BRAIN
ORIGINAL ARTICLE

Increases in amyloid-β_{42} slow cognitive and clinical decline in Alzheimer's disease trials

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Positive effects of new anti-amyloid-β (Aβ) monoclonal antibodies in Alzheimer's disease (AD) have been attributed to brain amyloid reduction. However, most anti-Aβ antibodies also increase the CSF levels of the 42-amino acid isoform ($A\beta_{42}$). We evaluated the associations of changes in CSF $A\beta_{42}$ and brain Aβ-PET with cognitive and clinical end points in randomized trials of anti-Aβ drugs that lowered (β- and γ-secretase inhibitors) or increased CSF $A\beta_{42}$ levels (anti-Aβ monoclonal antibodies) to test the hypothesis that post-treatment increases in CSF $A\beta_{42}$ levels are independently associated with cognitive and clinical outcomes.

From long-term (≥12 months) randomized placebo-controlled clinical trials of anti-Aβ drugs published until November 2023, we calculated the post-treatment versus baseline difference in ADAS-Cog (cognitive subscale of the Alzheimer's Disease Assessment Scale) and CDR-SB (Clinical Dementia Rate-Sum of Boxes) and z-standardized changes in CSF $A\beta_{42}$ and Aβ-PET Centiloids (CL). We estimated the effect size [regression coefficients (RCs) and confidence intervals (CIs)] and the heterogeneity (I^2) of the associations between AD biomarkers and cognitive and clinical end points using random-effects meta-regression models.

We included 25 966 subjects with AD from 24 trials. In random-effects analysis, increases in CSF $A\beta_{42}$ were associated with slower decline in ADAS-Cog (RC: −0.55; 95% CI: −0.89, −0.21, $P = 0.003$, $I^2 = 61.4\%$) and CDR-SB (RC: −0.16; 95% CI: −0.26, −0.06, $P = 0.002$, $I^2 = 34.5\%$). Similarly, decreases in Aβ–PET were associated with slower decline in ADAS-Cog (RC: 0.69; 95% CI: 0.48, 0.89, $P < 0.001$, $I^2 = 0\%$) and CDR-SB (RC: 0.26; 95% CI: 0.18, 0.33, $P < 0.001$, $I^2 = 0\%$). Sensitivity analyses yielded similar results.

Higher CSF $A\beta_{42}$ levels after exposure to anti-Aβ drugs are independently associated with slowing cognitive impairment and clinical decline. Increases in $A\beta_{42}$ may represent a mechanism of potential benefit of anti-Aβ monoclonal antibodies in AD.

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Introduction

The amyloid cascade hypothesis posits that the accumulation of amyloid-β (Aβ) is the first step of the Alzheimer's disease (AD) pathological process. This 30-year-old hypothesis, supported by the discovery that point mutations in Aβ-coding genes, *APP*, *PSEN1* and *PSEN2*, yield autosomal dominant forms of AD[1] has inspired a robust clinical trial pipeline since 2001. Eleven anti-Aβ drugs have significantly reduced brain amyloid without translating into cognitive or clinical benefits in over 30 different trials targeting mild or mild to moderate AD, mild cognitive impairment and pre-clinical AD (AN-1792, CAD106, semagacestat, avagacestat, verubecestat, lanabecestat, umibecestat, atabecestat, LY2886721, scyllo-inositol, solanezumab) (Fig. 1). The newer anti-Aβ monoclonal antibodies lecanemab and donanemab robustly decreased brain amyloid plaques and produced a statistically significant deceleration in cognitive and clinical decline. Most anti-Aβ monoclonal antibodies also increased the CSF levels of the 42-amino acid isoform of Aβ ($Aβ_{42}$). Lecanemab, in particular, increased CSF $Aβ_{42}$ by 288 pg/ml over 18 months of treatment.[2] Conversely, β-secretase and γ-secretase inhibitors, which inhibit the production of Aβ from the amyloid precursor protein (APP), lowered the CSF levels of $Aβ_{42}$ and consistently worsened cognition and functional performance in AD patients. Verubecestat, for example, lowered CSF $Aβ_{42}$ by 320 pg/ml over 18 months of treatment.[3]

In the pathophysiology of AD, CSF $Aβ_{42}$ becomes abnormally low in the earliest stages of AD, before amyloid-PET is abnormal and before neurodegeneration is detected.[4] As the soluble fraction of Aβ, the amyloidogenic $Aβ_{42}$, transforms into amyloid plaques, the CSF levels of $Aβ_{42}$ decrease while brain amyloid levels increase.[5] Whereas low CSF $Aβ_{42}$ is universally associated with AD (no one with AD has high $Aβ_{42}$ levels), high brain amyloid is not. Towards the end of a normal lifespan, by the age of 85, four-fifths of amyloid-positive individuals have normal cognition.[6] The ostensible paradox of brain amyloid positivity without dementia can in part be explained by the observation that high levels of CSF $Aβ_{42}$ are associated with normal cognition in amyloid-positive individuals,[7] a relationship longitudinally confirmed in carriers of *APP*, *PSEN1* and *PSEN2* pathogenic mutations.[8]

We tested the hypothesis that, after treatment with anti-Aβ antibodies, increases in CSF $Aβ_{42}$ levels are independently associated with cognitive and clinical outcomes.

Materials and methods

Search strategy

We performed a comprehensive search on PubMed to identify anti-Aβ drug trials in subjects with early or mild-to-moderated AD published from January 1985 to November 2023. We used a combination of the following search terms 'Alzheimer's Disease' AND 'clinical trial' AND 'cerebrospinal fluid' to screen for eligible articles. We cross-checked references to identify any missing pertinent studies from the initial search and to exclude overlapping studies. One of the authors (B.I.) also independently validated the eligible articles. We adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) 2020 checklist for the conduct of this analysis.[9]

Studies eligibility criteria

We included controlled trials of Aβ targeting drugs with a follow-up of at least 1 year that reported data on Aβ-PET and CSF $Aβ_{42}$ levels. In the original articles, Aβ-PET changes were reported as standardized uptake value ratio (SUVR) or Centiloids (CL). SUVR data were converted into CL, so that all the brain Aβ-PET data were expressed in CL (Supplementary Table 1). Studies must have included a cognitive or clinical performance measure, namely the cognitive subscale of the Alzheimer's Disease Assessment Scale (ADAS-Cog) or the Clinical Dementia Rate-Sum of Boxes (CDR-SB).

