Results from a Phase II Study to Assess the Clinical and Immunological Activity of AFFITOPE® AD02 in Patients with Early Alzheimer's Disease

A. Schneeberger[1], S. Hendrix[2], M. Mandler[1], N. Ellison[2], V. Bürger[1], M. Brunner[3], L. Frölich[4], N. Mimica[5], J. Hort[6,7], M. Rainer[8], D. Imarhiagbe[9], A. Kurz[10], O. Peters[11], H.-J. Gertz[12], L. Tierney[1], F. Mattner[1], W. Schmidt[1], B. Dubois[13] for the investigators of CT AFF006

1. AFFiRiS AG, Vienna, Austria; 2. Pentara Corporation, Salt Lake City, UT, USA; 3. Medizinische Universität Wien, Universitätsklinik für klinische Pharmakologie, Vienna, Austria; 4. Zentralinstitut für Seelische Gesundheit Abteilung Gerontopsychiatrie, Mannheim, Germany; 5. University Psychiatric Hospital Vrapče, University of Zagreb, School of Medicine, Zagreb, Croatia; 6. Memory clinic, Department of Neuorology, 2nd Faculty of Medicine, Charles University Prague, Motol University Hospital, Czech Republic; 7. International Clinical Research Center, St. Anne's University Hospital Brno, Brno, Czech Republic; 8. Memory Clinic and Karl Landsteiner Institute for Alzheimer-research in SMZEast, Vienna, Austria; 9. Landeskrankenhaus Hall, Gedächtnisambulanz, Hall in Tirol, Austria ; 10. Klinik und Poliklinik für Psychiatrie und Psychotherapie der Technischen Universität München, Munich, Germany; 11. Charite Universitätsmedizin Berlin, Campus Benjamin Franklin, Klinik und Hochschulambulanz für Psychiatrie und Psychotherapie, Berlin, Germany; 12. Arzneimittelforschung Leipzig GmbH, Studienzentrum, Leipzig, Germany; 13. Cognitive and behavioral disease center and Alzheimer´s institute, Research INSERM Unit "Cognition, Neuroimaging, and brain diseases, Salpêtrière University Hospital, Paris, France

Corresponding Author: Achim Schneeberger, AFFiRiS AG, Karl-Farkas-Gasse 22, 1030 Wien, Austria, Email: Achim.Schneeberger@affiris.com, Tel: +43-(1)-7981575-300, Fax +43-(1)-7981575-311

J Prev Alz Dis 2015;in press
Published online in process

Abstract

OBJECTIVES: The primary objective of this clinical trial was to assess the clinical activity of various doses and formulations of AFFITOPE® AD02 following its repeated s.c. administration to patients with early Alzheimer´s disease (AD), based on the evaluation of cognitive and functional domains.

DESIGN: It was designed as a randomized, placebo-controlled, parallel group, double blind, multicenter phase II trial with 10 regular outpatient visits and 6 telephone interviews.

SETTING: The trial was performed at 32 sites in six countries.

PARTICIPANTS: A total of 332 patients were enrolled and 265 patients completed the trial in 3 treatment groups with AD02 and 2 control groups with aluminum oxihydroxide, here named IMM-AD04. Patients were randomly assigned to 5 groups: two doses of IMM-AD04, 25μg AD02 (in two different formulations) and 75μg AD02.

INTERVENTION: At months 0, 1, 2, 3, 9 and 15, each patient received a single s.c. injection of the corresponding preparations of AFFITOPE® AD02 or the control, IMM-AD04.

MEASUREMENTS: Co-primary efficacy outcomes included a measure of cognition (adapted AD Assessment Scale cognitive [aADAS cog]), and a measure of function (adapted AD Cooperative Trial Activities of Daily Living [aADCS-ADL]). A primary composite score was the sum of these two scores.

RESULTS: Treatments were generally well tolerated and adverse events (AEs) were seen at similar rates across all treatment groups, with the exception that more injection site reactions were seen in the groups with a higher level of adjuvant. None of the AD02 groups showed a benefit over the IMM-AD04 controls for primary or exploratory efficacy outcomes. The control groups differed on aADCS-ADL and therefore couldn't be pooled (p=0.039). Unexpectedly, the 2mg IMM-AD04 showed statistically significant effects over the other groups on several clinical outcomes including: aADAS-cog, aADL, Composite, ADAS-cog, CDR-sb, and QOL-AD Caregiver as well as two biomarker outcomes: right and total hippocampal volume (all p<0.05). 48% of patients in the IMM-AD04 2mg group had no decline in the composite outcome over 18 months compared to 17%-31% in the other groups, which is consistent with historical placebo groups.

CONCLUSION: No significant treatment effects were seen for the investigational compound AD02. However, the IMM-AD04 2mg group showed statistically significant effects over all other groups on several clinical outcomes as well as a slowing of decline on right hippocampal volume. The data support further development of IMM-AD04 as a disease modifying agent in line with EMA/FDA definitions.

Key words: Aluminum, Alzheimer´s disease, vaccination, clinical trial, AFFITOPE®.

Introduction

Alzheimer's disease (AD), a chronic neurodegenerative disorder, is the most common cause of dementia currently affecting 44 million individuals worldwide (1). It is characterized by a cascade of events starting with a change in amyloid beta (Aβ) metabolism, which is followed by brain amyloidosis, tauopathy, brain atrophy and decreased glucose metabolism (2, 3). After these biological changes, cognitive impairment can be detected, which culminates in functional impairment and eventually dementia (4, 5). Innate immune mechanisms, activated in response to misfolded proteins such as aggregated Aβ, emerge as another crucial component of AD (6, 7) .

The multifactorial nature of AD and the limited specificity of diagnostic criteria, among others, have significantly hindered drug development. More than 100 agents have been assessed for their disease modifying potential in AD and not a single one has shown a drug-placebo difference in favor of active treatment (8).

Received February 16, 2015
Accepted for publication March 19, 2015

Clinical trials to date have primarily focused on Aβ, including four of the six most recent phase III studies (8). Active Aβ immunotherapy has been shown to diminish hallmark signs of the disease (e.g. microgliosis and astrocytosis) and improve cognitive function in numerous animal models (9-11). In contrast, no class of agents has been able to show efficacy for this target in clinical trials, bringing into question Aβ as a target, the immunotherapeutic approach, and the appropriate disease stage for intervention (12).

In this trial, we investigated AFFITOPE® AD02, a peptide-KLH conjugate vaccine where the peptide moiety mimics the N-terminal region of human Aβ. AD02 was developed for the treatment of patients with AD using AFFiRiS´ proprietary AFFITOME® technology (13, 14). The first-in-man trial of AD02 (ClinicalTrials.gov Identifier: NCT00633841) and follow-up studies encompassing a boost, demonstrated AD02 to be safe and provided evidence to suggest AD02 elicits Aβ aggregate-specific antibodies mediating a disease modifying clinical effect.

Aluminium salts are the major class of adjuvants used to enhance the immune response induced by vaccines due to their long-standing safety record. Aluminium salts amplify the adaptive immune response occurring upon vaccine administration by modulating the innate immune response to it. They enhance recruitment of inflammatory cells to the injection site (reviewed in (15)), modulate antigen presentation (16) and skew the ensuing adaptive immune response towards a type-2 helper (Th2) phenotype (reviewed in (17)). New technologies have revealed that locally administered aluminum salts spread systemically, even reaching the central nervous system (18).

Here we investigate the safety, tolerability, clinical and immunological activity of AD02 following its repeated subcutaneous (s.c.) administration at different doses to patients with early AD. Controls received Alum, in this paper referred to as IMM-AD04, at the two different doses that were also used in the active treatment groups.

Methods

Study Design

This study was a randomized, placebo controlled, parallel group, double blind, multicenter phase II trial to assess the clinical and immunological activity as well as the safety and tolerability of two doses (25μg and 75μg) of AFFITOPE® AD02 adsorbed to either 2 mg or 1 mg aluminum oxihydroxide (commonly known as "Alum" and here referred to as Alum when used in combination with AD02 or as IMM-AD04 when used separately), in patients with early AD. Groups were defined as follows: 25μg AFFITOPE® AD02 adsorbed to 2 mg Alum (= AD02 25/2); 75μg AFFITOPE® AD02 adsorbed to 2 mg Alum (= AD02 75/2), 25μg AFFITOPE® AD02 adsorbed to 1 mg Alum (= AD02 25/1); 1 mg IMM-AD04; and 2 mg IMM-AD04.

