Long-term effects of $A\beta_{42}$ immunisation in Alzheimer's disease: follow-up of a randomised, placebo-controlled phase I trial

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Lancet 2008; 372: 216–23

See Comment page 180

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Summary

Background Immunisation of patients with Alzheimer's disease with full-length amyloid-β peptide ($A\beta_{42}$) can clear amyloid plaques from the brain. Our aim was to assess the relation between $A\beta_{42}$ immune response, degree of plaque removal, and long-term clinical outcomes.

Methods In June, 2003, consent for long-term clinical follow-up, post-mortem neuropathological examination, or both, was sought from 80 patients (or their carers) who had entered a phase I randomised, placebo-controlled trial of immunisation with $A\beta_{42}$ (AN1792, Elan Pharmaceuticals) in September, 2000. The follow-up study was completed in September, 2006. Plaques were assessed in terms of the percentage area of the cortex with Aβ immunostaining (Aβ load) and in terms of characteristic histological features reflecting plaque removal. Survival of all 80 individuals until severe dementia or death was assessed with a Cox proportional hazard model.

Findings 20 participants—15 in the AN1792 group, five in the placebo group—died before follow-up started. A further 22 patients—19 in the AN1792 group, three in the placebo group—died during follow-up. Nine of the deceased patients, all in the AN1792 group, had given consent for post-mortem analysis; one of these who did not die with Alzheimer's disease was excluded. In the remaining eight participants who received immunisation and who were examined neuropathologically, mean Aβ load was lower than in an unimmunised control group that was matched for age at death (2·1% [SE 0·7] in treated participants *vs* 5·1% [0·9] in controls; mean difference 3·0%, 95% CI 0·6–5·4; p=0·02). Although there was considerable variation in Aβ load and degree of plaque removal among immunised participants, the degree of plaque removal varied significantly with mean antibody response attained during the treatment study period (Kruskal-Wallis p=0·02). Seven of the eight immunised patients who underwent post-mortem assessment, including those with virtually complete plaque removal, had severe end stage dementia before death. In the whole cohort, there was no evidence of improved survival (hazard ratio 0·93, 95% CI 0·43–3·11; p=0·86) or of an improvement in the time to severe dementia (1·18, 0·45–3·11; p=0·73) in the AN1792 group versus the placebo group.

Interpretation Although immunisation with $A\beta_{42}$ resulted in clearance of amyloid plaques in patients with Alzheimer's disease, this clearance did not prevent progressive neurodegeneration.

Funding Alzheimer's Research Trust, Medical Research Council.

Introduction

A major feature of Alzheimer's disease is the accumulation in the brain of an amyloid-β peptide (Aβ), which aggregates to form oligomers, plaques, and cerebrovascular deposits.[1] The putative key role of Aβ in the pathogenesis of Alzheimer's disease led to immunotherapeutic strategies[2–4] that aimed to reduce levels of Aβ in the brain. Active immunisation of mice genetically modified to develop Aβ plaques as they age with full-length Aβ ($A\beta_{42}$) resulted in a reduction of plaque burden and improved cognitive function.[2,5]

A phase I clinical trial of immunisation of patients with Alzheimer's disease with $A\beta_{42}$ (AN1792; Elan Pharmaceuticals, Dublin, Ireland) showed that there is a highly variable, largely dose-independent, antibody response to AN1792 in addition to variable clearance of amyloid plaques.[3,6–8] However, the relation between serum AN1792 antibody concentrations and the degree of plaque clearance is unknown. Although not designed to test efficacy, short-term clinical outcomes were disappointing, with three of the four exploratory measures of clinical efficacy showing no significant differences between the treatment and placebo group during the initial study period.[3]

A subsequent phase IIa study, halted when 6% of the patients developed meningoencephalitis,[9] showed no major differences in cognitive performance when antibody responders were compared with the placebo group at 1 year, despite evidence of high serum antibodies to $A\beta_{42}$ in a subset of those who received active treatment.[10] Whether active $A\beta_{42}$ immunisation results in longer-term cognitive or survival benefits is unknown. Additionally, although studies[9,11–14] have raised concerns about the long-term safety of these approaches,

no long-term data on the survival rates or the cause of death in study populations have been published. Our aim was to examine the relations between AN1792 drug dose, $A\beta_{42}$ antibody response, clinical outcomes after 6 years, and neuropathological evidence of Aβ plaque removal.

