AD04™ - modifying Alzheimer's disease by modulation of hippocampal lipid metabolism

ADVANTAGE THERAPEUTICS

P1-827

Carmela R Abraham, PhD, Achim Schneeberger, MD, Agustin F Santana III, Thomas Hoffmann, PhD, Anita Fischer, PhD and Sylvie Trembleau, PhD

Clinical and proteomics-based studies demonstrate the pleiotropic activity of AD04™ resulting in both immediate, symptomatic, and durable, disease-modifying effects and, as such, is unique. AD04™ may thus represent the first member of a new class of AD drugs. Beyond its pleiotropic activity, its safety profile is noteworthy. In particular, AD04™ did not trigger ARIA-E/H in the previous Phase 2 study. AD04™ treatment of aged mice restored the expression of proteins involved in lipid metabolism to the level of young mice. AD04™ Mechanism of Action elucidates the treatment effect observed in a previous phase 2 clinical study.

INTRODUCTION

We aim to repurpose the widely used adjuvant and immunomodulator Alhydrogel™ (AD04™) at a specific dose and schedule as a disease-modifying treatment for mild Alzheimer's disease (AD). AD04™ is a potent adjuvant commonly used for 100 years in vaccines and allergy immunotherapies to augment the magnitude and durability of the immune response (1-3). Our preliminary data come from a clinical Phase 2 study in which AD04™ served as a control arm. In this study, AD04™ showed promising results in delaying hippocampal atrophy and cognitive decline, as well as improving the quality of life in patients with mild AD (4). A global statistical test (GST) was used to compare the treatment effect of AD04™ to other drugs/drug candidates. GST used is a combination of ADAS-cog, ADCS-ADL and the CDR-sb. As such, it combines three important perspectives of three different aspects of AD progression. The effect size seen with AD04™ is comparable or better than that of most other available treatments (Fig 1). However, the precise mode of action of subcutaneous (s.c.) administration of AD04™ on the brain has not been deciphered. In cognitive and brain morphology measures, AD04™ outperformed the anti-amyloid monoclonal antibodies (5,6). Here we describe the results of a proteomics study aimed at identifying the mode of action of AD04™.

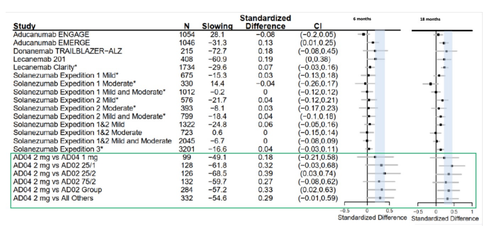

Figure 1. *Forest plot summarizing the GST analysis of efficacy of AD04 versus mAb studies show AD04 superiority at 6 and 18 months.*

METHODS

Aged (24 mo) C57BL/6 mice were injected s.c. with AD04™ or with PBS, three times, and 3-5 days after the last injection they were tested for behavior (contextual and cue fear conditioning). One day later, the mice were sacrificed, and hippocampi were subjected to proteomics. Young (10wks) mice were used as controls. Approximately 7000 gene products were analyzed. Lysates from mouse hippocampi were processed with the iST kit (Preomics) and the resulting peptides were analyzed using nano-LC-MS/MS (Orbitrap Exploris 480) with data-independent acquisition and label-free relative quantification. The data were processed using Spectronaut 18 (Biognosys) and statistically evaluated using the Msreport python library (Max Perutz Labs MS Facility).

RESULTS

In the fear conditioning test, AD04™-treated old mice, exhibited an improved freezing behavior compared with the PBS- injected mice (data not shown). Proteomics analysis of the hippocampi revealed that peripheral AD04™ administration modulated the production of proteins involved in lipid metabolism regulation including the homeostasis of microglial lipid droplets as seen by the restoration of some proteins to levels found in young mice (Figure 4). Abnormal lipid metabolism has been shown to occur in AD. For a review see (9).

