon, **Susan Abushakra, MD** – Chief Medical Officer of Alzheon.

Actionable Insights: Therapeutic agents that can selectively target amyloid oligomers can provide clinical efficacy without risk of vasogenic edema and microbleeds. Developing an assay that could detect and measure amyloid oligomers in the brain would greatly advance drug development efforts of such agents and serve as a tool for clinicians to help diagnose and better treat patients.

Key Points: (A) Analyses of clinical efficacy versus the binding profiles of drugs in recent late-stage AD trials suggests that efficacy outcomes correspond to level of amyloid oligomer binding, while risk of vasogenic edema and microbleeds corresponds with amyloid plaque binding. (B)The finding that soluble neurotoxic amyloid oligomers are important therapeutic targets is consistent with genetic findings in several familial AD mutations: The Osaka and Arctic mutations overproduce soluble amyloid oligomers (protofibrils) and develop early AD symptoms with minimal amyloid plaque. (C) Therapeutic agents that can selectively target amyloid oligomers can provide clinical efficacy without risk of vasogenic edema and microbleeds. Several such agents are in development and the most advanced is ALZ-801, which inhibits oligomer formation and is currently in a Phase 3 trial in AD patients with the APOE4/4 genotype.

5. *APOE4/4 Homozygotes with Early Alzheimer's Disease Show Accelerated Hippocampal Atrophy and Cortical Thinning that Correlates with Cognitive Decline*

Published in December 2020 in *Alzheimer's & Dementia: Translational Research & Clinical Interventions*

Susan Abushakra, MD – Chief Medical Officer of Alzheon, **John A. Hey, PhD** – Chief Scientific Officer of Alzheon, A. Porsteinsson, M. Sabbagh, L. Bracoud, J. Schaerer, **Aidan Power**, D. Scott, J. Suhy, **Martin Tolar MD, PhD** – Founder, President & CEO of Alzheon for the Alzheimer's Disease Neuroimaging Initiative.

Actionable Insights: Rates of hippocampal atrophy and cortical thinning in Alzheimer's Disease Neuroimaging Initiative (ADNI-1) cohort and tramiprosate Phase 3 trial were analyzed. APOE4/4 subjects with Mild Cognitive Impairment (MCI) or Mild Alzheimer's disease (AD) exhibited significantly smaller baseline hippocampal volumes and accelerated atrophy and cortical thinning rates over 12 months, compared to APOE3/3 subjects. Atrophy rates were significantly correlated with cognitive decline in MCI, but not in Mild AD. Hippocampal atrophy and cortical thinning seem to contribute to cognitive impairment early in the disease process and may be a harbinger of future cognitive decline.

Future Directions: Correlation of drug effects on hippocampal atrophy or cortical thinning to cognitive benefits in Early AD would support their use as potential surrogate outcomes in prevention trials of presymptomatic subjects, allowing trials of shorter duration. These imaging-cognitive correlations in MCI support the utility of these imaging biomarkers in Early AD trials. Rates of hippocampal atrophy or cortical thinning can be used as main outcomes in Phase 2 drug trials of ≥12 months, to predict clinical effects in Early AD.

Key Points: (A) APOE4/4 subjects with MCI were approximately 5 years younger and had significantly smaller hippocampal volumes and worse cognitive scores than APOE3/3 subjects. (B) APOE4/4 subjects with MCI and Mild AD showed significantly faster hippocampus atrophy and cortical thinning over 2 years compared to APOE3/3 subjects. (C) Atrophy rates of hippocampal volume and cortical thinning were significantly correlated with cognitive decline in MCI subjects, but not in Mild AD.