Methods

Patients

Patients who met National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria[15] for probable Alzheimer's disease with mild to moderate dementia (14–26 points on the mini-mental state examination [MMSE][16]) were eligible to enter this randomised, placebo-controlled, double-blind trial of active immunisation with an $A\beta_{42}$ synthetic peptide (AN1792).[3] 80 patients were enrolled, of whom 64 were randomly assigned to receive either 50 μg or 225 μg AN1792 with an adjuvant (QS-21); 16 individuals were randomly assigned to receive adjuvant alone. In a subsequent protocol extension phase, during which further injections were given of a modified formulation containing 0·4% polysorbate 80 with the aim of increasing solubility of the Aβ peptide,[3] 51 patients from the active treatment group received AN1792 in the modified formulation (25 patients received 50 μg, 26 received 225 μg) and 13 patients from the control group received adjuvant alone in the modified formulation. The total study treatment period was 84 weeks. Study enrolment, based at four sites in the UK (Southampton, Bath, Swindon, and Cardiff), began in April, 2000, and was completed by September, 2000, with the last study entrant finishing the study in June, 2002.

After obtaining ethical approval in June, 2003, all 80 patients (or their carers) enrolled in the original study were identified by the original study centre investigators and contacted in person. When study participants were reported as having died, confirmation of the date and cause of death was determined by access to public records from the UK General Register Office (Southport, UK). Consent for post-mortem neuropathological examination was sought from the carers of these patients. Surviving patients, or their carers, were approached for consent for further clinical follow-up, post-mortem neuropathological examination, or both.

Procedures

Patients who consented to participation in the follow-up phase were assessed at yearly intervals, whenever possible coincident with the month of their initial baseline assessment in the original treatment study, until the end of the follow-up study or until death. Clinical assessments were done by the same rater as in the original study and used the same clinical scales (the Alzheimer's disease assessment scale cognitive subscale [ADAS-Cog];[17] MMSE, and the disability assessment for dementia [DAD][18]). These cognitive and functional assessments were used as a basis for rating the severity of dementia in accordance with the International Classification of Diseases version 10 diagnostic criteria.[19] All participants defined as having severe dementia had MMSE scores of 10 points or less.

Because no patients in the placebo group had a post mortem during the follow-up study, we needed an alternative unimmunised control group of individuals with Alzheimer's disease for histological comparisons of Aβ load. Unimmunised control cases, closely matched for age at death with the immunised cases, were obtained from the neuropathology archives of Southampton General Hospital. All control cases had a history of progressive dementia and satisfied consensus criteria for Alzheimer's disease.[20] The formalin-fixed brains were examined macroscopically and samples taken for histology from frontal, temporal, parietal, and occipital lobes, striatum, thalamus, brainstem, and cerebellum. The brain tissue was fixed, processed for histology, and stained in the same laboratory. Routine neuropathological assessment was done on sections stained with haematoxylin and eosin, modified Bielschowsky silver impregnation, and immunostaining done for Aβ and tau.

Two different assessments were made of Aβ in the cerebral cortex. Percentage Aβ load—ie, the percentage area of the cortex with Aβ immunostaining (clone 6F/3D, an antibody that recognises aminoacid residues 8–17 of the Aβ peptide; Novocastra, Newcastle, UK)—was measured on consecutive 1·25× objective fields of frontal, parietal, and temporal neocortex, by use of the KS400 3.0 image analysis system (Carl Zeiss). This measurement of Aβ load has the advantage of being quantitative, but is known to have considerable variability in Alzheimer's disease[6] and we have no knowledge of the starting values before immunisation for each patient.

We also looked for specific histological evidence that plaques had been removed. Positive evidence of plaques having been removed was defined by the presence of a constellation of histological features as described previously,[7] including residual plaque cores in plaque-free areas, moth-eaten appearance of remaining plaques, phagocytosed Aβ within microglia, marked cerebral amyloid angiopathy, association of Aβ with capillaries in plaque-free areas, and resolution of tau-containing dystrophic neurites. Such evidence of plaque removal was assessed throughout all brain regions sampled for histology and was scored semi-quantitatively as minimal (ie, none or early process of removal), intermediate (ie, moderate or patchy), or very extensive (ie, virtually complete removal of plaques). This assessment was done in all cases, blind to cognitive function and antibody titres, by the