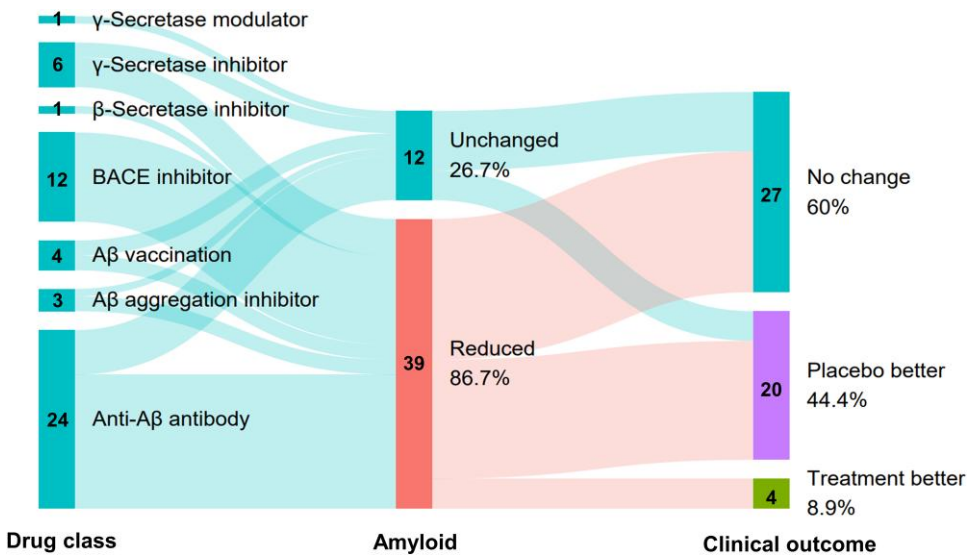

Figure 1 Sankey diagram of the distribution of clinical trials for Alzheimer's disease by drug class, their effects on amyloid-β-PET and clinical outcomes. Aβ = amyloid-β.

Data extraction

The primary outcome measures of efficacy were changes in the post- to pre-intervention in ADAS-Cog and CDR-SB between the intervention and placebo groups. The primary exposures were the difference between drug- and placebo-treated groups in the post- to pre-intervention changes in free (unbound) CSF $A\beta_{42}$ levels (pg/ml) and Aβ-PET levels (CL). For studies that did not report Aβ-PET levels in CL, we converted Aβ-PET SUVR to CL using tracer-specific formulas (Supplementary Table 1).[10] The exposure and outcome efficacy data were measured at the same follow-up time across studies. If free or unbound CSF $A\beta_{42}$ levels were not specified, the unspecified CSF $A\beta_{42}$ values were used. We standardized Aβ-PET CL and CSF $A\beta_{42}$ data with z-transformation using the formula $z = (x - \text{mean})/\text{standard deviation (SD)}$, where x is Aβ-PET CL or CSF $A\beta_{42}$.

All data were extracted from tables or graphs using the online software WebPlotDigitalizer by Automeris.[11] We also collected type of AD patient population, intervention, doses, sample sizes for each treatment group, assessment time of exposures, outcomes from each treatment arm within each study, and the type of assay used for the measurement of CSF $A\beta_{42}$, their range and detection antibody. The resulting Excel spreadsheet was independently double-checked by three study authors (J.A., A.D. and B.I.).

Quality assessment

We used the Study Quality Assessment Tool for controlled interventional studies from the National Heart Lung Blood Institute (NHLBI) for methodological and reporting appraisal of study quality (https://www.nhlbi.nih.gov/health-topics/study-quality-assessment-tools).[12] Two authors (J.A. and A.D.) independently graded the selected articles for their quality. Any disagreements were discussed among all authors to achieve consensus.

Statistical analysis

We documented the post-to-pre-intervention mean change in ADAS-Cog and CDR-SB and the standard error (SEM) between treatment groups. Wherever the mean change between groups was not reported, it was computed from each pre- and post-intervention group. If the SEM for the mean change of outcome was not reported, we calculated it from the 95% confidence interval (CI). The mean change in ADAS-Cog and CDR-SB from baseline in the placebo group and treatment group was computed for each study. We defined the placebo-adjusted mean change as the difference in the mean change in ADAS-Cog and CDR-SB from baseline between treatment and placebo groups. Similarly, we calculated the placebo-adjusted mean change in CSF $A\beta_{42}$ and Aβ-PET. In the primary analysis, we evaluated the association between placebo-adjusted changes in each cognition outcome with placebo-adjusted changes in CSF $A\beta_{42}$ and Aβ-PET levels using a restricted maximum likelihood (REML) random effects meta-regression analyses.[13,14] The REML method computes the pooled association after weighting each study according to the inverse variance of the outcome estimate for each study. The overall variance is the sum of variances estimated within each study and across studies for each outcome separately.[13] For each study a weight was assigned using the inverse variance of the placebo-adjusted mean differences in each outcome. The overall mean and SD of the changes in CSF $A\beta_{42}$ and Aβ-PET levels across studies were used to compute the z-transformed CSF $A\beta_{42}$ and Aβ-PET levels using the formula, $z = (x - \text{mean})/SD$. This allows the direct comparison of the effect sizes, i.e. regression coefficients (RCs) associated with Aβ-PET CL and CSF $A\beta_{42}$ levels. RC values estimated by meta-regression analysis represent the change in cognitive or clinical outcome associated with 1 SD increase in the placebo-adjusted changes in each biomarker. We used the I^2 statistic to assess heterogeneity in the RC estimates. Funnel plots and Begg's test were used to determine the presence of publication bias and small sample size effects on the reported estimates for each cognition outcome. A non-significant P-value from Begg's test indicates the absence of a small sample size effect. Various sensitivity analyses were performed to validate the findings obtained from random effects meta-regression analysis. These sensitivity analyses were conducted after removing studies (i) where only one biomarker was available; (ii) without early AD subjects; (iii) with increased heterogeneity in the associations; or (iv) considering only studies testing monoclonal antibodies using fixed effects analysis; or (v) using fixed effects regression analysis. In a further sensitivity analysis (vi), we controlled assay variability and other sources of variability by standardizing the placebo-adjusted changes in CSF $A\beta_{42}$ and Aβ PET CL using Cohen's d method within each study dataset. Cohen's d was computed by dividing the placebo–adjusted changes of the two biomarkers by the pooled SD within each study dataset. The pooled SD was computed as standard error multiplied by 1/square root of $[1/n_1 + 1/n_2]$ in which n_1 and n_2 are sample sizes for the placebo and drug groups, respectively.