At trial weeks 0, 4, 8, 12, 40, and 65 (Visits 2, 3, 4, 5, 7, and 9, respectively), each patient received a single s.c. injection of the corresponding preparation of AFFITOPE® AD02 or the control IMM-AD04. In total, each patient received six injections over a 65 week treatment period. The primary objective of this clinical trial was to assess the clinical activity of various doses and formulations of AFFITOPE® AD02 following its repeated s.c. administration, based on the evaluation of the cognitive and functional domains. The secondary objective was to assess the immunological activity, treatment effect on the volume of defined brain regions, biological markers of brain atrophy, impact on health related quality of life (QOL) and behavior. Safety and tolerability were additionally assessed. The study took place in 32 sites in six European countries: France (8 sites), Germany (8 sites), Austria (7 sites), Croatia (5 sites), Czech Republic (2 sites), and Slovakia (2 sites) between 01 Oct 2010 (first patient screened) and 06 Dec 2013 (last patient visit).

Vaccine Application

All six injections were administered at the trial site. Injections were applied s.c. to the external surface of the upper arm alternating the injection arm. Before vaccine administration, an intravenous access was placed to enable rapid therapy of AEs such as allergic reactions. Following application, the patient was observed for at least 1 hour. A 30-day post-treatment window following the study was included, covering an appropriate timeframe for possible late AEs.

This trial was performed in compliance with Good Clinical Practice (GCP), the Declaration of Helsinki (2013), and local legal and regulatory requirements and applicable international regulations.

Patient Population

The trial was performed in patients with early AD, defined by the following criteria: probable AD as defined by National Institute of Neurological and Communicative Disorders and Stroke/AD and Related Disorders Association (NINCDS/ADRDA) criteria; a MMSE score ≥20; an amnestic syndrome of the hippocampal type defined by a Free and Cued Selective Reminding Test (FCSRT) (19, 20) result of total recall ≤40 or free recall ≤17, in favor of AD in its typical form (21). The result of a centrally read MRI of a patient's brain must have been compatible with the diagnosis AD, in particular, presence of a medial temporal lobe atrophy (Scheltens Score ≥2) or in the case of a Scheltens score of 0 or 1, the presence of Aβ42 reduced in CSF, reflecting cerebral amyloidosis, and with elevated tau or phospho tau, reflecting neuronal injury, based on cutoffs established at the local labs.

Other major inclusion criteria were as follows:

Hachinski Ischemia Scale (22) score of ≤4, an informed consent capability as assessed by an independent professional, availability of a partner/caregiver, availability of ApoE genotype, stable treatments for AD and/or hypothyroidism for 3 months prior to study inclusion.

Major exclusion criteria included: pregnancy, participation in the active treatment phase of another clinical trial within 3 months before Visit 1, and history and/or presence of an allergy to the vaccine or autoimmune disease, contraindication for MRI imaging, presence of ≥4 microhemorrhages at baseline, recent history of cancer (exceptions: basal cell carcinoma, intraepithelial cervical neoplasia), presence/history of immunodeficiency, significant systemic illness, prior and/or current treatment with experimental AD immunotherapies and/or immunosuppressive drugs.

All study subjects provided voluntary written informed consent. The trial protocol, patient information, informed consent, and all other required trial documents were submitted to independent ethics committees (IEC). Notification in writing of approval was obtained by the IEC by the investigator before trial initiation.

Safety Assessment

The safety assessments included a complete physical examination, standard neurological examination, spontaneously reported AEs. An extensive laboratory (hematology, chemistry, serology, urinalysis, etc.) assessment was performed at visits 1-10 or upon early termination. Grading of local injection site reactions was performed according to the FDA guidance for industry toxicity scale for healthy adult and adolescents patients enrolled in preventative vaccine clinical trials (2007).

At visits 1, 4 (France only), 6, 8 and 10 or early termination visit (ETV), an MRI of the patient's brain was performed. The results of the MRI were used to evaluate differences from baseline for both safety and efficacy assessment including volumetric analyses. Vaccination safety was based on the assessment of microhemorrhages (ARIA-H), vasogenic edema (ARIA-E) and any other abnormalities including meningoencephalitis (ME).

Co-primary endpoints

Primary endpoints were an adapted ADAS-cog (aADAS-cog) and an adapted ADCS-ADL (aADCS-ADL), and a combined composite score that was the sum of the aADAS-cog and aADCS-ADL. For description, refer to Hendrix et al. (23).

Secondary and exploratory endpoints

The secondary endpoint was the Clinical Dementia Rating 'sum of boxes' (CDR-sb) (24). Exploratory endpoints included the Neuropsychiatric Inventory (NPI) (25), Alzheimer's Disease Assessment Scale-cognitive (ADAS-cog) 11 item (26) and 13 item (27), Alzheimer's Disease Cooperative Trial-Activities of Daily Living Standard (ADCS-ADL) (28) and the patient Quality of Life (QOL-AD) reported by patients and caregivers.

The NPI and ADCS-ADL outcomes were measured at visits 2, 5, 6, 7, 8, 9, and 10 or ETV. The ADAS-cog was measured at these time points and at visits 1 and 3. The QOL-AD was only measured at visits 1, 6, 8, and 10 or EDV. MRI brain volumes were also assessed for efficacy.

Immune Response

Four titers of IgG response were analyzed including monomeric and aggregated Aβ, AD02 and KLH. Titers of IgG antibodies specific for the immunizing peptide, N-terminal part of Aβ, Aβ itself, and KLH were measured at visits 1 through 10 or ETV. Antibody (to aggregated Aβ, AD02 and KLH (IgG)) responder and non-responder groups were compared on each of the primary and secondary outcomes at the end of the trial.

Statistical analysis

All significance testing was 2-tailed using $\alpha = 0.05$. Tests were declared statistically significant if the calculated p-value was ≤ 0.05. The primary trial hypothesis was deemed satisfied if the aADAS-cog scores showed improvements in the active treatment antibody responder (aggregated Aβ IgG) group relative to the control that were significant at the 0.05 level. All analyses were conducted with SAS® software version 9.4 (Cary, NC).

Study size was determined using a power calculation (simple t-test on the change from baseline on adapted ADAS-cog scores at 18 months) for detecting a difference on the aADAS-cog score, comparing antibody responders (aggregated Aβ, AD02 and KLH IgG) in all active groups (vaccine) versus control-treated patients. A population of 290 patients with early AD was targeted to complete the clinical trial (69 patients in each of 3 treatment groups; 83 in the control groups). A 10% dropout rate was assumed.

For the primary efficacy analyses, SAS PROC MIXED was used to fit an MMRM, with CFB of each of the efficacy outcomes (e.g., adapted ADAS cog) as the response variable and the following covariates and fixed effects, as well as each of these interacting with visit:

- Age (covariate);
- Level of Education (fixed effect split into categories of ≤12 years, >12 years);
- Gender (fixed effect);
- Baseline Test Score of Efficacy Parameter (covariate);
- Center (fixed effect);
- Treatment (fixed effect);
- APOEe4 status (fixed effect, positive or negative);
- Use of AChEI (fixed effect, determined from

medications);
- Visit (fixed effect);
- Visit by [ALL OF THE ABOVE TERMS] interactions.

The covariance structure for this model was ARH(1) (first-order heterogeneous autoregressive). If ARH(1) did not converge for the model, a heterogeneous compound symmetry covariance structure was applied. If both heterogeneous covariance structures failed to converge, the model was simplified to exclude some terms. A similar model was fit for secondary efficacy analyses and subgroup analyses.