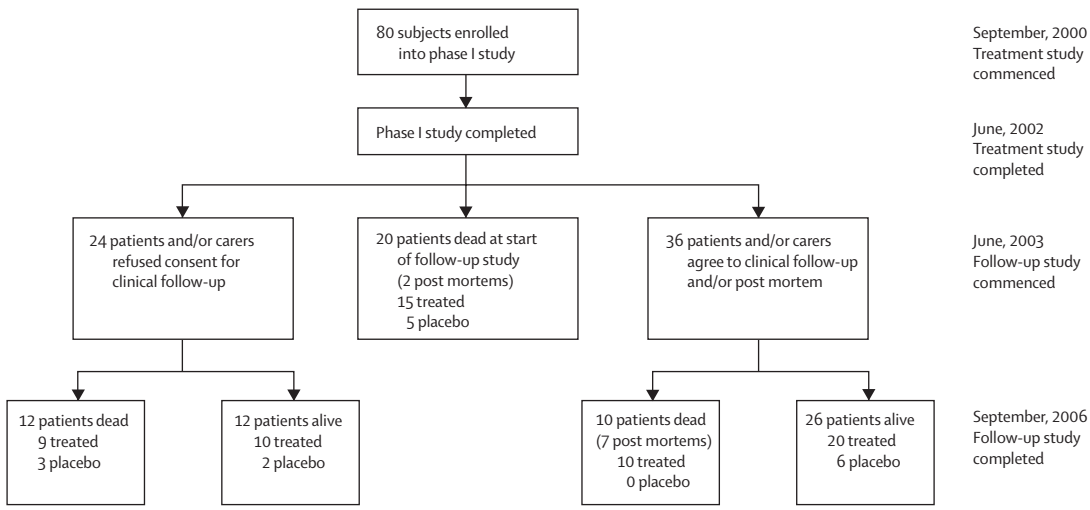

Figure 1: **Trial profile**

same neuropathologist (JARN). Although this assessment is semi-quantitative, it relies on the presence of histological features that are specific features of the response to Aβ immunisation in Alzheimer's disease.[7]

Mean anti-AN1792 antibody titres were derived from the original study data, supplied by Elan Pharmaceuticals, and were defined as the total serum anti-AN1792 antibody titre in ELISA units[3] over the treatment period for each participant divided by the number of assays done for that individual.

Additionally, blood samples were obtained from 15 consenting AN1792-treated survivors at 5 or 6 years after their baseline assessment and from an age and cognitively (MMSE score) matched non-immunised Alzheimer's disease (NINCDS-ADRDA probable criteria) control group (n=15), since we had too few samples from individuals who received placebo in the original trial. Total serum anti-Aβ antibody titres were determined with an ELISA assay developed in our laboratory. Values described are for combined bound and free levels of anti-$Aβ_{42}$ antibody. The concentration of anti-$Aβ_{42}$ antibody (in μg/mL) was calculated with a four-parameter curve-fit equation for the anti-$Aβ_{42}$ antibody (clone 21F12, Elan Pharmaceuticals) standard curve as described previously.[21]

Statistical analysis

Time from the first immunisation dose (with AN1792 or placebo) to death, loss to clinical follow-up, or to the data censoring date—Sept 1, 2006, 6 years after the last patient had been entered into the trial—was analysed with a multivariable Cox proportional-hazards model. Randomisation was determined on the date of immunisation. Age and baseline cognitive state (ADAS-Cog score) at the start of the treatment study were entered as predictive variables. Differential survival was examined with dose of vaccine (placebo; 50 μg or 225 μg AN1792) and, in a separate analysis, mean anti-AN1792

	Baseline MMSE (points)	AN1792 dose (μg)	Mean antibody response (ELISA units)	Evidence of Aβ plaque removal*	Aβ load	Braak tau stage†	Survival time (months)	MMSE before death (points)
1‡	16	50	<1:100	None	2·76%	V	4	16
2	15	225	<1:100	None	2·52%	VI	41	0
3	21	50	1:119	Intermediate	0·75%	VI	20	0
4	16	225	1:4072	Intermediate	6·65%	VI	44	0
5	25	50	1:1707	Intermediate	2·19%	VI	57	0
6	21	225	1:491	Intermediate	1·18%	VI	63	0
7	23	50	1:4374	Very extensive	0·12%	VI	60	0
8	20	50	1:6470	Very extensive	0·35%	VI	64	0

MMSE=mini-mental state examination. *None=none or early process of plaque removal; intermediate=moderate/patchy removal of plaques; very extensive=virtually complete removal of plaques. †Of the unimmunised controls, seven were Braak stage VI, one was stage V. ‡Patient died suddenly after a ruptured abdominal aortic aneurysm.