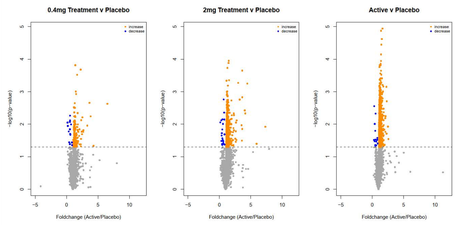

Figure 2*. Volcano plot of proteins differentially modified in response to 0.4 or 2 mg AD04™. In the left two panels proteomic results of 5 treated mice at two dosages and 5 untreated mice are shown. In the right panel, the results of 10 treated vs. 10 untreated mice are shown. Because of the small number of mice, p-values of <0.05, <0.1 and <0.2 were tested. See Table below.*

Enrichment Analyses: Lipid Metabolism

System Name	P-Value Cut-off	N Differentially Abundant Proteins	Pathway N	Empirical P-Value
Fatty Acid Biosynthesis	0.1	8	10	0.003
Fatty Acid Biosynthesis	0.2	9	10	0.084
Steroid Biosynthesis	0.01	1	8	0.0258
Glycerolipid Metabolism	0.05	5	12	0.0463

Enrichment Analyses: Lipid Metabolism Fatty Acid Biosynthesis

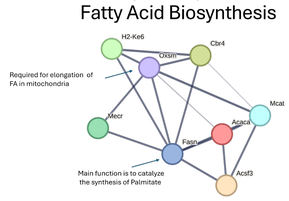

Figure 3*. Network analysis demonstrates interrelations of significantly upregulated proteins within lipid metabolism pathways. Shown here, fatty acid biosynthesis: 8 out of the 10 proteins in this pathway were stimulated by AD04™. For further details on the node proteins ACACA and Fasn, see refs. 10 and 11.*

RESULTS cont.

Proteins involved in lipid metabolism were the most significantly modulated in response to AD04™: among others, LMF1, ACSL1 and BSCL2. LMF1 is involved in the breakdown of lipids by inducing lipoprotein lipase (LPL). ACSL1 and BSCL2 are needed for the metabolism of lipid droplets, which are important storage organelles. Accumulation of lipid droplets are found in aged microglia, the immune cells of the brain. The role of microglia is to ingest and destroy toxic proteins (phagocytosis), such as the AD-related misfolded proteins amyloid and tau, viruses, bacteria and dead cells debris. Microglia that are overfilled with lipid droplets cannot fulfill their role.

Figure 4*. AD04™ treatment restores levels of ACSL1 and BSCL2 to the levels seen in young mice.*

The aged mouse (24month-old) has dysregulation of brain cells as found in AD patients. This involves several populations of microglial cells:
A microglia phenotype found in the aged mouse as in AD patients is positive for the markers: Trem2, ApoE, and Clec7a. It is called the Disease-Associated Microglia (DAM) (7).
Another microglia phenotype is termed lipid-droplet-accumulating microglia, LDAM (8).
Other brain cells in the aged mouse like in AD patients also accumulate lipid-droplet and/or have a dysregulated lipid metabolism.

The abundant „DAM" microglia phenotype, expressing Clec7a, Trem2, and ApoE, found in aged mice was not modulated by AD04™, as measured by qPCR (Clec7a, Trem2) and histology (ApoE) (data not shown).

However, our results suggest that the lipid-droplet accumulating microglia, LDAM, was modulated by AD04™.

Proteins of interest modulated by AD04™.

BSCL2 plays a crucial role in the formation of lipid droplets (LDs) which are storage organelles at the center of lipid and energy homeostasis. Also required for growth and maturation of small nascent LDs into larger mature LDs . Mediates the formation and/or stabilization of endoplasmic reticulum-lipid droplets (ER-LD) contacts, facilitating protein and lipid delivery from the ER into growing LDs. Binds anionic phospholipids including phosphatidic acid

ACSL1 Ligase that catalyzes the conversion of long-chain fatty acids to their active form acyl-CoAs for both synthesis of cellular lipids, and degradation via beta-oxidation. Preferentially uses palmitoleate, oleate and linoleate.