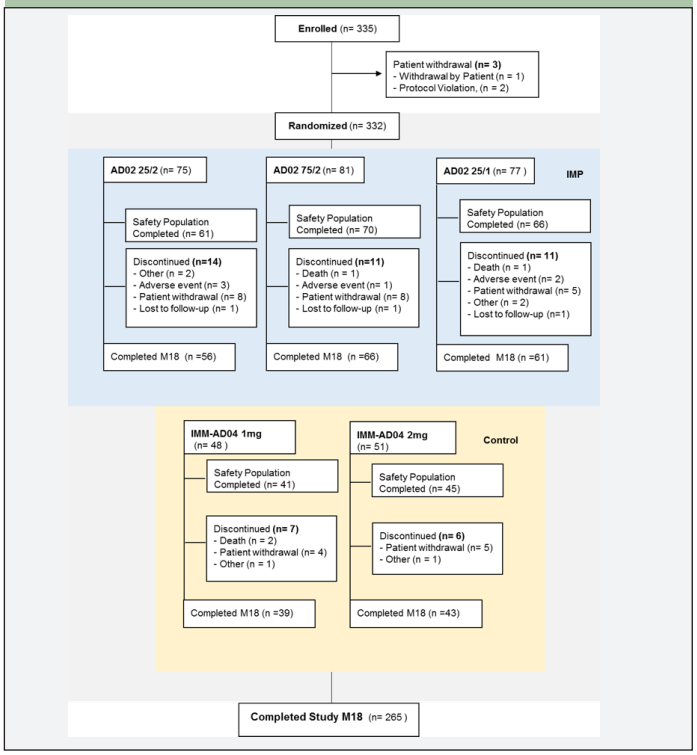
Results

Study participants

Individuals between 50-80 years old with a probable early degree AD as defined in the Materials and Methods were included in the trial. Trial subjects (n= 332) were randomized in five study groups: AD02 25/2 (n=75), AD02 75/2 (n=81), AD02 25/1 (n=77), which received AD02 at different doses/formulations and IMM-AD04 1mg (n =48) and IMM-AD04 2mg (n=51), which received different doses of IMM-AD04 (Figure 1).

The majority of patients were Caucasian (99.7%) and of nonsmoking status (90.7%) and approximately

Figure 1. Flow diagram of subject disposition



The safety population included all patients who received at least one dose of AFFITOPE® AD02 or IMM-AD04. Patients were included in the treatment groups according to the highest dose received in cases of incorrect treatment administration. Patients in the safety population were analyzed based on the treatment they actually received and not necessarily the one to which they were randomized. M: Month.

even numbers of men (49.1%) and women (50.9%) were enrolled. Overall, patients had a mean age of 70.4(±7.1) years, a mean education level of 12.1(±3.7) years, mean weight of 70.1(±12.8) kg, mean height of 168.0(±9.4) cm, and a mean BMI of 24.8(±3.6) kg/m2. Approximately 59% of patients were taking AChEI alone at baseline, another 3% were taking Memantine alone, 9% were taking both and 28% were not on either concomitant AD treatment. Groups were well balanced on these baseline characteristics (all p-values were >0.079) with the following exceptions: 1) the IMM-AD04 2mg was shorter on the average (164.6±10.2) than 3 groups, IMM-AD04 1mg (168.9±7.7, p=0.021), AD02 25/1mg (169.8±9.1, p=0.003), AD02 25/2mg (168.6±9.6, p=0.029), but not different from AD02 75/2mg (167.4±9.6, p=0.111) and (2) the IMM-AD04 2mg had more females (63%) compared to fewer females (42%) in the IMM-AD04 1mg (p=0.045) (Table 1).

Patients in the treatment arms received six injections of AD02 absorbed to Alum and the control groups received six injections of IMM-AD04. Each patient received injections over a 65-week treatment period during trial weeks 0, 4, 8, 12, 40, and 65.

AD02 does not show improvement in primary or secondary efficacy endpoints

The primary objective of this clinical trial was to assess the clinical activity of various doses and formulations of AD02, therefore a series of primary and secondary efficacy endpoints were evaluated. Analyses were performed on a primary single endpoint measuring global function (Composite), co-primary endpoints measuring cognition and function (Adapted ADAS-cog and Adapted ADL), secondary endpoint measuring global function (CDR-sb) and exploratory traditional scales measuring cognition and function (ADAS-cog 11, ADCS-ADL). Additionally, patient QOL assessed by patient (patient QOL) and caregiver (caregiver QOL), behavior (NPI) and MRI brain volumes were also assessed.

The initial study plan included the pooling of the control groups assuming that they were not significantly different on key efficacy parameters. However, as differences were found with the control groups early on in the analysis, they were not pooled. The primary analysis was therefore a comparison of all five groups. A statistically significant treatment difference was seen between IMM-AD04 1mg and IMM-AD04 2mg showing an effect size of 46% (p-value 0.0392) for the adapted ADCS-ADL; this effect size corresponds to a reduced decline in the IMM-AD04 2mg group for this primary endpoint in the model that only included the two control groups.

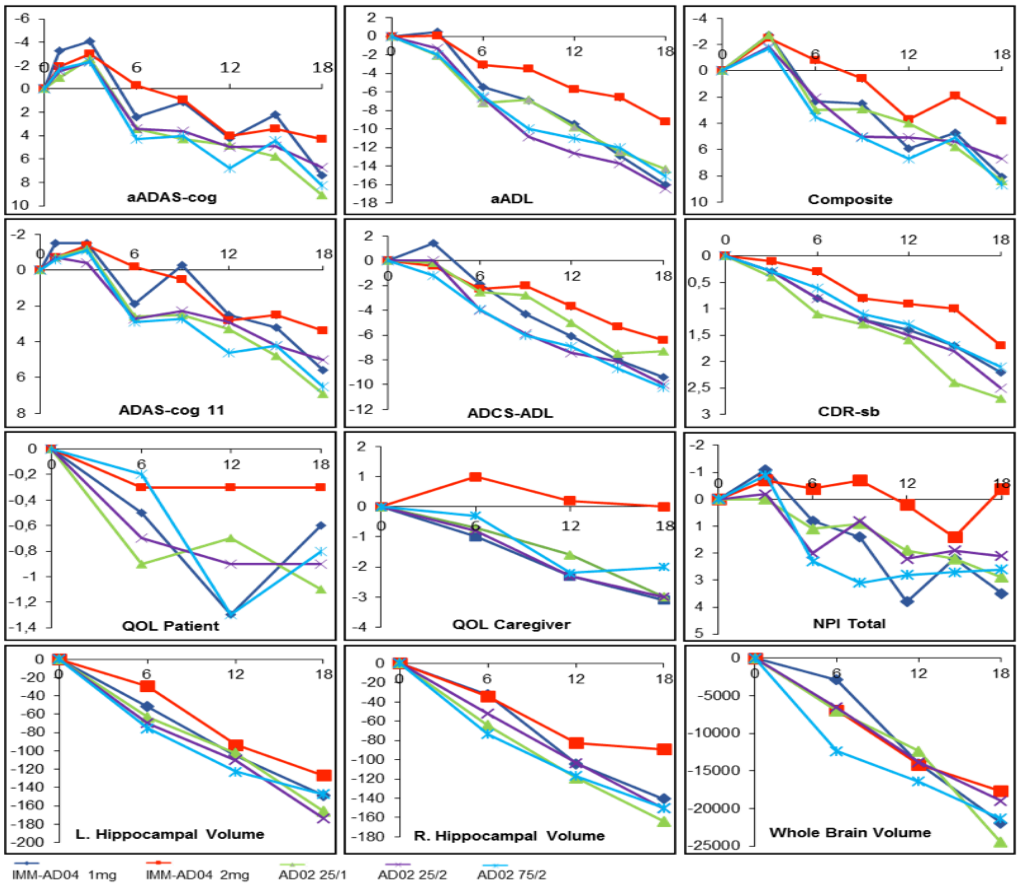
None of the groups treated with AD02 showed benefit over the IMM-AD04 2mg group for primary or

Table 1: Patient baseline characteristics (Safety population). Max: maximum; Min: minimum; SD: standard deviation.