Table 1: **Aβ plaque removal and clinical characteristics of participants who had received AN1792 and who had post-mortem neuropathology**

antibody titres in ELISA units found over the treatment study period (0–84 weeks) (placebo; <1:100; 1:100 to 1:4000, and >1:4000 ELISA units). This second analysis was based on the neuropathological findings that an antibody titre of less than 1:100 ELISA units was associated with no completed plaque removal, an antibody titre over 1:100 ELISA units was associated with the presence of areas of completed plaque removal, and an antibody titre over 1:4000 ELISA units was associated with very extensive plaque removal. This analysis was deemed to be exploratory in view of the small numbers involved. Similar analyses were done examining the time from the start of immunisation (with AN1792 or placebo) to the development of severe dementia or to the point of data censoring.

Assessment of normality of the variables Aβ load, anti-AN1792 antibody titre, change in ADAS-Cog, DAD, MMSE, and age at death were determined by quantile–quantile plots of the residuals. SPSS software (version 14) was used for all analyses.

Role of the funding source

Neither the funders of the follow-up study, nor the original phase I clinical trial, had a role in the design or conduct of the study, or in the collection, analysis, or interpretation of the data. All authors had full access to all the data; the corresponding author had final responsibility for the decision to submit for publication.

Results

The trial profile is shown in figure 1. The mean age at baseline of the 20 participants who had died before the start of follow-up was 73·8 (SD 5·3) years; their mean ADAS-Cog at baseline was 28·8 (11·6) points. The mean age at baseline of the 24 participants who refused consent to long-term clinical follow-up was 73·5 (6·9) years and their mean ADAS-Cog score at baseline was 22·3 (11·0) points. The mean age at baseline of the 36 participants who consented to long-term clinical follow-up was 73·1 (8·6) years; their mean ADAS-Cog score at baseline was 22·2 (9·6) points. By the data censoring date, a further 22 participants had died; 38 individuals were confirmed as survivors (figure 1). There was no loss to mortality or clinical follow-up.

By the data censoring date, nine of the participants who had consented to post-mortem examination had died and all had been examined neuropathologically. All nine had received AN1792 in the phase I study. In one participant, who had received 50 μg AN1792, the neuropathological assessment indicated a diagnosis of progressive supranuclear palsy, on the basis of neuronal tangles mainly in the brainstem and basal ganglia. Neuronal tangles were sparse in the cerebral neocortex and absent from the hippocampus. The neuropathological diagnosis was supported on review of the clinical records. This participant was excluded from further neuropathological analysis. The remaining eight cases satisfied the criteria for Braak stage V/VI consistent with a diagnosis of Alzheimer's disease (table 1).

There was no difference in age at death between the eight immunised participants who were examined neuropathologically and the unimmunised histological controls (75·3 [SE 3·1] years *vs* 79·1 [2·4] years; mean difference 3·8 years, 95% CI –4·5 to 12·2; *t* test p=0·3).