6. *Aducanumab, Gantenerumab, BAN2401, and ALZ-801 — the First Wave of Amyloid-Targeting Drugs for Alzheimer's Disease with Potential for Near Term Approval*

Published in August 2020 in *Alzheimer's Research & Therapy*

Authors: **Martin Tolar MD, PhD** – Founder, President & CEO of Alzheon, **Susan Abushakra, MD** – Chief Medical Officer of Alzheon, **John A. Hey, PhD** – Chief Scientific Officer of Alzheon, A. Porsteinsson, M. Sabbagh

Actionable Insights: Four anti-amyloid agents with potential for near term approval share a common characteristic of targeting soluble Aβ oligomers, albeit to varying degree. The degree of selectivity for Aβ oligomers and brain exposure drive the magnitude and onset of clinical efficacy, while the clearance of plaques is associated with vasogenic brain edema. Only the highest doses of aducanumab and BAN2401 show modest efficacy, as higher doses are limited by increased risk of vasogenic edema, especially in APOE4 carriers. These limitations

can be avoided, and efficacy improved by small molecule agents that selectively inhibit the formation or block the toxicity of Aβ oligomers without clearing amyloid plaques. The most advanced highly selective anti-oligomer agent is ALZ-801, an optimized tablet oral prodrug of tramiprosate, which demonstrated efficacy in homozygous APOE4/4 AD subjects. ALZ-801 selectively and fully inhibits the formation of Aβ42 oligomers at the clinical dose, without evidence of vasogenic edema, and will be evaluated in a Phase 3 trial in homozygous APOE4/4 patients with Early AD.

Key Points: (A) Targeting of amyloid oligomers drives clinical and biomarker efficacy. (B) Time to peak brain levels is an important factor for achieving optimal target engagement and, together with peak brain exposure, drives clinical benefit. (C) Selectivity for Aβ oligomers and the individual pharmacokinetic properties underlie the different safety profiles of the anti-amyloid agents, namely risk of brain edema, ARIA-E, and microhemorrhage, ARIA-H. (D) The consistency of effects of anti-amyloid antibodies on phosphorylated tau, total tau, and neurofilament light chain protein measured in cerebrospinal fluid, supports the important role of these biomarkers in future AD trials. (E) The most advanced selective anti-oligomer agent is ALZ-801, entering Phase 3 for the treatment of Early AD, in a genetically defined high-risk population.

7. *The Path Forward in Alzheimer's Disease Therapeutics: Reevaluating the Amyloid Cascade Hypothesis*

Published in January 2020 in *Alzheimer's & Dementia*

Authors: **Martin Tolar MD, PhD** – Founder, President & CEO of Alzheon, **Susan Abushakra, MD** – Chief Medical Officer of Alzheon, M. Sabbagh

Actionable Insights: Substantial evidence from clinical trials, as well as basic science, now supports primary initiating role of soluble Aβ oligomers in Alzheimer's. Soluble Aβ oligomers, rather than insoluble fibrils or plaques, cause synaptic toxicity and neurodegeneration. Inhibiting the formation of soluble toxic oligomers, or their removal, is the key to clinical efficacy of anti-amyloid treatments.

Key Points: (A) Prioritize development of therapeutic agents, which are highly selective for Aβ oligomers, efficiently cross the blood-brain barrier and achieve sustained brain levels that prevent oligomer toxicity. (B) Test treatments in AD populations enriched for Aβ oligomers, such as carriers of APOE4 genotype. (C) Poor brain penetration of anti-amyloid antibodies as a class results in suboptimal drug levels in the brain, below the sustained levels needed to continuously prevent the formation or effective removal of Aβ oligomers. (D) ALZ-801, an oral drug with favorable long-term safety and high brain penetration that fully inhibits Aβ

oligomer formation, will be evaluated in a Phase 3 trial in patients with Early AD who are homozygous for APOE4 genotype.

8. *Discovery and Identification of an Endogenous Metabolite of Tramiprosate and Its Prodrug ALZ-801 that Inhibits Beta Amyloid Oligomer Formation in the Human Brain*

Published August 2018 in *CNS Drugs*

Authors: **John A. Hey, PhD** – Chief Scientific Officer of Alzheon, P. Kocis, J. Hort, **Susan Abushakra, MD** – Chief Medical Officer of Alzheon, **Aidan Power**, M. Vyhnálek, J. Y. Yu, **Martin Tolar MD, PhD** – Founder, President & CEO of Alzheon

Actionable Insights: Discovery that 3-SPA, the primary metabolite of tramiprosate and Alzheon clinical lead ALZ-801, occurs naturally in human brain, and has Aβ anti-oligomer activity comparable to that of tramiprosate. Study shows that 3-SPA may contribute to the clinical activity of tramiprosate and its prodrug ALZ-801 in AD. The potential protective role of endogenous 3-SPA on normal brain function, and in the pathogenesis of AD, warrants further investigation.