	IMP			Control		Total
*Demographic Characteristics	AD02 25/2 (n = 75)	AD02 25/1 (n = 77)	AD02 75/2 (n = 81)	IMM-AD04 1mg (n = 48)	IMM-AD04 2mg (n = 51)	(n = 332)
Age (yrs)						
Mean ± SD	70.8 ± 7.20	70.3 ± 6.92	71.3 ± 6.56	70.3 ± 6.56	68.9 ± 8.36	70.4 ± 7.11
Min, Max	52, 83	50 , 81	52 , 81	57, 80	50, 80	50, 83
Height (cm)						
Mean ± SD	168.6±9.6	169.8 ± 9.1	167.4 ± 9.6	168.9±7.7	164.6±10.2	168.0±9.4
Min, Max	150, 189	142, 187	147, 192	150, 183	144, 185	142, 192
BMI (kg/m^2)						
Mean ±SD	25.0 (3.9)	24.8 (3.6)	24.5 (3.4)	24.7 (2.9)	24.8 (3.9)	24.8 (3.6)
Min, Max	18.0, 39.2	16.2, 37.7	17.2, 32.7	17.8, 31.2	18.2, 35.4	16.2, 39.2
Education						
Mean ± SD	12.5 (3.73)	12.0 (3.92)	12.1 (3.43)	12.3 (4.03)	11.8 (3.18)	12.1 (3.66)
Min, Max	4 , 20	4 , 22	4 , 20	8 , 26	6 , 22	4 , 26
Sex, n (%)						
Male	37 (49.3)	41 (53.2)	38 (46.9)	28 (58.3)	19 (37.3)	163 (49.1)
Female	38 (50.7)	36 (46.8)	43 (53.1)	20 (41.7)	32 (62.7)	169 (50.9)
Race, n (%)						
White	75 (100.0)	77 (100.0)	81 (100.0)	48 (100.0)	50 (98.0)	331 (99.7)
Asian	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.0)	1 (0.3)
Stratification Group						
AChEI only	56 (74.7%)	47 (58.0%)	38 (49.4%)	26 (54.2%)	30 (58.8%)	197 (59.3%)
Memantine only	1 (1.3%)	3 (3.7%)	4 (5.2%)	1 (2.1%)	1 (2.0%)	10 (3.0%)
Both AChEI and Memantine	0 (0.0%)	8 (9.9%)	8 (10.4%)	9 (18.8%)	6 (11.8%)	31 (9.3%)
Neither AChEI nor Memantine	18 (24.0%)	23 (28.4%)	27 (35.1%)	12 (25.0%)	14 (27.5%)	94 (28.3%)
CDR-sb Total Score						
Mean ± SD	3.9 ± 1.99	4.1 ± 2.41	4.7 ± 2.65	4.2 ± 2.37	3.9 ± 2.34	4.2 ± 2.37
Min, Max	1, 11	1 , 13	1 , 14	1, 10	1, 10	1, 14
NPI Total Score						
Mean ± SD	9.1 ± 11.60	9.0 ± 9.22	8.0 ± 9.17	8.6 ± 8.38	9.0 ± 9.78	8.9 ± 9.72
Min, Max	0 , 59	0 , 38	0 , 42	0, 30	0 , 45	0 , 59
ADAS-Cog13 Total Score						
Mean ± SD	31.5 ± 9.20	31.6 ± 9.06	33.3 ± 10.55	31.7 ± 9.19	30.2 ± 8.74	31.8 ± 9.44
Min, Max	10 , 54	15 , 52	15 , 64	18 , 58	13 , 59	10 , 64
ADCS-ADL Total Score						
Mean ± SD	65.4 ± 7.70	62.3 ± 12.28	62.7 ± 11.63	62.1 ± 11.79	64.5 ± 9.36	63.4 ± 10.74
Min, Max	46 , 78	23 , 78	16 , 78	21 , 76	37 , 78	16 , 78
MMSE Total Score						
Mean ± SD	23.3 ± 2.59	23.4 ± 2.73	23.1 ± 2.68	23.0 ± 2.61	23.9 ± 2.95	23.3 ± 2.71
Min, Max	**18 , 29	19 , 30	17 , 30	18 , 27	16 , 29	16 , 30

*Groups were well balanced on these baseline characteristics (all p-values were >0.079) with the following two exceptions: (1) the IMM-AD04 2mg was shorter (164.6±10.2) than 3 groups, IMM-AD04 1mg (168.9±7.7, p=0.021), AD02 25/1mg (169.8±9.1, p=0.003), AD02 25/2mg (168.7±9.6, p=0.025), but not different from AD02 75/2mg (167.4±9.6, p=0.119) and (2) the IMM-AD04 2mg had more females (63%) compared to fewer females (42%) in the IMM-AD04 1mg (p=0.045). **Two MMSE scores per patient were obtained before the first immunization. The one at the screening visit was used for the inclusion decision (data not shown). The one at the visit of the first vaccination is shown in the table. The mean of the two was defined as baseline for the statistical analysis.

Figure 2. Efficacy outcomes



Outcomes are shown as change over time using the least squares means from mixed model. ADAS-Cog: Alzheimer Disease Assessment Scale-cognitive; ADCS-ADL: Alzheimer Disease Cooperative Trial-Activities of Daily Living; CDR-sb: Clinical Dementia Rating 'sum of boxes; NPI: Neuropsychiatric Inventory; QOL-AD: Quality of Life-Alzheimer's Disease. R: right. L: left. a: Adapted.

exploratory efficacy outcomes (Figure 2).

Higher rates of clinical response (improvement or stabilization on a clinical outcome) were seen for the IMM-AD04 2mg compared to the other treatment groups for the composite outcome, with similar direction of effects in the co-primary, secondary and exploratory endpoints (Table 2). The percentage of patients who were rated as "same" or "better" at month 18 was nearly double in the IMM-AD04 2mg compared to all other groups for the composite.

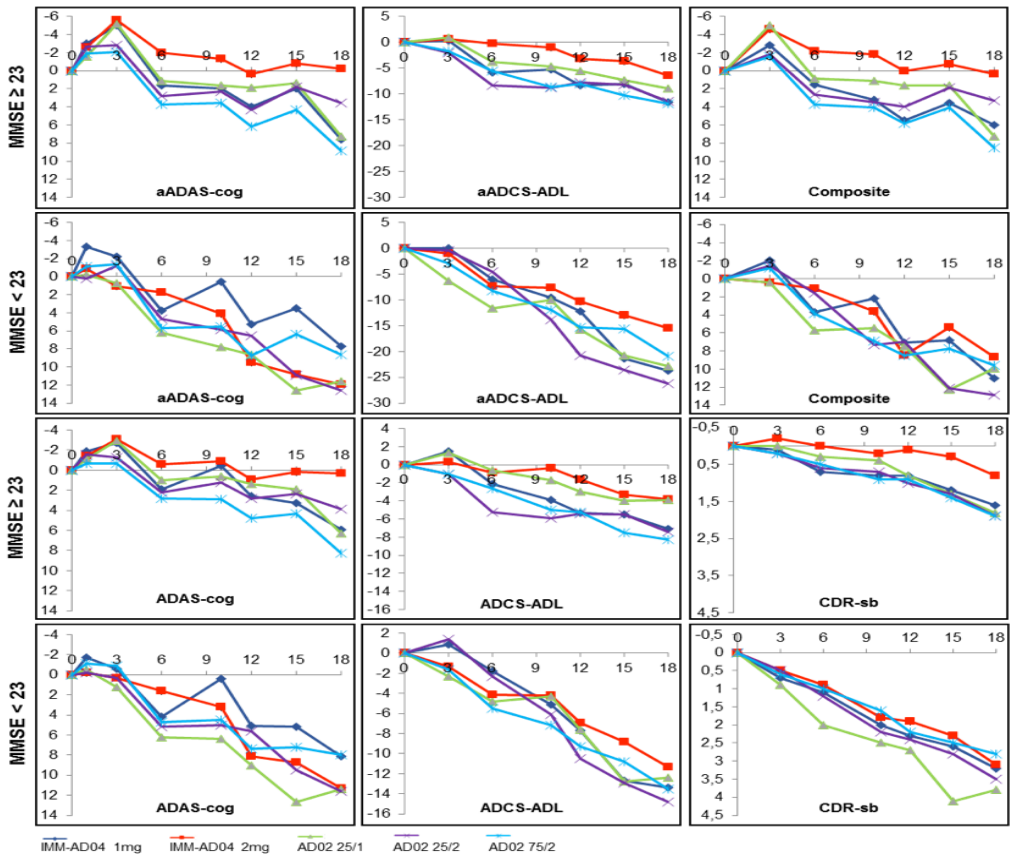
IMM-AD04 has beneficial effects on cognition and function

Unexpectedly, the IMM-AD04 2mg group showed a slowing of cognitive, functional and global decline as shown on primary and secondary outcomes including adapted and traditional scales (Figure 2). Exploratory outcomes (NPI, QOL patient and QOL caregiver) and MRI brain volumes also showed some significant effects in the IMM-AD04 2mg group compared to the other 4 groups. Although least squares means are shown in the figures, means show very similar patterns, so the covariates did not substantially affect the results of the study.