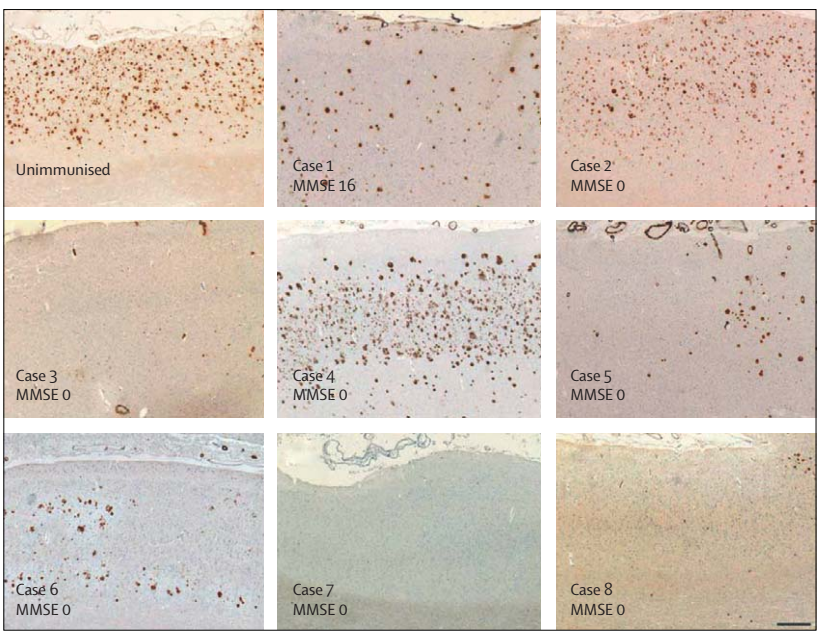

Figure 2: **Histological patterns of Aβ in the temporal lobe neocortex after immunisation with AN1792**
An unimmunised control (top left) has a high density of plaques. Cases 1–8 are all patients who were immunised with $Aβ_{42}$. Case 1 died 4 months after the first immunisation dose and showed an early stage of Aβ removal. Cases 2–8 survived 20–64 months after first immunisation dose. Case 2 did not develop anti-Aβ antibodies and showed no evidence of plaque clearance. Cases 3–6 showed an intermediate range of plaque clearance. Cases 7 and 8 showed very extensive (case 8) to nearly complete (case 7) removal of Aβ plaques throughout the cerebral cortex. All the long-term survivors (ie, cases 2–8) continued to have progressive dementia with cognitive function declining to an unrecordable level (ie, MMSE=0) before death. Scale bar=0·5 mm.

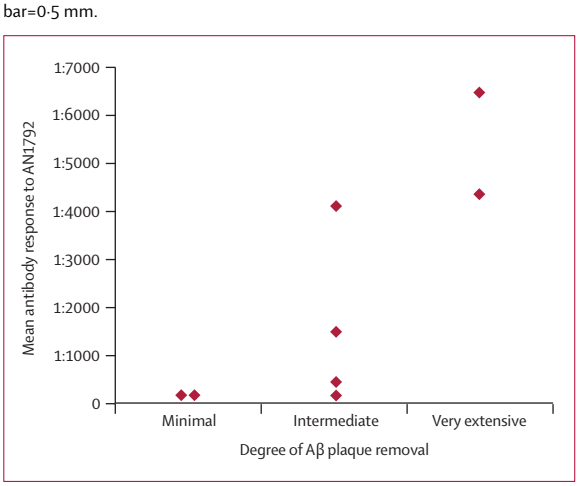

Figure 3: **Mean antibody response to AN1792 and Aβ plaque removal**

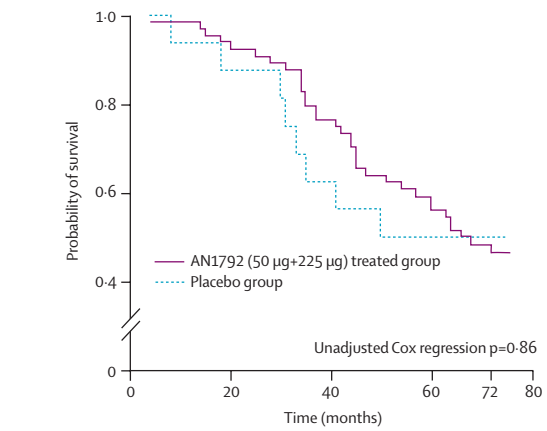

Figure 4: **Kaplan-Meier estimates of survival time to death by treatment group**

	Survival time hazard ratio (95% CI)*	p value	Time to severe dementia hazard ratio (95% CI)*	p value
AN1792 treatment dose group (n=16 in placebo group)				
50µg (n=32)	0·84 (0·36–1·95)	0·7	1·43 (0·49–4·20)	0·5
225µg (n=32)	0·78 (0·33–1·85)	0·6	0·96 (0·35–3·10)	0·9
Mean antibody response† (n=16 in placebo group)				
<1:100 (n=25)	0·80 (0·33–1·96)	0·6	1·03 (0·32–3·31)	1·0
1:100 to ≤1:4000 (n=33)	0·72 (0·30–1·70)	0·5	1·15 (0·40–3·30)	0·8
>1:4000 (n=6)	1·50 (0·45–5·04)	0·5	3·07 (0·7–13·45)	0·1

*Cox regression analysis adjusted for baseline cognitive state (ADAS-Cog) and age. †Mean antibody response in ELISA units.