We also compared the placebo-adjusted mean differences in each efficacy outcome by categorizing biomarker data into two groups according to the mean increase in CSF $A\beta_{42}$ ($A\beta_{42} \geq 0$ versus $A\beta_{42} < 0$) and the mean decrease in brain Aβ-PET ($CL < -10$ versus $CL \geq -10$) using REML random effects meta-regression analyses. The cut-off of 10 CL was selected based on the finding that 12 CL marks the transition from the absence of pathology to subtle pathology,[15] meaning that Aβ-PET changes lower than 10 CL are not radiologically significant. To be consistent with the CSF $A\beta_{42}$ categorization, we also analysed the categorical mean change in brain Aβ-PET in $CL < 0$ versus $CL \geq 0$ classes. To summarize the association between changes in CSF $A\beta_{42}$ and Aβ-PET levels and changes in efficacy outcomes, we calculated the weighted correlation coefficient (r) from the weighted fixed effects linear regressions.[16] The weight was assigned for each study using the proportion of the respective sample sizes. The fixed effects analysis produces the coefficient of determination (R^2) between independent predictor and outcome. The square root of R^2 (with RC sign) provides the Pearson's correlation coefficient between each marker and the clinical and cognitive outcomes.[17] Fixed effects weighted regression analyses were also performed to evaluate the correlation between AD biomarkers and efficacy outcomes among studies on monoclonal antibodies and tested associations in studies reporting data on both AD biomarkers or studies yielding a significant decline in brain Aβ-PET ($CL < -10$). An adjusted fixed effects model involving both AD biomarkers was also conducted for each efficacy outcome separately. The results of fixed or random effects meta-regression analyses were summarized with RC, 95% CI and P-values. All the statistical analyses were performed using STATA 17.0. applying statistical checklists.[12,18] The statistical analysis codes and the dataset are provided in the Supplementary material.

Results

The initial screening eligibility criteria yielded 281 articles (Supplementary Fig. 1). After excluding studies with observational design, small sample size, not reporting CSF $A\beta_{42}$ levels and examining drugs not directly targeting A, 22 articles encompassing 24

Table 1 Main characteristics of the studies included in this systematic analysis

Drug class and name	Dose regimen (mg)	No. randomized subjects		Weeks	Placebo-adjusted mean change from baseline			
		Placebo	Drug		CSF $A\beta_{42}$ (pg/ml)	Aβ-PET-CL	ADAS-Cog	CDR-SB
Gamma-secretase inhibitors								
Semagacestat[19,20]	100/day	501	506	76	−51.14	NA	1.10	0.40
Semagacestat[19,20]	140/day	501	527	76	−47.46	NA	1.40	0.70
Avagacestat[21]	50/day	131	132	104	0.92	NA	0.63	−0.20
BACE inhibitors								
Verubecestat[22]	12/day	653	652	78	−270.30	−2.43	0.20	0.00
Verubecestat[22]	40/day	653	652	78	−331.30	−4.85	0.40	0.00
Verubecestat[3]	12/day	485	485	104	−231.10	−6.07	1.10	0.07
Verubecestat[3]	40/day	485	484	104	−320.20	−7.28	1.30	0.44
Anti-Aβ monoclonal antibodies								
Bapineuzumab[23,24]	0.5/kg/13 weeks	432	658	78	NA	−9.60	−0.20	0.20
Bapineuzumab[23,24]	0.5/kg/13 weeks	493	314	78	NA	8.95	−0.30	0.00
Bapineuzumab[23,24]	1.0/kg/13 weeks	493	307	78	NA	6.52	0.40	0.20
Crenezumab[25]	300/2 weeks	62	122	73	120.16	NA	−0.04	−0.69
Crenezumab[25]	15/kg/4 weeks	84	165	73	170.50	NA	−1.78	−0.08
Crenezumab[26]	300/2 weeks	13	26	69	127.10	−1.99	0.15	1.41
Crenezumab[26]	15/kg/4 weeks	17	35	69	94.51	9.21	−0.70	−0.23
Crenezumab[27]	60/kg/4 weeks	409	409	105	278.24	−1.99	0.26	0.17
Crenezumab[27]	60/kg/4 weeks	399	407	53	308.27	−3.79	−1.74	−1.30
Solanezumab[28]	400/4 weeks	506	506	80	−25.80	NA	−1.40	0.10
Solanezumab[28]	400/4 weeks	519	521	80	36.10	NA	−1.60	−0.30
Solanezumab[29,30]	400/4 weeks	1072	1057	80	−28.00	NA	−0.80	−0.34
Solanezumab[31]	1600/4 weeks	591	578	240	NA	−7.70	NA	0.12
Gantenerumab[32]	105/4 weeks	266	271	104	0.89	3.61	−0.62	0.10
Gantenerumab[32]	225/4 weeks	266	260	104	42.79	−12.64	−0.27	0.18
Gantenerumab[33]	1200/4 weeks	40	52	208	207.50	−59.65	NA	−0.27
Lecanemab[34]	2.5/kg/2 weeks	247	52	79	NA	−17.69	0.67	−0.27
Lecanemab[34]	5.0/kg/4 weeks	247	51	79	NA	−24.37	0.84	0.21
Lecanemab[34]	5.0/kg/2 weeks	247	92	79	NA	−36.28	−0.40	−0.04
Lecanemab[34]	10/kg/4 weeks	247	253	79	205.60	−41.34	−0.28	−0.25
Lecanemab[34]	10/kg/2 weeks	247	161	79	205.60	−55.96	−2.31	−0.40
Lecanemab[2]	10/kg/2 weeks	875	859	79	285.87	−59.12	−1.44	−0.45
Aducanumab[35]	3–6/kg/4 weeks	548	543	78	178.83	−32.49	−0.70	−0.26
Aducanumab[35]	10/kg/4 weeks	548	547	78	317.06	−48.74	−1.40	−0.39
Aducanumab[35]	3–6/kg/4 weeks	545	547	78	86.07	−31.05	−0.58	−0.18
Aducanumab[35]	10/kg/4 weeks	545	555	78	197.47	−42.24	−0.59	0.03
Donanemab[36]	700/4 weeks[a]	126	131	76	NA	−85.06	−1.86	−0.36
Donanemab[37]	700/4 weeks[a]	876	860	76	NA	−86.33	−1.35	−0.7
Gantenerumab[38]	510/2 weeks	485	499	116	−0.97	−1.29	−0.26	−0.42
Gantenerumab[38]	510/2 weeks	477	498	116	−0.97	−1.07	−0.26	−0.34