MRI brain volumes showed some statistically significant effects for right hippocampal volume with the IMM-AD04 2mg declining at a slower rate than other groups (Table 2). Total hippocampal volume showed similar, but less significant effects. No statistically significant differences were seen for other MRI brain volumes. Statistically significant correlations were seen between change in hippocampal volumes and change in aADAS-cog and the composite but not for change in aADL (data not shown).

Historical efficacy data from comparable populations in ADNI and two ADCS studies were assessed to understand how the observed clinical decline rates compare to historical placebo declines. The IMM-AD04 1mg declined at a similar rate to the historical control for the adapted ADCS-ADL with an estimated mean to standard deviation ratio in the IMM-AD04 1mg of approximately 0.72, compared to the 0.70 ratio that was based on the pooled historical control group that was used to power the study. This result supports a positive effect of IMM-AD04 2mg on the rate of disease progression.

Figure 3. Efficacy outcomes in mild and less mild patients



Patients with a score of MMSE ≥ 23 compared to those with an MMSE < 23 at Baseline. Outcomes are shown as change over time using the least squares means from mixed model. R: right. L: left. a: Adapted.

The lack of an effect in the AD02 groups that also included 2 mg Alum was consistent with an observed negative statistical interaction between AD02 and Alum. This negative synergy was seen for the aADAS-cog as well as other outcomes.

Vaccine induced IgG type antibodies to aggregated Aβ, AD02 and KLH are depicted in Table 3. Patients who had a sufficient increase (using pre-defined cut-off values) in these markers compared to baseline, were classified as antibody responders. We detected a robust IgG antibody response to KLH and AD02. The level of antibody response to aggregated Aβ was low.

Comparisons between antibody responders and non-responders showed a somewhat slower cognitive decline in the non-responder population for all three parameters (aggregated Aβ, AD02 and KLH IgG) but differences were not statistically significant.

A dose response relationship was found for Alum across all endpoints at 18 months, with the groups including 2mg Alum performing better than those including 1mg Alum. AD02 however was found to have a negative dose response relationship across most endpoints at 18 months with the best response in the 0 mg group, and the 25ug and 75ug groups performing similarly to one another (data not shown). The apparent effect of Alum 2mg is not observed when there is an aggregated Aβ IgG immune response to AD02, which occurs in the majority of patients when AD02 is given. The lack of an effect in the AD02 groups that also included 2 mg Alum was consistent with a negative statistical interaction between AD02 and Alum in the adapted ADAS-cog and other outcome measures.

Analysis of MMSE ≥ 23 and MMSE <23

The most significant effects of IMM-AD04 2mg were seen in the patients who were milder at baseline (MMSE ≥ 23) (Figure 3). This difference was seen across all endpoints. The IMM-AD04 2mg patients who were less mild at baseline (MMSE<23) showed less decline in function (aADCS-ADL), but minimal separation from the other four groups on the other outcomes.

AD02 and IMM-AD04 are safe and well tolerated

The majority of AEs reported in the trial were mild or moderate in severity (Table 4). Treatments were generally well tolerated as indicated by the low dropout rate of

Table 2: Primary and exploratory efficacy results

	AD02 25/2		AD02 25/1		AD02 75/2		IMM-AD04 1mg		
	Effect Size	**P-Value**	**Effect Size**	**P-Value**	**Effect Size**	**P-Value**	**Effect Size**	**P-Value**	
Primary efficacy measures									
aADAS cog	36%	0.2543	53%	0.0220	48%	0.0553	42%	0.1899	
aADL	44%	0.0414	36%	0.1512	39%	0.0885	43%	0.0850	
Composite	43%	0.2266	55%	0.0484	56%	0.0315	53%	0.0996	
ADAS-cog 11	32%	0.3431	51%	0.0351	48%	0.0603	39%	0.2396	
ADCS-ADL	36%	0.1108	12%	0.6863	37%	0.0803	32%	0.2320	
CDR-sb	32%	0.1168	37%	0.0459	19%	0.3436	23%	0.3315	
Exploratory efficacy measures									
NPI	119%	0.1718	114%	0.0708	115%	0.0969	111%	0.0514	
QOL Caregiver	100%	0.0053	100%	0.0040	100%	0.0485	100%	0.0085	
QOL Patient	67%	0.4694	73%	0.3711	63%	0.5241	50%	0.7253	
R.Hippocampal Volume	41%	0.0354	46%	0.0095	41%	0.0328	37%	0.1080	
L.Hippocampal Volume	27%	0.1316	24%	0.2036	14%	0.5015	15%	0.5232	
	AD02 25/2		**AD02 25/1**		**AD02 75/2**		**IMM-AD04 1mg**		**IMM-AD04 2mg**
	Effect Size	**P-Value**	**Effect Size**	**P-Value**	**Effect Size**	**P-Value**	**Effect Size**	**P-Value**	**Effect size**
% Improved on Composite and P-value vs. IMM-AD04 2mg									
Same or Better Over 18 Months	26%	0.0955	23%	0.0151	31%	0.0484	17%	0.0067	48%

Results are shown for month 18 in comparison to the IMM-AD04 2mg group. ADAS-cog: Alzheimer's disease Assessment Scale-cognitive; ADL: Activities of daily living; CDR-sb: Clinical Dementia Rating 'sum of boxes.' Neuropsychiatric Inventory; QOL: Quality of life. L: Left, R: Right, a: Adapted.

Table 3: Characterization of the vaccine-induced IgG type antibody response

	AD02 25/2	AD02 25/1	AD02 75/2	IMM-AD04 1mg	IMM-AD04 2mg
A. ELISA analysis					
AD02 (baseline)	39.9 (69.54)	24.7 (15.02)	26.4 (22.77)	25.9 (26.21)	24.6 (14.75)
AD02 (week 12)	1820.0 (3335.89)	837.8 (1896.66)	1890.3 (5361.39)	24.6 (25.13)	33.5 (74.97)
KLH (baseline)	297.9 (551.88)	270.3 (459.46)	295.2 (462.25)	561.7 (1558.79)	305.9 (648.04)
KLH (week 12)	29840.0 (32496.84)	14460.0 (23178.79)	27573.0 (30771.12)	506.7 (1507.21)	328.8 (656.71)
B. FACS analysis					
aggregated Aβ (baseline)	39.1 (19.83)	39.1 (19.77)	40.4 (23.10)	43.5 (32.11)	43.5 (31.37)
aggregated Aβ (week 12)	53.4 (78.58)	41.7 (19.27)	42.3 (20.26)	44.3 (31.55)	45.1 (34.52)
C. Responder Rate					
AD02	85.1%	68.8%	79.0%	2.1%	2.0%
KLH	93.2%	81.8%	92.6%	0.0%	0.0%
aggregated Aβ	45.9%	32.5%	30.9%	27.1%	23.5%

A. Reciprocal titers of AD02 and KLH were measured at baseline and after 3 immunizations by ELISA. Values are expressed as the mean titer (± SD). B. The Ab response to aggregated Aβ was measured at baseline and after 3 immunizations by a FACS-based assay. Mean fluorescence intensity values are given as the mean (± SD).C. Subjects who have a ratio at post-baseline that is greater than the defined cut-off were classified as an antibody responder for the particular immune response. Antibody responder cut-off values were defined as follows: aggregated Aβ ≥1.2x baseline, AD02 (end titer) ≥9x baseline, KLH (end titer) ≥9x baseline.