Table 2: **Long-term survival and cognitive outcomes by treatment dose and mean antibody titre compared with placebo group**

In the eight immunised participants, mean Aβ load was lower than in the unimmunised controls (2·1% [SE 0·7] in the treated participants *vs* 5·1% [0·9] in the controls; mean difference 3·0%, 95% CI 0·6–5·4; *t* test p=0·02). However, there was considerable variation both in the Aβ load and in the degree of plaque removal among the immunised participants (table 1 and figure 2). There was no evidence of a relation between AN1792 dose and Aβ load or plaque removal (AN1792 dose comparison with Aβ load Mann-Whitney *U* p=0·3; AN1792 dose comparison with Aβ plaque removal χ^2 0·7, df 2, p=0·6; table 1). However, the degree of plaque removal varied significantly with mean antibody response attained during the treatment study period—ie, up to 84 weeks after the first dose (Kruskal-Wallis p=0·02, one-tailed; figure 3). Two participants with the least evidence of plaque removal had a mean antibody response below the detectable range of the assay (<1:100; table 1). However, one of these individuals, despite the absence of a documented IgG antibody response during the study period, and a fairly high Aβ load, had clear evidence that acute mobilisation of plaque Aβ had started by the time he died.[7] Overall, the mean antibody response during the treatment period showed a non-significant inverse correlation with post-mortem Aβ load (Spearman rank –0·52, p=0·09, one tailed; table 1).

All eight immunised participants had mild to moderate dementia at the start of the study (table 1). One of them, with a mean antibody response below the detectable range of the assay (<1:100), died suddenly after rupture of an abdominal aortic aneurysm 4 months after the study had begun, with a last recorded MMSE score of 16. All of the other seven recipients, including the two with post-mortem evidence of almost complete Aβ plaque removal, had severe end stage dementia in the absence of any pre-terminal acute confusional state (MMSE score 0) at their last examination before death (table 1 and figure 2).

Of the 42 (53%) participants who had died by the data censoring date, the most common causes of death cited from all listed causes, in addition to dementia, were bronchopneumonia (13 patients, 31%), cerebrovascular accident (five, 12%), and myocardial infarction (two, 5%). Other causes of death included a ruptured aortic aneurysm, pulmonary embolism, carcinoma of the breast, carcinoma of the bronchus, and carcinoma of the pancreas (one death each). No patients died from meningoencephalitis. Only one patient had clinical features of meningoencephalitis similar to those described in the phase II trial.[4]

By the data censoring date, 34 (53%) of the 64 individuals in the treated group and eight (50%) of the 16 individuals in the placebo group had died (χ^2 0·1, p=0·8). Median survival time was 66 (IQR 41–91) months for the AN1792 treated group and 50 (31–69) months for

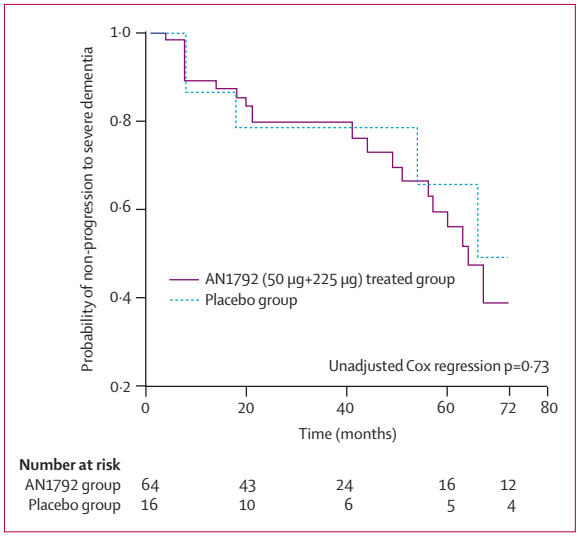

Figure 5: **Kaplan-Meier estimates of time to severe dementia by treatment group**

the placebo group. There was no evidence of differences in survival time between the treated and placebo groups (hazard ratio 0·93, 95% CI 0·43–3·11; p=0·86; figure 4). Likewise, there was no difference in survival time after correction for cognitive state and age at the start of the study (0·81, 0·37–1·77; p=0·6). Cox regression analyses of survival time, with correction for cognitive state and age at the start of the study, showed no evidence of differences in survival time by treatment dose group or by mean antibody response compared with the placebo group (table 2).

15 participants—12 in the AN1792 group and three in the placebo group—had progressed to severe dementia by the start of the follow-up study; a further 13 had progressed by the data censoring date. Thus, by treatment group, 23 (36%) of 64 individuals in the AN1792 treated group (13 in the 50 µg group, ten in the 225 µg group) and five (31%) of 16 (31%) individuals in the placebo group had progressed to severe dementia by the end of the follow-up period (χ^2 0·1, p=0·7). There was no difference in the time to severe dementia between the treated and placebo groups (hazard ratio 1·18, 95% CI 0·45–3·11; p=0·73; figure 5). Likewise, there was no difference in time to severe dementia after correction for cognitive state and age at the start of the study (1·22, 0·46–3·27; p=0·7). Cox regression analysis of time to severe dementia, with correction for cognitive state and age at start of study, showed no evidence of differences in time to severe dementia by treatment group or by mean antibody titre compared with the placebo group (table 2).