LMF1 The most statistically significant protein upregulated by AD04™ has been confirmed by qPCR.

CONCLUSIONS

Omics-based studies, done in various settings (old mice: AD04™ vs buffer; young vs. old mice), yielded results consistent with the key findings of the Phase 2 study.

- Proteomic studies unraveled AD04™ effects across various pathways (not all shown here) demonstrating the AD04™ effect to be pleiotropic in nature. This aligns well with the broad activity of AD04™ seen in the previous Phase 2 trial, where effects were seen across all clinical endpoints and the biomarker hippocampal volume.
- In the clinical study, effects (cognition, function, biomarker) were seen early during the study and were dose dependent. Results of the proteomics study confirmed both findings. Pleiotropic effects were detected one week after the 3rd administration and their extent was dose dependent.
- The clinical activity of AD04™ has a unique pattern: effects are seen early, which is pointing to a symptomatic nature, but they are also durable and fulfill characteristics/statistical definitions of a disease-modifying drug (curves remained separated after AD04™ was stopped/paused). Proteins and pathways identified by the proteomic analysis readily explain these uncommon clinical effects of AD04™. Modulation of the expression of proteins involved in mitochondrial function (energy supply) or the function of neurons (signaling), not shown here, have the potential to immediately improve various aspects of the disease. Their long-term normalization will further contribute to brain health. Moreover, other proteins/pathways addressed by AD04™, such as BACE, APP metabolism, but also lipid metabolism may add disease-modifying activities.
- AD04 treatment of aged mice restored the expression of Acsl1 and Bscl2 towards the level of young mice, suggesting restoration of normal lipid droplet metabolism and modulation of microglia to a phagocytic type.
- AD04 Mechanism of Action elucidates the treatment effect observed in a previous phase 2 clinical study.

category	system_name	pvalue_cutoff	cutoff	num_samples	empirical_pvalue	sig||
Lipid metabolism	Fatty acid biosynthesis	0,1	8	10	0,003	Y
Lipid metabolism	Fatty acid biosynthesis	0,2	9	10	0,0084	Y
Lipid metabolism	Steroid biosynthesis	0,01	1	8	0,0258	Y
Lipid metabolism	Glycerolipid metabolism	0,05	5	12	0,0463	Y
Neurodegenerative disease	Neurodegenerative disease	0,05	66	210	0,0052	Y
Neurodegenerative disease	Neurodegenerative disease	0,1	104	210	0,015	Y
Neurodegenerative disease	Neurodegenerative disease	0,2	142	210	0,0241	Y
Neurodegenerative disease	Alzheimer disease	0,05	25	79	0,0479	Y
Neurodegenerative disease	Parkinson disease	0,05	16	44	0,022	Y
Neurodegenerative disease	Amyotrophic lateral sclerosis	0,05	32	83	0,0013	Y
Neurodegenerative disease	Amyotrophic lateral sclerosis	0,1	43	83	0,035	Y
Neurodegenerative disease	Huntington disease	0,05	19	58	0,0494	Y
Neurodegenerative disease	Pathways of neurodegeneration	0,05	42	130	0,0124	Y
Neurodegenerative disease	Pathways of neurodegeneration	0,1	68	130	0,0094	Y
Neurodegenerative disease	Pathways of neurodegeneration	0,2	92	130	0,0102	Y

Proteins involved in lipid metabolism and neurodegenerative diseases are the most differentially modified in response to AD04™.