Key Points: (A) We describe the discovery and identification of an endogenous Aβ anti-oligomer substance, 3-sulfopropanoic acid (3-SPA), in the human brain. (B) 3-SPA is the primary metabolite of ALZ-801, which is in late-stage clinical development for the treatment of AD. (C) 3-SPA penetrates the brain and in a tramiprosate Phase 3 trial achieved brain concentrations associated with prevention of Aβ oligomer formation and clinical benefit in AD patients carrying the APOE4 genotype. (D) The levels of 3-SPA were up to 12.6-fold greater in patients with AD receiving tramiprosate than in drug-naïve patients.

9. *Clinical Pharmacokinetics and Safety of ALZ-801, a Novel Prodrug of Tramiprosate in Development for the Treatment of Alzheimer's Disease*

Published in October 2017 in *Clinical Pharmacokinetics*

Authors: **John A. Hey, PhD** – Chief Scientific Officer of Alzheon, J. Y. Yu, M. Versavel, **Susan Abushakra, MD** – Chief Medical Officer of Alzheon, P. Kocis, **Aidan Power,** P. L. Kaplan, J. Amedio, **Martin Tolar MD, PhD** – Founder, President & CEO of Alzheon

Actionable Insights: ALZ-801 showed excellent oral safety and tolerability in healthy adults and elderly volunteers, with significantly improved PK characteristics over oral tramiprosate. The clinical dose of ALZ-801 administered 265 mg twice daily, achieves AUC exposure of 150 mg of tramiprosate twice daily, which had previously shown positive cognitive and functional improvements in APOE4 homozygous AD patients. These bridging data support advancement of ALZ-801 to Phase 3 in early AD patients homozygous for APOE4.

Key Results: (A) We summarize the Phase 1 bridging program to evaluate the safety, tolerability, and pharmacokinetics of ALZ-801 in healthy volunteers. (B) ALZ-801 is an orally available, valine-conjugated prodrug of tramiprosate with substantially improved pharmacokinetics properties and gastrointestinal tolerability compared with the parent compound. (C) Oral ALZ-801 represents an advanced and markedly improved clinical candidate for the treatment of Alzheimer's disease.

10. Clinical Effects of Tramiprosate in APOE4/4 Homozygous Patients with Mild Alzheimer's Disease Suggest Disease Modification Potential

Published 2017 in *Journal of Prevention of Alzheimer's Disease*

Authors: **Susan Abushakra, MD** – Chief Medical Officer of Alzheon, A. Porsteinsson, P. Scheltens, C. Sadowsky, B. Vellas, J. Cummings, S. Gauthier, **John A. Hey, PhD** – Chief Scientific Officer of Alzheon, **Aidan Power**, P. Wang, L. Shen; **Martin Tolar MD, PhD** – Founder, President & CEO of Alzheon

Actionable Insights: The Mild subgroup of APOE4/4 AD patients (MMSE 22-26) showed larger benefits on high dose tramiprosate than the overall mild to moderate group. Consistent with its preclinical effects on Aβ oligomers, tramiprosate stabilized cognitive performance over the 78-week trial duration, supporting its disease modification potential, and had clinically meaningful benefit on both function and disability. Confirmatory studies using ALZ-801, an improved pro-drug formulation of tramiprosate, will target APOE4/4 patients with Early/Mild AD.