At the data censoring date, the 26 surviving participants who consented to long-term clinical follow-up had a mean ADAS-Cog score of 43·7 (SD 22·8) points, a mean MMSE score of 12·5 (8·9), and a mean DAD score of 30·4 (27·1), compared with mean scores at baseline at the start of the treatment study of 19·6 (5·8), 22·3 (2·7), and 76·9 (16·8), respectively. There was no evidence of a difference in decline over the 6-year follow-up period for any of these outcome measures between the treatment group and the placebo group (table 3).

A blood serum sample for anti-Aβ antibody assays was obtained in 15 (75%) of the 20 AN1792-treated survivors at 5 or 6 years (mean 63 [SD 4] months) after baseline assessment and compared with 15 control individuals with Alzheimer's disease matched by age and MMSE (age 77·1 [SD 8·6] years; MMSE 14·0 [8·4] points). Eight cases had been treated with 225 µg and seven with 50 µg AN1792. The mean anti-Aβ antibody titre was raised in those treated with AN1792 compared with the control individuals (232 [SE 56] µg/mL *vs* 79 [26] µg/mL; *t* test p=0·02). Anti-Aβ antibody titres at long-term follow-up showed a modest positive correlation with the mean anti-AN1792 antibody titres found during the initial treatment study period (Spearman rank 0·52, p=0·048). No correlations were found between anti-Aβ antibody titres at long-term follow-up and rate of decline as measured by ADAS-Cog; MMSE, or DAD at 6-year follow-up (all cases Spearman rank p>0·1).

	n	Baseline observed mean (SD)	6 year follow-up		
			Observed mean change from baseline (SD)	Difference in mean (95% CI)	p value
ADAS-Cog					
Placebo	6	18·8 (2·2)	23·3 (18·9)	–1·0 (–19·7 to 17·8)	0·9
AN1792	20	19·8 (6·6)	24·3 (19·6)		
MMSE					
Placebo	6	23·2 (2·3)	–11·7 (9·4)	–2·4 (–10·5 to 5·8)	0·5
AN1792	20	22·1 (2·8)	–9·3 (8·2)		
DAD					
Placebo	6	81·8 (16·5)	–43·7 (31·9)	3·6 (–23·6 to 30·9)	0·8
AN1792	20	75·4 (17·0)	–47·3 (27·3)		

ADAS-Cog=Alzheimer's disease assessment scale—cognitive subscale. DAD=disability assessment for dementia. MMSE=mini-mental state examination.

Table 3: **Effect of AN1792 or placebo on exploratory measures in the assessable population at 6 year follow-up**

Discussion

These data show that immunisation of patients with Alzheimer's disease with $A\beta_{42}$ (AN1792) is associated with a long-term reduction in Aβ load and a variable degree of plaque removal compared with unimmunised control individuals. Although the degree of plaque removal was variable, immunisation seems to initiate a long-term process, with post-mortem evidence of plaque removal 5 years after the last injection; further, in the survivors, there is evidence of persistently raised serum antibodies to Aβ correlating with the initial mean antibody response. The limited number of autopsy cases means that the correlations found between the measures of Aβ load, plaque removal, and mean antibody response to AN1792 should be viewed with caution. However, the two patients who had almost complete elimination of plaques, and the lowest Aβ loads, also had the highest mean AN1792 antibody response during the treatment phase of the study.

Despite the evidence of disease modification, there is little evidence to suggest that there is any major effect on cognitive function. All but one of the individuals who died during the follow-up phase had clear end stage dementia before death, including the two individuals with the highest mean antibodies to Aβ and almost complete elimination of plaques. These findings imply that progressive neurodegeneration can occur in Alzheimer's disease despite removal of plaques.

Cox regression analysis showed no evidence of immunisation having any effect on long-term survival or clinical outcomes. However, the small numbers of participants enrolled in the initial study greatly limit the

power of this study and a larger trial might have shown some small benefit that could not be detected with the cohort size examined here. Caution is also required in the interpretation of the cognitive outcomes of the cohort of consenting survivors at 6 year follow-up. Although baseline age and cognitive function were comparable, a greater proportion of those who did not consent to long-term follow-up died during the follow-up period than of those who consented (12 [50%] of 24 *vs* 10 [28%] of 36). The cognitive and functional decline of the consenting survivors might thus represent an underestimate of overall decline and, potentially, of possible differences between groups. Similar considerations apply to the generalisability of the long-term antibody titres in the consenting survivors, since determining whether non-survivors and those who did not consent also had a persistent antibody response was not possible.