$A\beta_{42}$ = 42-amino acid isoform of amyloid-β; ADAS-Cog = cognitive subscale of the Alzheimer's Disease Assessment Scale; BACE = β-site amyloid precursor protein cleaving enzyme; CDR-SB = Clinical Dementia Rate-Sum of Boxes; CL = Centiloids; NA = not available; SD = standard deviation.
[a]Then 10 mg/kg/4 weeks.

trials of 10 anti-Aβ treatments were eligible for this analysis. Of these, 37 datasets reported at least one outcome measure of efficacy were included in the final analyses yielding 25 996 AD subjects; 14 853 allocated to treatment and 11 143 to placebo (Table 1; full version in Supplementary Table 2). In the quality assessment, all studies were graded fair or good. Most studies had dropouts > 20% during the follow-up period (Supplementary Table 3). We found no evidence of small sample size or publication bias for ADAS-Cog (Begg's test $P = 0.42$) or CDR-SB (Begg's test $P = 0.38$) (Supplementary Fig. 2). Of 37 datasets, 28 reported CSF $A\beta_{42}$ levels and 29 reported Aβ-PET CL (Supplementary Fig. 3). Eighteen of 28 datasets that published CSF $A\beta_{42}$ levels reported an increase in this biomarker. Four out of 29 datasets reported an increase in Aβ-PET of up to 9.2 Centiloids compared to controls (Fig. 2).

Associations between efficacy outcomes and Alzheimer's disease biomarkers

In random-effects meta-regression analyses using the dataset of Table 1, increased CSF $A\beta_{42}$ was associated with a slower decline in ADAS-Cog (RC: −0.55; 95% CI: −0.89, −0.21, $P = 0.003$, $I^2 = 61.4\%$) and CDR-SB (RC: −0.16; 95% CI: −0.26, −0.06, $P = 0.002$, $I^2 = 34.5\%$). Similarly, decreased Aβ-PET was associated with a slower decline in ADAS-Cog (RC: 0.69; 95% CI: 0.48, 0.89, $P < 0.001$, $I^2 = 0\%$) and CDR-SB (RC: 0.26; 95% CI: 0.18, 0.33, $P < 0.001$, $I^2 = 0\%$) (Fig. 3). These associations were unchanged after (i) removing heterogeneous studies (Supplementary Table 4); (ii) using fixed effects regression analysis (Supplementary Table 5); (iii) restricting the analysis to studies reporting both CSF $A\beta_{42}$ and Aβ-PET data (Supplementary Table 6); (iv) standardizing the CSF $A\beta_{42}$ and

