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Brain volume change following amyloid-beta immunotherapy for Alzheimer's disease: amyloid-removal related pseudo-atrophy

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Brain volume change following amyloid-beta immunotherapy for Alzheimer's disease: amyloid-removal related pseudo-atrophy

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Summary

Progressive cerebral volume loss on MRI is a hallmark of Alzheimer's disease and has been widely used as an outcome in Alzheimer trials, with the prediction that disease-modifying treatments would slow loss. However, multiple anti-amyloid immunotherapy trials reported *excess* volume loss with treatment. Explanations for this range from reduced amyloid-beta plaque burden and related inflammatory changes, through to treatment-induced toxicity. We review these hypotheses and their compatibility with data arising from amyloid immunotherapy trials and histopathological findings. We conclude that these excess volume changes are characteristic of *only* those immunotherapies that achieve amyloid-beta lowering; *are* compatible with plaque removal; and that evidence to date does *not* suggest an association with harm. Understanding the causes, and consequences, of these changes is important to enable informed decisions about treatments. Patient-level analyses of trials is urgently needed along with longitudinal follow-up and imaging to determine the longer-term trajectory of volume changes and clinical correlates. Post-mortem examination of cerebral tissue from treated patients and correlation with antemortem imaging is a priority. Based on current evidence, we propose the provisional term "amyloid-removal related pseudo-atrophy (ARPA)" to describe this phenomenon.

Introduction

Progressive cerebral volume loss, often referred to as atrophy, is a characteristic and diagnostic feature of Alzheimer's disease (AD) and an accepted biomarker of neurodegeneration (Panel 1).[1] Measurement of global and regional brain volume changes from serial magnetic resonance imaging (MRI) has been widely used in trials of candidate disease-modifying treatments (DMTs), the presumption being that effective treatment would, in time, slow neurodegeneration and lead to a reduction in rates of brain volume loss.[7,8]

However, in the first trial of immunization against amyloid-beta (Aβ) using the agent AN1792, *excess* volume loss was observed in patients on active drug – considered "paradoxical" at the time.[10] A similar phenomenon was seen subsequently in several other immunotherapies directed at Aβ,[11] including recently reported phase 3 trials of gantenerumab, lecanemab and donanemab.[9,12,13] The cause of this "paradoxical" volume loss is not well understood, but has led to concerns that it might represent accelerated neurodegeneration and so lead to deleterious long term outcomes.[11,14] Other proposed explanations include that the excess volume loss is due to removal of Aβ plaques, reduction in plaque-associated inflammatory changes, or alterations in cerebrospinal fluid (CSF) dynamics.[15]

One of the difficulties in disentangling causation is that therapies that are effective in removing Aβ also cause the potentially serious side effect of amyloid-related imaging abnormalities with oedema/effusions (ARIA-E) or microhaemorrhages (ARIA-H),[16] which in turn might influence brain volume.

Given that some of these treatments are now in clinical use and others are in or entering clinical trials, it is vital to understand whether these volume changes are a signal of harm, efficacy, or neither. In this review, we examine the potential explanations, their plausibility and fit with available data, and propose areas for further evaluation.

Summary of current evidence

Volume loss in anti-amyloid-beta immunotherapy

Immunotherapies designed to stimulate the removal of Aβ from the brain and so to slow AD progression have been a major focus of therapeutic development over the last 25 years. These efforts started with the AN1792 trial of active immunization against full length Aβ1-42 peptide, the phase II trial of which was stopped after 6% of those on active treatment developed meningoencephalitis.[17] Despite early termination, strong antibody titre-dependent excess brain volume reduction and ventricular enlargement was seen compared with placebo over ~11 months follow-up.[10] Notably, individuals in the highest titre group did *better* cognitively despite having greatest brain volume reductions; this group also had disproportionately greater ventricular volume increase relative to brain loss – a deviation from the normal balance of volume changes seen in AD.[7,10]

Excess brain volume reduction has been observed in many, but not all, subsequent amyloid-beta immunotherapy trials, dependent largely on their abilities to remove amyloid-beta (Table 1 and Figure 1). Notably, despite influencing plasma and CSF amyloid-beta, solanezumab and crenezumab neither achieved significant amyloid reduction on amyloid-PET nor were they associated with excess volume changes.[21-24]