The results of this study suggest that plaque removal is not enough to halt progressive neurodegeneration in Alzheimer's disease and prompt some intriguing challenges to the amyloid hypothesis. There are a number of possible explanations for these findings. First, the presence of Aβ plaques might be necessary to initiate, but not to maintain, progressive neurodegeneration. Previous studies have shown a poor correlation between Aβ plaque load and the presence of dementia. For example, a substantial proportion of elderly individuals have Aβ plaques in equivalent densities to patients with Alzheimer's disease but remain cognitively intact.[22,23] Furthermore, recent reports in early Alzheimer's disease show that amyloid load as measured with an in-vivo imaging probe does not change as the clinical condition deteriorates.[24]

Second, although some animal studies suggest that Aβ plaque removal can occur within days of the injection of anti-Aβ antibodies into the brain,[25] other studies suggest that plaque removal occurs progressively over a period of months.[26] The removal of plaques after AN1792 immunisation could thus be a slow process in human beings with Alzheimer's disease. Although there was evidence that Aβ mobilisation could start within 4 months of immunisation, very extensive plaque removal was only present in those patients who survived to 60 months or longer after immunisation.

Third, much attention has been paid to the role of oligomeric Aβ, rather than fibrillar Aβ in plaques, as the immediate cause of synaptic dysfunction and dementia in Alzheimer's disease.[27,28] Immunisation could fail to reduce the concentration of oligomeric Aβ and the concentration might even be increased during the active phase of disintegration of Aβ plaques.[29] According to this view, aggregated Aβ in the form of plaques is harmless, or could even be protective, and therefore the process of removing them might be counterproductive.

Fourth, vaccination with full-length Aβ could result in over-activation of the innate immune system.[30,31] Both AN1792 and its adjuvant QS-21 have been shown to elicit a pro-inflammatory Th1 response[32] that might thereby compromise any potential improvements that plaque removal could bring. Indeed, although not statistically significant, participants in this study with high antibody titres had a more rapid clinical progression than did those with moderate antibody titres. Whether these participants also had a marked Th1 response has not yet been examined.

On the basis of the results of this long-term follow-up study of patients with Alzheimer's disease who were immunised with $A\beta_{42}$, it is likely that the modified immunisation protocols currently in clinical trials will also result in removal of plaques from brains affected by Alzheimer's disease and could prove to be safer strategies. However, our findings suggest that removal of Aβ plaques might not be sufficient to prevent the progressive neurodegeneration in Alzheimer's disease.

Contributors

CH participated in the design, writing, analysis, and organisation of the study, and in the recruitment of patients. DP participated in the writing and analysis of the study, and did post-mortem histological examinations. DW took part in the design and writing of the study, and in the recruitment of patients. GY provided statistical advice. VH took part in the design and organisation of the study, and recruited patients. AB enrolled patients, participated in clinical follow-up and data collection, and to the writing of the paper. RWJ took part in the design and writing of this study, and recruited patients. RB took part in the design and writing of this study, and recruited patients. SL contributed to the writing of the report, did post-mortem histological examinations, and recruited patients. JWN helped write the paper, recruited patients, and did post-morten neuropathological examinations. EZ helped write the paper and did antibody assays. JARN took part in the design and writing of the study, did post-mortem histological examinations, and recruited patients.

Conflict of interest statement

CH, DP, GY, VH, RB, SL, JWN, EZ declare that they have no conflict of interest. DW has received research grant support from Elan for involvement in the original vaccine study and two questionnaire validation trials. AB has received financial support from Elan/Wyeth for clinical research. RWJ has received honoraria for one lecture and attended an advisory board for Elan. JARN has received travel expenses from Elan to attend meetings to present findings in relation to this study.

Acknowledgments

The Alzheimer's Research Trust funded this study. D Boche is funded by the Medical Research Council (G0501033). We thank Elan Pharmaceuticals for access to phase I AN1792 treatment study data and AN1792 antibody. We also thank all patients and carers who took part in the study; J Gifford, Helen Cartwright, Carol Hall and other clinical research staff at Southampton, Cardiff, Swindon, and Bath; neuropathology laboratory staff; staff of the Histochemistry Research Unit and Biomedical Imaging Unit at Southampton General Hospital; M Esiri and C Joachim at Oxford University.

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