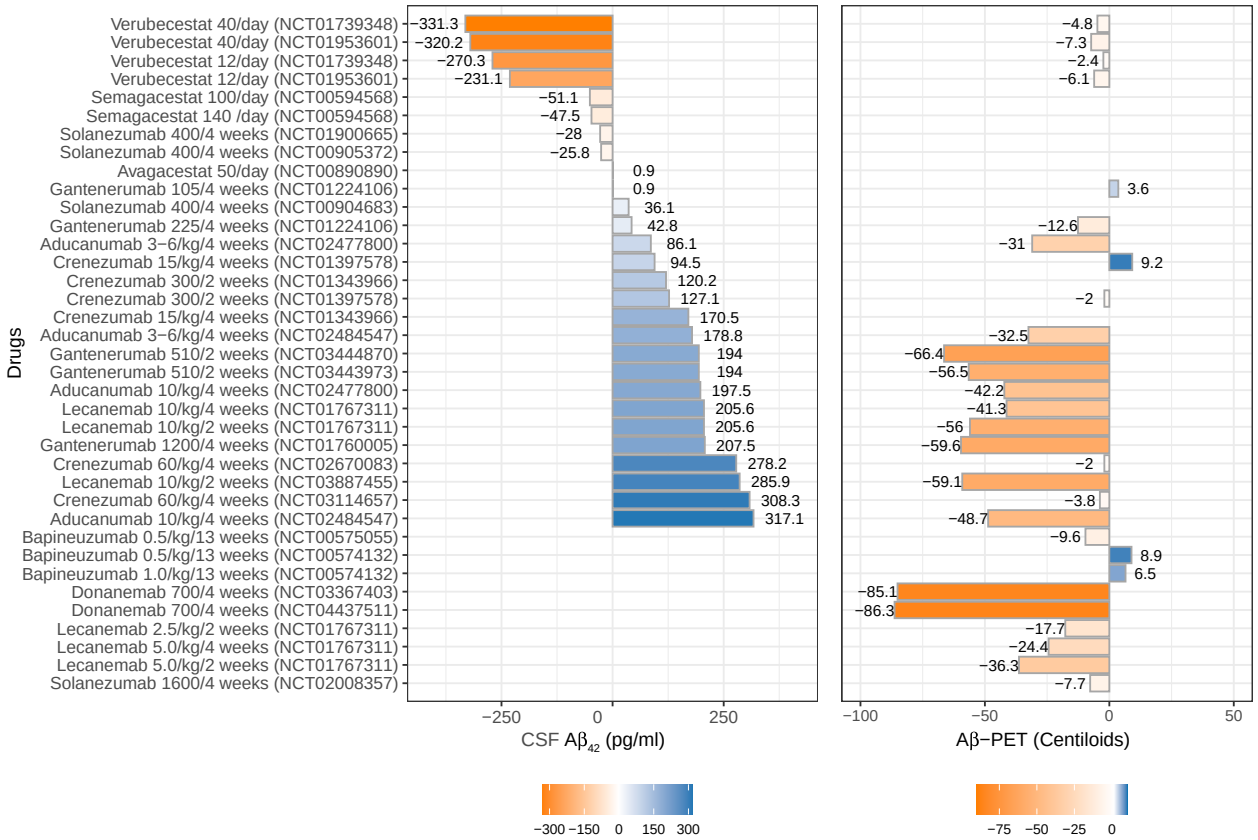

Figure 2 Placebo-adjusted changes from baseline in CSF amyloid-β $(Aβ)_{42}$ and Aβ-PET. Empty rows denote studies in which no data were reported for the specific biomarker. Darker shades denote higher magnitude of reductions and increases.

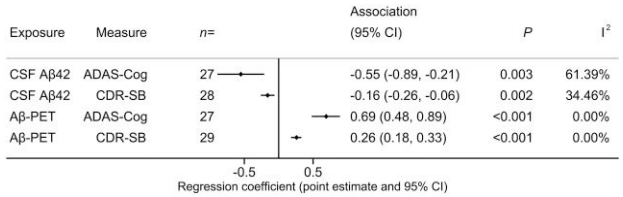

Figure 3 Random-effects analysis for evaluating the associations between placebo-adjusted changes in CSF amyloid-β $(Aβ)_{42}$ and Aβ-PET and changes in ADAS-Cog and CDR-SB. $Aβ_{42}$ = 42-amino acid isoform of amyloid-β; ADAS-Cog = cognitive subscale of the Alzheimer's Disease Assessment Scale; CDR-SB = Clinical Dementia Rate-Sum of Boxes; CI = confidence interval; I^2 = heterogeneity.

Aβ-PET data within each study set using Cohen's *d* method (Supplementary Table 7); or (v) restricting the analysis to early AD studies only (Supplementary Table 8). Meta-regression analysis restricted to studies of anti-Aβ monoclonal antibodies found a significant association between Aβ-PET and efficacy outcomes but not for CSF $Aβ_{42}$ (Supplementary Table 9). This is likely because only two studies (both on solanezumab) of the 17 studies carried out on monoclonal antibodies reported a decrease in CSF $Aβ_{42}$ compared to placebo (−25 and −28 pg/ml, respectively), thus severely limiting the efficiency of the regression analyses with such a reduced scale width of the independent variable. In weight-adjusted fixed effects regression analyses, the magnitude of association between changes in CSF $Aβ_{42}$ and changes in ADAS-Cog ($r = -0.678$, $P < 0.001$) was similar to that of Aβ-PET and ADAS-Cog changes ($r = 0.711$, $P < 0.001$) (Fig. 4). The association between CSF $Aβ_{42}$ and CDR-SB changes ($r = -0.532$, $P = 0.004$) was also similar to that of Aβ-PET and CDR-SB changes ($r = 0.564$, $P = 0.001$) (Fig. 5). Finally, in adjusted regression analyses involving both AD markers simultaneously, changes in CSF $Aβ_{42}$ were significantly associated with changes in cognitive and clinical outcomes (ADAS-Cog, RC: −0.46; 95% CI: −0.73, −0.19, $P = 0.002$ and CDR-SB, RC: −0.18; 95% CI: −0.33, −0.03, $P = 0.024$) whereas changes in Aβ-PET were not (ADAS-Cog, RC: 0.32; 95% CI: −0.08, 0.73, $P = 0.111$ and CDR-SB, RC: −0.03; 95% CI: −0.26, 0.20, $P = 0.777$) (Supplementary Table 5).