Key Results: (A) In APOE4/4 homozygotes receiving tramiprosate 150mg twice daily, efficacy in Mild AD patients (MMSE 20-26) was higher than the overall group (MMSE 16-26), and efficacy in very mild patients (MMSE 22-26) was highest of all. (B) Tramiprosate benefits compared to placebo on ADAS-cog, CDR-SB, and DAD were 125%, 81% and 71%, respectively ($p<0.02$). (C) The very mild subgroup (MMSE 22-26) showed cognitive stabilization with no decline over 78 weeks, and both ADAS-cog and DAD effects increased over time. (D) Tramiprosate safety in APOE4/4 patients was favorable over 1.5 years, with

no events of vasogenic edema. The most common adverse events were gastrointestinal and mild or moderate in severity.

11. Elucidating the Aβ42 Anti-Aggregation Mechanism of Action of Tramiprosate in Alzheimer's Disease: Integrating Molecular Analytical Methods, Pharmacokinetic and Clinical Data

Published in October 2017 in *CNS Drugs*

Authors: P. Kocis, **Martin Tolar MD, PhD** – Founder, President & CEO of Alzheon, J. Yu, W. Sinko, S. Ray, K. Blennow, H. Fillit, **John A. Hey, PhD** – Chief Scientific Officer of Alzheon

Actionable Insights: We have identified the molecular mechanism for the observed clinical efficacy of tramiprosate in patients with APOE4/4 homozygous AD. Additionally, we showed the feasibility of modulating and controlling Aβ42 molecular conformation (shape) by a small molecule, causing favorable, clinically relevant inhibition of oligomer formation. This novel enveloping MOA of tramiprosate has potential utility in the development of disease-modifying therapies for AD and other neurodegenerative diseases caused by misfolded proteins.

Key Results: (A) We have elucidated and characterized the molecular mechanism of action of tramiprosate. (B) Tramiprosate modulates conformational flexibility of amyloid beta Aβ42, and thus prevents amyloid oligomer formation. (C) Translational analysis shows alignment of the molecular effects on Aβ42 with pharmacokinetic and published clinical data.

12. Clinical Benefits of Tramiprosate in Alzheimer's Disease are Associated with Higher Number of APOE4 Alleles: The APOE4 Gene Dose Effect"

Published in October 2016 in *Journal of Prevention of Alzheimer's Disease*

Authors: **Susan Abushakra, MD** – Chief Medical Officer of Alzheon, A. Porsteinsson, B. Vellas, J. Cummings, S. Gauthier, **John A. Hey, PhD** – Chief Scientific Officer of Alzheon, **Aidan Power**, S. Hendrix, P. Wang, L. Shen, J. Sampalis, **Martin Tolar MD, PhD** – Founder, President & CEO of Alzheon

Actionable Insights: The phenomenon of increased tramiprosate efficacy in patients with one or two copies of the APOE4 gene is likely explained by the high prevalence of amyloid pathology in APOE4 carriers, leading to much higher diagnostic accuracy in these patients

compared with APOE3/3. Recent studies have shown that to up 38% of APOE3/3 patients may be amyloid negative, while almost all APOE4/4 patients are amyloid positive. In APOE4/4 patients, high dose tramiprosate showed favorable safety and clinically meaningful efficacy when added to standard of care treatment with cholinesterase inhibitors. Prospective confirmation of these promising efficacy findings in APOE4/4 patients with AD, together with the favorable safety profile and convenience of oral ALZ-801, could provide a major therapeutic advance for this genetically defined, high unmet medical need population.

Key Results: (A) In APOE4/4 subjects, tramiprosate showed dose-dependent and statistically significant effects on ADAS-cog and positive trends on CDR-SB, 40-66% and 25-45% benefit compared to placebo, respectively. (B) Highest efficacy was observed in APOE4/4 homozygotes receiving tramiprosate 150 mg twice daily. (C) APOE4 heterozygotes showed intermediate efficacy, and non-carriers showed no benefit. (D) In 426 patients with serial MRI scans, no cases of treatment-emergent vasogenic edema were observed. (E) Across the three APOE subgroups treated for up to 1.5 years, the most common adverse events were nausea, vomiting, and decreased weight. (F) Oral tramiprosate showed clinically meaningful efficacy in symptomatic APOE4/4 patients with Mild to Moderate AD at a dose with favorable long-term safety, and no observed brain edema.