18.7% by the end of the study.

AEs were seen at similar rates across all treatment groups with slightly higher incidence rates of injection site reactions in the groups with 2 mg Alum. The most common local reactions were erythema, swelling, warmth, induration, pain, and pruritus.

The percentages of patients with an AE considered related to the study drug were similar for the AD02 25/2 (90.7%) and the IMM-AD04 2mg control group (86.3%). In comparison, percentages were lower for the AD02 75/2 (79.0%), the AD02 25/1 (71.4%) and the IMM-AD04 1mg (70.8%). AEs leading to discontinuation were reported by two patients in IMM-AD04 1mg (4.2%), no patients in IMM-AD04 2mg, three patients in AD02 25/2

Table 4: Adverse Events

	IMP			Control	
Assessment	**AD02 25/2 (n =75)**	**AD02 25/1 (n=77)**	**AD02 75/2 (n =81)**	**IMM-AD04 1mg (n =48)**	**IMM-AD04 2mg (n =51)**
Patients with 0 AEs, n (%)	4 (5.3)	9 (11.7)	10 (12.3)	7 (14.6)	4 (7.8)
Patients with at least 1 AE, n (%)	71 (94.7)	68 (88.3)	71 (87.7)	41 (85.4)	47 (92.2)
Total number of AEs	1017	742	949	336	649
Serious adverse events					
Patients with a serious AEs n (%)	14 (18.7)	10 (13.0)	16 (19.8)	5 (10.4)	7 (13.7)
Number of serious AEs	23	11	23	6	10
Patients with an SAE considered related, n (%)	2 (2.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Number of SAEs considered related	4	0	0	0	0
Patients who discontinued due to an AE*, n (%)	2** (4.2)	1 (1.3)	0 (0.0)	3 (4.0)	0 (0.0)
Adverse events (preferred terms or body systems of events occurring in at least 10% of patients)					
General disorders and administration site conditions	66 (88.0)	56 (67.5)	63 (77.8)	34 (70.8)	43 (84.3)
Injection site erythema	59 (78.7)	42 (54.5)	51 (63.0)	27 (56.3)	37 (72.5)
injection site swelling	48 (64.0)	36 (46.8)	44 (54.3)	13 (27.1)	26 (51.0)
Injection site warmth	40 (53.3)	31 (40.3)	43 (53.1)	18 (37.5)	26 (51.0)
Injection site induration	34 (45.3)	26 (33.8)	35 (43.2)	13 (27.1)	14 (27.5)
Injection site pruritus	32 (42.7)	22 (28.6)	30 (37.0)	4 (8.3)	10 (19.6)
Injection site pain	30 (40.0)	22 (28.6)	23 (28.4)	14 (29.2)	31 (60.8)
Injection site nodule	13 (17.3)	12 (15.6)	18 (22.2)	4 (8.3)	11 (21.6)
Infections and infestations	21 (28.0)	19 (24.7)	22 (27.2)	15 (31.3)	12 (23.5)
Nasopharyngitis	9 (12.0)	9 (11.7)	5 (6.2)	9 (18.8)	2 (3.9)
Nervous system disorders	17 (22.7)	24 (31.2)	21 (25.9)	13 (27.1)	10 (19.6)
Headache	5 (6.7)	10 (13.0)	8 (9.9)	3 (6.3)	3 (5.9)
Psychiatric disorders	9 (12.0)	19 (24.7)	17 (21.0)	8 (16.7)	8 (15.7)
Investigations	14 (18.7)	21 (27.3)	15 (18.5)	9(18.8)	12 (23.5)
Injury, poisoning and procedural complications	11 (14.7)	10 (13.0)	14 (17.3)	2 (4.2)	5 (9.8)
Gastrointestinal disorders	13 (17.3)	13 (16.9)	11 (13.6)	9 (18.8)	6 (11.8)
Diarrhea	3 (4.0)	8 (10.4)	3 (3.7)	5 (10.4)	0 (0.0)
Vascular disorders	9 (12.0)	8 (10.4)	5 (6.2)	1 (2.1)	7 (13.7)
Musculoskeletal and connective tissue disorders	11 (14.7)	10 (13.0)	10 (12.3)	6 (12.5)	7 (13.7)

AE: adverse event; SAE: serious adverse event; * Two additional patients, 11-003 (75ug/2mg) and 62-011 (25ug/1mg) had drug withdrawn due to AE and also discontinued the study, but are not included in these numbers; ** One of these patients, 32-003, had actually completed the study within 30 days prior to experiencing the AE that would have resulted in withdrawal

(4.0%), one in AD02 25/1 (1.3%), and by no patients in the AD02 75/2. Of these, only one patient in AD02 25/2 discontinued because of an AE that was considered related to the vaccine.

A total of 73 serious adverse events (SAEs) were reported during the trial; however, only four of these in AD02 25/2 were considered by the investigator to be related to treatment. A lower percentage of patients reported AEs in the control groups (range: 4.2% to 5.9%) compared with the active treatment groups (range: 7.8% to 9.9%). Four patient deaths were reported during the trial distributed among the treatment and control groups. Deaths were caused by respiratory failure, disease progression of a prior condition, and esophageal and bronchial carcinoma. The investigators assessed all four events to be unrelated to the study drug.

Safety lab results showed no confirmed signal for autoimmunity surrogates. There was no evidence of meningoencephalitis or vasogenic edema (ARIA-E). Microhemorrhages (ARIA-H) were in the expected range with no differences between treatment groups.

Laboratory analysis did not reveal any clinically relevant abnormalities in blood cell counts. There was also no evidence for hepatotoxicity or nephrotoxicity, and no impact on vital signs was observed.

Discussion

This phase II trial failed to show a treatment benefit of AD02, a vaccine targeting aggregated Aβ. In contrast to the results of the phase I trial, we were unable to show consistent results between antibody responders to aggregated Aβ and to non-responders across evaluated endpoints, possibly due to the low level immune response to aggregated Aβ. Unexpectedly, the IMM-AD04 2mg control group showed a slowing of decline on primary, secondary and exploratory scales relative to the other four groups. We additionally observed that the decline in hippocampal volume is slowed relative to the other four groups following treatment with IMM-AD04 2mg, which is supportive of a disease modifying effect.

The slowing of decline in the control group, IMM-AD04 2mg, raises the concern that in our study it is not clear which, if any, of our groups has a true placebo decline rate. Several potential scenarios could explain the slower decline in the IMM-AD04 2mg group and the relatively faster decline in the other four groups. The first scenario is that the result is due to the IMM-AD04 2mg group having no effect (i.e. reflecting a true placebo decline), and the other four groups having a similar size negative effect. A second scenario is that a true placebo group would have declined at an even faster rate than all other groups, potentially pointing to a treatment effect of similar magnitude in all other groups and an even larger effect for IMM-AD04 2mg. These two scenarios are improbable due to the requirement that the other four groups would need to have a similar negative or positive effect relative to a true placebo. Comparison of the results of this study to a historical control group based on the ADCS studies and ADNI revealed an even faster decline than the four other groups. The explanation that the population in this study was inherently slower declining than the historical groups, potentially due to a milder disease state, with no positive or negative treatment effect in all other groups, would be a more rational explanation. Even if we assume no treatment effect for the four other groups, the treatment effect observed in the IMM-AD04 2mg could be due to chance, or due to minor baseline imbalances in the randomized groups. For example, there were slight imbalances in baseline age, gender distribution and height of study participants. However, the p-values take into account this chance possibility, and the primary model was corrected for baseline covariates such as age and baseline scores that are known to affect decline rates.