Association between efficacy outcomes and categorized Alzheimer's disease biomarkers

In the analysis with categorized changes in AD biomarkers using the dataset of Table 1, CSF $Aβ_{42}$ changes greater than 0 pg/ml were associated with a slower decline in ADAS-Cog (RC: −1.19; 95% CI: −1.97, −0.41; $P = 0.004$, $I^2 = 60.3\%$) and CDR-SB (RC: −0.33; 95% CI: −0.57, −0.10; $P = 0.008$, $I^2 = 44.3\%$) compared with CSF $Aβ_{42}$ changes below 0 (Figs 6 and 7). Similarly, decreases in Aβ-PET greater than 10 CL were associated with a slower decline in ADAS-Cog (RC: −1.29; 95% CI: −1.70, −0.89, $P < 0.001$, $I^2 = 0\%$) and CDR-SB (RC: −0.38; 95% CI: −0.57, −0.19, $P < 0.001$, $I^2 = 25.9\%$) compared to increases above 10 CL (Figs 6 and 7). These findings were confirmed after removing heterogeneous studies (Supplementary Table 10). Fixed effects regression analyses for the differences between categorized changes in CSF $Aβ_{42}$ and brain Aβ-PET levels and changes in efficacy outcomes yielded similar results to those of the primary

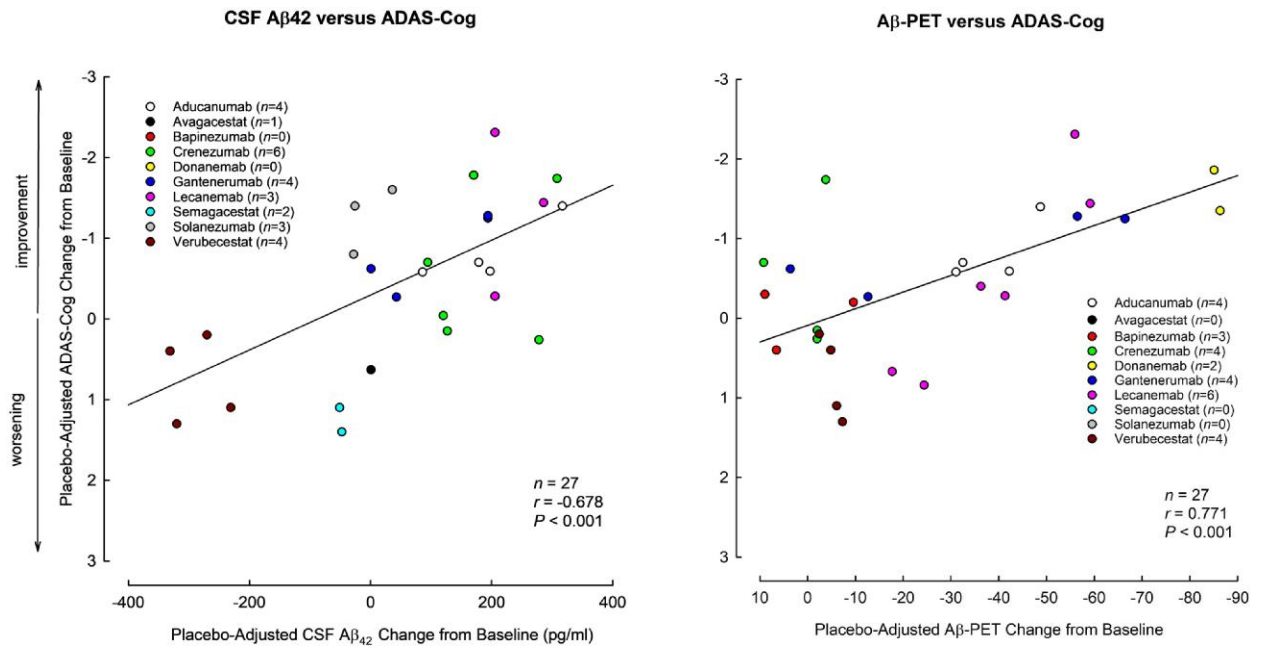

Figure 4 Weight-adjusted correlations between CSF amyloid-β ($Aβ)_{42}$ and Aβ-PET and placebo-adjusted changes in ADAS-Cog across 24 trials of 10 anti-Aβ treatments. Effects of individual studies on the cognitive subscale of the Alzheimer's Disease Assessment Scale (ADAS-Cog) are reported. Inverse Aβ-PET scale was used to compare the associations in the same direction. $Aβ_{42}$ = 42-amino acid isoform of amyloid-β.