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REFERENCES

1. Marrack, P., McKee, A. S., & Munks, M. W. (2009). Towards an understanding of the adjuvant action of aluminium. In *Nature Reviews Immunology* (Vol. 9, Issue 4, pp. 287–293).
2. Oleszycka, E., & Lavelle, E. C. (2014). Immunomodulatory properties of the vaccine adjuvant alum. In *Current Opinion in Immunology* (Vol. 28, Issue 1, pp. 1–5).
3. Pulendran, B., S. Arunachalam, P., & O'Hagan, D. T. (2021). Emerging concepts in the science of vaccine adjuvants. In *Nature Reviews Drug Discovery* (Vol. 20, Issue 6, pp. 454–475). Nature Research.
4. Schneeberger A, Hendrix S, Ellison N, Bürger V, Dubois B (2015) Results from a Phase II Study to Assess the Clinical and Immunological Activity of AFFITOPE® AD02 in Patients with Early Alzheimer's Disease. *J Prev Alzheimer's Dis* 11:276–276.
5. Mintun MA, Lo AC, Duggan Evans C, Wessels AM, Ardayfio PA, Andersen SW, Shcherbinin S, Sparks J, Sims JR, Brys M, Apostolova LG, Salloway SP, Skovronsky DM. (2021) Donanemab in Early Alzheimer's Disease. *N Engl J Med*. 6;384(18):1691-1704.
6. van Dyck CH, Swanson CJ, Aisen P, Bateman RJ, Chen C, Gee M, Kanekiyo M, Li D, Reyderman L, Cohen S, Froelich L, Katayama S, Sabbagh M, Vellas B, Watson D, Dhadda S, Irizarry M, Kramer LD, Iwatsubo T. (2023) Lecanemab in Early Alzheimer's Disease. *N Engl J Med*. 388(1):9-21.
7. Krasemann S, Madore C, Cialic R, Baufeld C, Calcagno N, El Fatimy R, Beckers L, O'Loughlin E, Xu Y, Fanek Z, Greco DJ, Smith ST, Tweet G, Humulock Z, Zrzavy T, Conde-Sanroman P, Gacias M, Weng Z, Chen H, Tjon E, Mazaheri F, Hartmann K, Madi A, Ulrich JD, Glatzel M, Worthmann A, Heeren J, Budnik B, Lemere C, Ikezu T, Heppner FL, Litvak V, Holtzman DM, Lassmann H, Weiner HL, Ochando J, Haass C, Butovsky O. (2017) The TREM2-APOE Pathway Drives the Transcriptional Phenotype of Dysfunctional Microglia in Neurodegenerative Diseases. *Immunity*. 47(3):566-581.
8. Marschallinger J, Iram T, Zardeneta M, Lee SE, Lehallier B, Haney MS, Pluvinage JV, Mathur V, Hahn O, Morgens DW, Kim J, Tevini J, Felder TK, Wolinski H, Bertozzi CR, Bassik MC, Aigner L, Wyss-Coray T. (2020) Lipid-droplet-accumulating microglia represent a dysfunctional and proinflammatory state in the aging brain. *Nat Neurosci*. 23(2):194-208. Erratum in: *Nat Neurosci*. 2020 23(10):1308.
9. Yin F. (2023) Lipid metabolism and Alzheimer's disease: clinical evidence, mechanistic link and therapeutic promise. *FEBS J*. 290(6):1420-1453.
10. Dong J, Li M, Peng R, Zhang Y, Qiao Z, Sun N. (2024) ACACA reduces lipid accumulation through dual regulation of lipid metabolism and mitochondrial function via AMPK- PPARα- CPT1A axis. *J Transl Med*. 23;22(1):196.
11. Knobloch M, Braun SM, Zurkirchen L, von Schoultz C, Zamboni N, Araúzo-Bravo MJ, Kovacs WJ, Karalay O, Suter U, Machado RA, Roccio M, Lutolf MP, Semenkovich CF, Jessberger S. (2013) Metabolic control of adult neural stem cell activity by Fasn-dependent lipogenesis. *Nature*. 493(7431):226-30.

CONTACT

Dr. Carmela Abraham, CSO
ADvantage Therapeutics, Inc.
US:+1-781-424-7090
carmela@advantagetherapeutics.com

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