The original intention to include Alum instead of inert saline in the control groups was to preserve blinding, as solutions with Alum have a different appearance and would be expected to induce more local reactions. Although blinding was preserved, the two IMM-AD04 doses showed a different rate of cognitive decline, the control groups for the study were no longer able to be pooled for comparison, resulting in weaker study power than originally planned. The two control groups have only 48 and 51 subjects, respectively, compared to 75, 77 and 81 subjects in the AD02 groups, resulting in mean scores that have more uncertainty than if the controls could have been pooled.

The apparent treatment effect of IMM-AD04 2mg comes unexpectedly, as a link has been suggested between increasing human exposure to aluminum and the rise in chronic diseases including cancers and neurodegenerative diseases such as AD (reviewed in (29)). However, scientists have been unable able to definitively show a connection between aluminum exposure and the development of chronic neurodegenerative diseases (reviewed in (30)). Additionally, the route of exposure (subcutaneous vs. ingested) will significantly influence aluminum´s toxicity in the body. It is important to note that the amount of Alum included in vaccines addressed in this trial would only be expected to increase the normal metabolic clearance rate of aluminum by about 2%.

The question arises, why should Alum have an effect when administered alone (as IMM-AD04) but not when present in formulations with AD02 at doses of 25μg and 75μg? IMM-AD04 and complexes of Alum/AD02 are inherently different entities. Aspects in which they differ include their fate upon injection and the type of immune reactions they induce. Aluminum has been found in the brains of mice at dose dependent levels upon s.c. injection of aluminum alone (18). It is conceivable that distribution upon injection is different if Alum is administered alone or as a complex with large proteins such as KLH. When administered alone, Alum primarily triggers a response of the innate immune system. In addition, it may modulate specific immune responses that are ongoing in the host organism. By contrast, AD02 adsorbed to Alum triggers a different immune response. The AD02 peptide protein component may blunt the stimulation of the innate immune response elicited by "naked" Alum. Moreover, the AD02 peptide protein conjugate elicits antibodies to vaccine components and the target as well as carrier-specific T cells, which may modulate the effect of Alum. These differences between the two entities may explain their distinct abilities to hinder AD progress. Comparing aggregated Aβ IgG responders and non-responders within the AD02 treated groups revealed that Aβ non-responders decline less. This suggests that the aggregated Aβ response (IgG) in the AD02 groups may eliminate or reduce the effect of the Alum. Either of these explanations, the blunting of the innate immune response by protein coating and the potential induction of antibodies with a negative effect, may contribute to the lack of effect seen when Alum is applied along with AD02. Either or both of these possibilities are consistent with the observed statistically significant negative interaction between Alum and AD02 for the composite outcome.

Initial studies suggest adjuvants have effects beyond

their classic role as amplifiers of specific immune responses. Adjuvant-activated phagocytes have been suggested to be able to clear toxic molecules, including Aβ (31). This was further supported by the demonstration that Monophosphoryl Lipid A (MPL) reduces Aβ plaques in the APP/PS1 transgenic mouse model (32). Interestingly, a Phase III clinical study involving 450 chronic hepatitis B patients showed a higher seroconversion rate (defined in this study as the loss of hepatitis B surface antigen and presence of anti-hepatitis antibody at the end of the treatment) in the aluminum only group compared to the antigen/aluminum-containing vaccine group (33). The authors speculate that this may be related to the response of the pro-inflammatory cytokine IL-17 that was observed in the aluminum-only group. Innate immune mechanisms, triggered by misfolded/aggregated proteins through pattern recognition receptors, have recently emerged in the literature as a crucial component in the pathophysiology of neurodegenerative diseases including AD (5, 6). Neuroinflammation and lifestyle conditions associated with elevated inflammatory mediators in the periphery (e.g., history of systemic infection, obesity, reduced physical activity) are risk factors for AD (6, 34, 35). Moreover, inflammatory pathways resulting in increased levels of complement components, eicosanoids, chemokines and cytokines, are activated in AD as shown by different approaches in several studies (6, 36-38). It is therefore tempting to speculate that IMM-AD04 acts by beneficially modulating inflammatory processes associated with AD, either centrally upon arrival in the brain and interaction with microglia and/or peripherally through stimulation of innate immune cells at the injection site.

The failure to show a treatment benefit of AD02 in this phase II study precludes further development of AD02, although it is acknowledged that the desired immune response was not achieved. The unexpected positive signal for 2mg of IMM-AD04 supports further study to both identify a potential mechanism of action and to confirm the clinical and biomarker signal that was observed.

Acknowledgments: We would like to thank all the investigators of AFF006 for their significant contribution to the study: Austria: Markus Müller, Peter Dal-Bianco, Siegfried Kasper (Medizinische Universität Wien, Vienna); Michael Rainer (Memory Clinic, Vienna); Wolfgang Staffen (Universitätsklinik für Neurologie, Salzburg); Douglas Imarhiagbe (Landeskrankenhaus Hall Gedächtnisambulanz, Hall in Tirol); Christine Hertl (Landesnervenklinik Wagner-Jauregg, Linz). Croatia: Ninoslav Mimica (University Psychiatric Hospital Vrapče, Zagreb); Branko Malojčić, Vladimir Sabljić (Klinčki Bolnički Centar Rijeka, Rijeka); Marinko Bilušić (Poliklinika za kliničku farmakologiju i toksikologiju, Zagreb); Spomenka Kidemet-Piskac (General Hospital Varaždin, Varaždin). Czech Republic: Jakub Hort (Memory Clinic, Prague; International Clinical Research Center, Brno); Robert Rusina (Thomayer University Hospital, Prague). France: Bruno Dubois (Cognitive and Behavioral Disease Center and Alzheimer's Institute, Paris); Olivier Rouaud (Service de Neurologie et Laboratoire d´Explorations du Système Nerveux, Dijon); Pierre-Jean Ousset (Centre de Recherche Gérontopole, Toulouse); Bernard Croisile (Laboratoire de Neuropsychologie, Bron); Sophie Auriacombe (CMRR Hôpital Pellegrin, Bordeaux); Serge Belliard (CMRR de Haute Bretagne, Rennes); Karim Bennys (Centre Mémoire de Ressources et de Recherche, Montpellier). Germany: Lutz Frölich (Zentralinstitut für Seelische Gesundheit Abteilung Gerontopsychiatrie, Mannheim); Günter Niklewski (Klinik für Psychiatrie und Psychotherapie, Nürnberg); Klaus-Christian Steinwachs (Studienzentrum PD Dr. Steinwachs, Nürnberg); Alexander Kurz (Klinik und Poliklinik für Psychiatrie und Psychotherapie der Technischen Universität München, Munich); Oliver Peters (Charite Universitätsmedizin Berlin, Berlin); Hermann-Josef Gertz (Arzneimittelforschung Leipzig GmbH, Studienzentrum, Leipzig); Herbert Schreiber (NeuroPoint GmbH, Ulm/Donau); Holger Jahn (Universitätsklinikum Hamburg-Eppendorf, Klinik für Psychiatrie und Psychotherapie, Hamburg). Slovak Republic: Monika Biackova (Psychiatric Hospital Michalovce, Michalovce); Eva Palova (EPAMED, s.r.o., Kosice). We would also like to thank the DSMB board for their feedback on the study.

Competing interests: AS, MM, VB, LT, FM, WS are employees of AFFiRiS, the company that commercializes the AFFITOPE® technology described in the manuscript. SH and NE are consultants for AFFiRiS through Pentara Corporation. MB, LF, NM, JH, MR, DI, AK, OP, HG, BD declare no conflict of interest.

Data and materials availability: This trial is registered at www.clinicaltrials.gov. Identifier: NCT01117818.

Ethical standards: This trial was performed in compliance with Good Clinical Practice (GCP), the Decleration of Helsinki (2013), and local legal and regulators requirements and applicable international regulations.