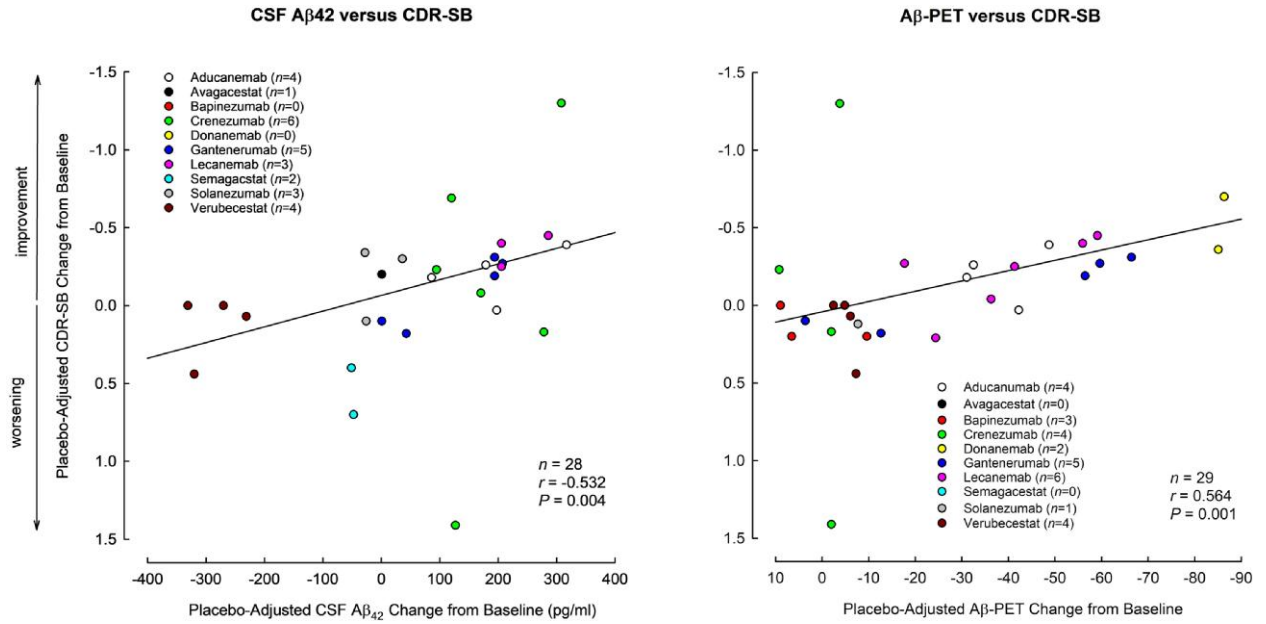

Figure 5 Weight-adjusted correlations between CSF amyloid-β ($Aβ)_{42}$ and Aβ-PET and placebo-adjusted changes in CDR-SB across 24 trials of 10 anti-Aβ treatments. Effects of individual studies on cognitive subscale of the Alzheimer's Disease Assessment Scale (ADAS-Cog) are reported. Inverse Aβ-PET scale was used to compare the associations in the same direction. $Aβ_{42}$ = 42-amino acid isoform of amyloid-β.

analysis (Supplementary Table 11). There was no significant association between Aβ-PET and clinical or cognitive outcomes when this biomarker was categorized as <0 and ≥0 (Supplementary Fig. 4).

Meta-regression analysis restricted to studies of anti-Aβ monoclonal antibodies found a significant association between Aβ-PET and efficacy outcomes but not for CSF $Aβ_{42}$ (Supplementary Table 11). This is likely because only two studies (both on solanezumab) of the 17 studies carried out on monoclonal antibodies reported a decrease in CSF $Aβ_{42}$ compared to placebo (−25 and −28 pg/ml, respectively), thus severely limiting the efficiency of the regression analyses with such a reduced scale width of the independent variable.

Discussion

This analysis of 24 trials evaluating 10 anti-Aβ interventions in AD showed that changes in CSF $Aβ_{42}$ and Aβ-PET levels mirrored positive and negative cognitive and clinical effects of different classes

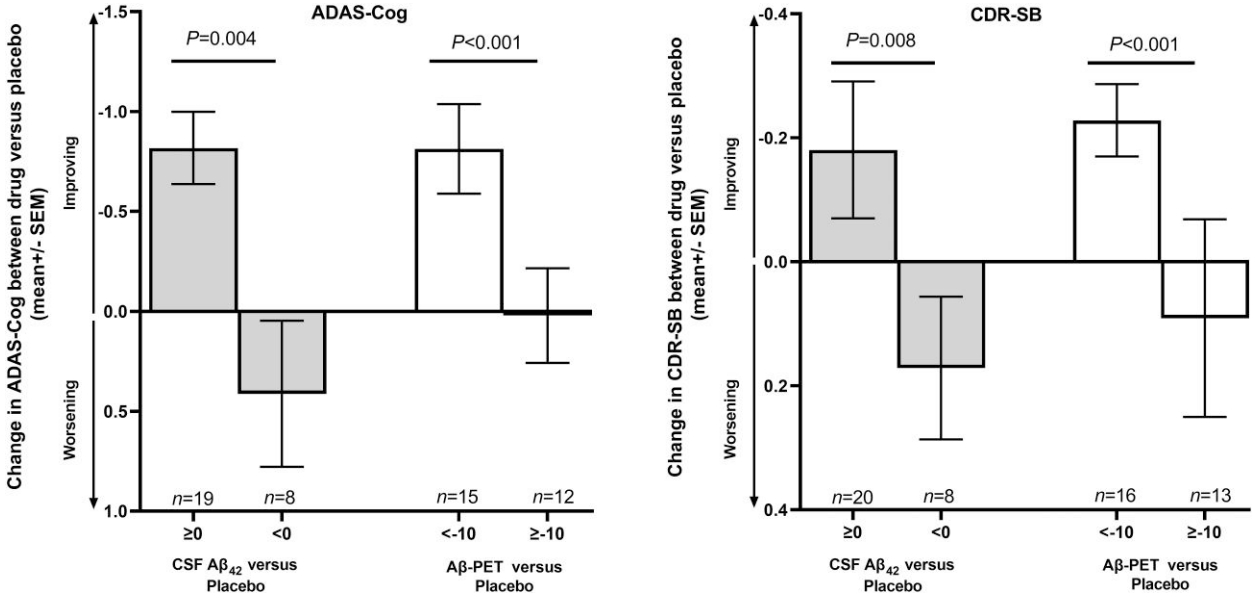

Figure 6 Mean (± SEM) drug versus placebo differences in ADAS-Cog and CDR-SB by placebo-adjusted CSF amyloid-β (Aβ)$_{42}$ and Aβ-PET classes. ADAS-Cog = cognitive subscale of the Alzheimer's Disease Assessment Scale; CDR-SB = Clinical Dementia Rate-Sum of Boxes; Aβ$_{42}$ = 42-amino acid isoform of amyloid-β; SEM = standard error of the mean.

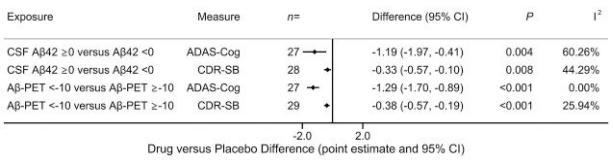

Figure 7 Placebo-adjusted differences in ADAS-Cog and CDR-SB by categorized changes in CSF amyloid-β (Aβ)$_{42}$ and brain Aβ-PET levels. Aβ$_{42}$ = 42-amino acid isoform of amyloid-β; ADAS-Cog = cognitive subscale of the Alzheimer's Disease Assessment Scale; CDR-SB = Clinical Dementia Rate-Sum of Boxes; CI = confidence interval; I^2 = heterogeneity.

of drugs targeting Aβ, namely β- and γ-secretase inhibitors (negative) and anti-Aβ monoclonal antibodies (positive). These results support a unified AD model whereby the soluble and insoluble fractions of the Aβ protein are inversely correlated with cognition. Indeed, potent secretase inhibitors which depleted Aβ$_{42}$ levels accelerated cognitive and clinical decline whereas potent anti-Aβ monoclonal antibodies increased Aβ$_{42}$ levels and improved cognition.

Considering the individual studies, the 18-month, phase 3 trial of lecanemab in early AD showed that an increase of about 300 pg/ml in CSF Aβ$_{42}$ was associated with beneficial effects.[2] The baseline CSF Aβ$_{42}$ levels for this study were not reported, but hypothesizing values of around 400–500 pg/ml on average, patients treated with lecanemab may have had CSF Aβ$_{42}$ levels of about 700–800 pg/ml by the end of the 18-month treatment. These values are very similar to the hypothetical 'compensatory' threshold (within the low end of the physiological range) of CSF Aβ$_{42}$ identified in amyloid-positive but cognitively normal subjects (864 pg/ml).[7] Conversely, most individuals with CSF Aβ$_{42}$ levels below 800 pg/ml by ELISA (INNOTEST Aβ$_{42}$; Innogenetics) fulfill criteria for dementia.[39]

How monoclonal antibodies may increase CSF Aβ$_{42}$ is not clear. One potential mechanism involves the action of microglia, which leads to the phagocytosis of Aβ mediated by Fc receptors although this hypothesis is not supported by analysing the effects of these drugs on CSF levels of soluble triggering receptor expressed on myeloid cells 2 (sTREM2), a marker of microglia activation.[40] Another hypothesis is a direct action of the anti-Aβ monoclonal antibodies on Aβ plaques, fibrils, protofibrils, or oligomers with a destabilization of aggregated species.[41] Indeed, anti-Aβ antibodies may directly bind to Aβ and either prevent oligomerization and fibril formation of Aβ or dissolve Aβ aggregates.[42] An *in vivo* animal study has shown that monoclonal antibodies bind to Aβ aggregates leading to disaggregation of the fibrils and partial restoration of the Aβ solubility.[43]

Aβ$_{42}$ is an evolutionarily conserved protein throughout the animal kingdom,[44] with neuroprotective roles uncovered in knockdown and knockout animal studies,[45] critical to the growth and plasticity of synapses.[46] Furthermore, Aβ$_{42}$ is known to exert strong antioxidant and neuroprotective effects, with roles in enhancing synaptic plasticity and memory.[47-53] Below 1 nM, which is closer to physiological concentration than most toxicity models, Aβ protects against oxidative damage in CSF samples.[54] Therefore, the depletion of soluble Aβ$_{42}$ during the process of amyloid formation may be more detrimental to the brain than the accrual of its insoluble counterpart.[55,56] Although an association between reductions in Aβ-PET and slower decline in CDR-SB has been suggested,[57] such analysis did not statistically quantify the association. At the individual level, the coefficient of determination between amyloid lowering and CDR-SB in the phase 2b trial of lecanemab is only 0.014 (not statistically significant), meaning that lowering brain amyloid alone explains only about 1.4% of the variance in CDR-SB.[34]

There are several limitations related to this work, the most important among which is the lack of individual data from each of the source trials. The restrictions to data access are widespread and were renewed by the Eisai/Biogen partnership in the lecanemab trial: no data sharing permitted. As such, we could only extract the CSF Aβ$_{42}$ levels from the figures in the Supplementary material of each of the trial reports. Also, neither the phase 2 nor phase 3 trials of donanemab included CSF Aβ$_{42}$ collection,

precluding our ability to calculate the relative contribution of the changes in soluble versus insoluble fraction of the protein in that study.[36] In addition, Aβ-PET and CSF $Aβ_{42}$ data were not consistently reported in some trials. However, our analysis was based on direct reporting of placebo-adjusted mean differences in exposures and outcomes with their 95% CIs, limiting the noise expected from data extraction and variation in outcome dispersion measures. It is important to note that we had limited statistical power to conduct analysis for disease subgroups beyond mild AD or to account for individual differences in disease progression, genetic factors and treatment responses, all of which reduce the precision of the estimated associations as well as the generalizability of the results. In a sensitivity analysis considering only studies testing monoclonal antibodies, CSF $Aβ_{42}$ was not significantly associated with slowing ADAS-Cog or CDR-SB decline. We believe this represents an artefact because this class of drugs, unlike secretase inhibitors, only resulted in increases in CSF $Aβ_{42}$; therefore, the regression analyses did not include 'negative' (decreased) CSF $Aβ_{42}$ values. Lastly, by virtue of the research question we were seeking to answer, we did not include tau PET data, which is known to exhibit a higher correlation with dementia than Aβ-PET.[58] Caveats above notwithstanding, our analysis benefits from a large number of trials with large samples followed over at least one year, minimal heterogeneity for ADAS-Cog and CDR-SB outcomes, and consistent results validated using multiple sensitivity analyses for continuous and categorized expressions of CSF $Aβ_{42}$ and brain Aβ-PET changes.

In conclusion, changes in CSF $Aβ_{42}$ levels are associated with cognitive and clinical effects of anti-Aβ drugs. The increase in CSF $Aβ_{42}$ levels may be an overlooked but relevant mechanism by which anti-Aβ monoclonal antibodies may exert a positive effect. As recently supported by double knockout and APP/PS1/Tau triple transgenic mice experiments,[59] strategies to restore the levels of $Aβ_{42}$ above a threshold of compensation may be desirable to evaluate for the treatment of patients with AD, who universally have low CSF $Aβ_{42}$ levels.[60]

Data availability

The data of this study and statistical analysis codes with STATA 17 software are available in Appendix A of the Supplementary material.

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Competing interests

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Supplementary material

Supplementary material is available at *Brain* online.

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