References

1. World Alzheimer Report 2014. Dementia and Risk Reduction. http://www.alz.co.uk/research/world-report-2014. Accessed on 10 February 2015.
2. Gong CX, Iqbal K. Hyperphosphorylation of microtubule-associated protein tau: a promising therapeutic target for Alzheimer disease. Curr Med Chem 2008; 15: 2321-2328.
3. Mandler M, Walker L, Santic R, Hanson P, Upadhaya AR, et al. Pyroglutamylated amyloid-beta is associated with hyperphosphorylated tau and severity of Alzheimer's disease. Acta Neuropathol 2014; 128: 67-79.
4. Davies L, Wolska B, Hilbich C, Multhaup G, Martins R, et al. A4 amyloid protein deposition and the diagnosis of Alzheimer's disease: prevalence in aged brains determined by immunocytochemistry compared with conventional neuropathologic techniques. Neurology 1988; 38: 1688-1693.
5. Heneka MT, O'Banion MK, Terwel D, Kummer MP. Neuroinflammatory processes in Alzheimer's disease. J Neural Transm 2010; 117: 919-947.
6. Heneka MT, Kummer MP, Latz E. Innate immune activation in neurodegenerative disease. Nat Rev Immunol 2014; 14: 463-477.
7. Masters CL, Simms G, Weinman NA, Multhaup G, McDonald BL, et al. Amyloid plaque core protein in Alzheimer disease and Down syndrome. Proc Natl Acad Sci 1985; 82: 4245-4249.
8. Cummings JL, Morstorf T, Zhong K. Alzheimer's disease drug-development pipeline: few candidates, frequent failures. Alzheimers Res Ther 2014; 6: 37.
9. Janus C, Pearson J, McLaurin J, Mathews PM, Jiang Y, et al. A beta peptide immunization reduces behavioural impairment and plaques in a model of Alzheimer's disease. Nature 2000; 408: 979-982.
10. Morgan D, Diamond DM, Gottschall PE, Ugen KE, Dickey C, et al. A beta peptide vaccination prevents memory loss in an animal model of Alzheimer's disease. Nature 2000; 408: 982-985.
11. Schenk D, Barbour R, Dunn W, Gordon G, Grajeda H, et al. Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. Nature 1999; 400: 173-177.
12. Wilcock DM The usefulness and challenges of transgenic mouse models in the study of Alzheimer's disease. CNS Neurol Disord Drug Targets 2010; 9: 386-394.
13. Mandler M, Santic R, Gruber P, Cinar Y, Pichler D, et al. Tailoring the antibody response to aggregated aβ using novel Alzheimer-vaccines. PLoS One 10: e0115237.
14. Schneeberger A, Mandler M, Mattner F, Schmidt W. AFFITOME® technology in neurodegenerative diseases: the doubling advantage. Hum Vaccin 2010; 6: 948-952.
15. De Gregorio E, Tritto E, Rappuoli R. Alum adjuvanticity: unraveling a century old mystery. Eur J Immunol 2008; 38: 2068-2071.
16. Mannhalter JW, Neychev HO, Zlabinger GJ, Ahmad R, Eibl MM. Modulation of the human immune response by the non-toxic and non-pyrogenic adjuvant aluminium hydroxide: effect on antigen uptake and antigen presentation. Clin Exp Immunol 1985; 61: 143-151.
17. Marrack P, McKee AS, Munks MW. Towards an understanding of the adjuvant action of aluminium. Nat Rev Immunol 2009; 9: 287-293.
18. Khan Z, Combadiere C, Authier FJ, Itier V, Lux F, et al. Slow CCL2-dependent translocation of biopersistent particles from muscle to brain. BMC Med 2013; 11: 99.
19. Sarazin M, Berr C, De Rotrou J, Fabrigoule C, Pasquier F, et al. Amnestic syndrome of the medial temporal type identifies prodromal AD: a longitudinal study. Neurology 2007; 69: 1859-1867.
20. Sarazin M, Chauvire V, Gerardin E, Colliot O, Kinkingnehun S, et al. The amnestic syndrome of hippocampal type in Alzheimer's disease: an MRI study. J Alzheimers Dis 2010; 22: 285-294.

21. Wagner M, Wolf S, Reischies FM, Daerr M, Wolfsgruber S, et al. Biomarker validation of a cued recall memory deficit in prodromal Alzheimer disease. Neurology 2012; 78: 379-386.
22. Rosen WG, Terry RD, Fuld PA, Katzman R, Peck A. Pathological verification of ischemic score in differentiation of dementias. Ann Neurol 1980; 7: 486-488.
23. Hendrix S, Ellison N, Stanworth S, Tierney L, Mattner F, Schmidt W, Dubois B, Schneeberger A. Methodological Aspects of the Phase II Study AFF006 Evaluating Amyloid-beta -Targeting Vaccine AFFITOPE® AD02 in Early Alzheimer's Disease – Prospective Use of Novel Composite Scales. J Prev Alz Dis inpress.
24. Morris JC. The Clinical Dementia Rating (CDR): current version and scoring rules. Neurology 1993; 43: 2412-2414.
25. Cummings JL, Mega M, Gray K, Rosenberg-Thompson S, Carusi DA, et al. The Neuropsychiatric Inventory: comprehensive assessment of psychopathology in dementia. Neurology 1994; 44: 2308-2314.
26. Rosen WG, Mohs RC, Davis KL. A new rating scale for Alzheimer's disease. Am J Psychiatry 1984; 141: 1356-1364.
27. Mohs RC, Knopman D, Petersen RC, Ferris SH, Ernesto C, et al. Development of cognitive instruments for use in clinical trials of antidementia drugs: additions to the Alzheimer's Disease Assessment Scale that broaden its scope. The Alzheimer's Disease Cooperative Study. Alzheimer Dis Assoc Disord 1997; 11 Suppl 2: S13-21.
28. Galasko D, Bennett D, Sano M, Ernesto C, Thomas R, et al. An inventory to assess activities of daily living for clinical trials in Alzheimer's disease. The Alzheimer's Disease Cooperative Study. Alzheimer Dis Assoc Disord 1997; 11 Suppl 2: S33-39.
29. Shaw CA, Seneff S, Kette SD, Tomljenovic L, Oller JW, Jr., et al. Aluminum-induced entropy in biological systems: implications for neurological disease. J Toxicol 2014; 491316.
30. Willhite CC, Karyakina NA, Yokel RA, Yenugadhati N, Wisniewski TM, et al. Systematic review of potential health risks posed by pharmaceutical, occupational and consumer exposures to metallic and nanoscale aluminum, aluminum oxides, aluminum hydroxide and its soluble salts. Crit Rev Toxicol 2014; 44 Suppl 4: 1-80.
31. Frenkel D, Maron R, Burt DS, Weiner HL. Nasal vaccination with a proteosome-based adjuvant and glatiramer acetate clears beta-amyloid in a mouse model of Alzheimer disease. J Clin Invest 2005; 115: 2423-2433.
32. Michaud JP, Halle M, Lampron A, Theriault P, Prefontaine P, et al. Toll-like receptor 4 stimulation with the detoxified ligand monophosphoryl lipid A improves Alzheimer's disease-related pathology. Proc Natl Acad Sci 2013; 110: 1941-1946.
33. Xu DZ, Wang XY, Shen XL, Gong GZ, Ren H, et al. Results of a phase III clinical trial with an HBsAg-HBIG immunogenic complex therapeutic vaccine for chronic hepatitis B patients: experiences and findings. J Hepatol 2013; 59: 450-456.
34. Holmes C, Cunningham C, Zotova E, Woolford J, Dean C, et al. Systemic inflammation and disease progression in Alzheimer disease. Neurology 2009; 73: 768-774.
35. Scarmeas N, Luchsinger JA, Schupf N, Brickman AM, Cosentino S, et al. Physical activity, diet, and risk of Alzheimer disease. JAMA 2009; 302: 627-637.
36. Cagnin A, Brooks DJ, Kennedy AM, Gunn RN, Myers R, et al. In-vivo measurement of activated microglia in dementia. Lancet 2001; 358: 461-467.
37. Lambert JC, Heath S, Even G, Campion D, Sleegers K, et al. Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. Nat Genet 2009; 41: 1094-1099.
38. Zhang B, Gaiteri C, Bodea LG, Wang Z, McElwee J, et al. Integrated systems approach identifies genetic nodes and networks in late-onset Alzheimer's disease. Cell 2013; 153: 707-720.