Bapineuzumab was the first amyloid-beta antibody tested in a phase 3 trial. *APOE*-ε4 carriers and non-carriers were enrolled in separate phase 3 trials, with different maximal doses,[25] all of which were relatively low compared to those used in more recent studies. Within each of the trials, relative to placebo, those on treatment showed small or equivocal effects on amyloid burden, accompanied by significant increases in ventricular enlargement, and small (1-2mL) but not statistically significant increases in brain volume loss (Table 1 and [26]). *APOE*-ε4 non-carriers receiving 1mg/kg bapineuzumab (the highest dose) had greater brain and hippocampal volume declines and ventricular enlargement compared with pooled carriers and non-carriers on placebo.[26]

In the ENGAGE and EMERGE phase 3 trials of aducanumab, both of which showed pronounced amyloid removal (54-62 centiloids (CL) from a baseline of 76-77 CL in high dose groups), a dose-dependent increase in ventricular volume was seen in all active treatment arms compared to placebo, with an excess of ~2·6mL

at 78 weeks in the high dose groups; no significant differences in brain or hippocampal volumes were observed.[27]

In the GRADUATE I and II phase 3 trials of gantenerumab, treatment was evaluated out to 116 weeks; in GRADUATE I, treated patients had greater brain volume reduction (3·0% of baseline vs. 2·7% in placebo; an excess of 0·32% or 4·2mL) with proportionally greater reduction in cortical volumes (0·64% of baseline, 3·3mL).[9] A greater expansion in ventricular volume compared to placebo (5mL) was also observed. Very similar changes were seen in GRADUATE II. Gantenerumab did not demonstrate statistically significant clinical benefit in its primary endpoint, though there was robust amyloid removal (56-66 CL reduction relative to placebo from a baseline of 94-96 CL).[9]

The phase 3 studies of lecanemab[12] and donanemab[13] were both positive, achieving their primary outcomes as well as showing robust amyloid removal. Lecanemab treatment reduced amyloid burden from a mean baseline of 78 CL to 23 CL. The MRI outcomes were not initially published,[12] but were presented at Clinical Trials on Alzheimer's Disease conference (CTAD) in 2022.[28] At a dose of 10mg/kg fortnightly, after 79 weeks, there was greater brain volume reduction compared with placebo (21·8mL vs 17·7mL; a difference of 4·1mL, equivalent to 0·4% of baseline brain volume);[12,28] there was also a greater increase (1·8mL) in ventricular volume. Hippocampal volume, however, declined 0·02mL (0·3% of baseline) *less* in the treated compared to the placebo group.

In the donanemab phase 3 trial, participants were stratified by baseline tau-PET and a prespecified analysis was performed examining those with low-medium levels of tau deposition ("low-medium tau population") as well as the full study population ("combined" population). A very similar pattern to lecanemab was seen, with donanemab-treated patients showing a very significant reduction in amyloid burden (a mean of 87 CL, from 103 CL to 16 CL) accompanied by excess brain volume reduction (27·5mL vs 20·8mL; 6·7mL difference, equivalent to ~0·7% of baseline) and ventricular enlargement (3mL).[13] As with lecanemab, there was *less* hippocampal volume loss in treated patients (0·02mL over 76 weeks; $p<0·01$ in the full combined population), although in the low-medium tau population this was not statistically significant. Additional imaging outcome measures for the phase 2 trial of donanemab reported on clinicaltrials.gov shows that there were similar excess volume changes observed in that population with again proportionally greater loss in cortex than in whole brain.[29]

In summary, trials of anti-amyloid monoclonal antibodies that have achieved successful amyloid removal have consistently shown excess brain volume changes (Table 1 and Figure 1A,B) – of a magnitude less than 1% of brain volume. A reasonably consistent pattern of volume change emerges, with proportionally greater excess volume change in the ventricular system than the brain, and in the cortex compared to the brain as a whole.[9,29] Importantly, there is no consistent evidence for excess hippocampal volume loss – indeed in trials showing slowing of cognitive decline there was if anything slight attenuation of hippocampal volume loss.[13,28] All amyloid removing antibodies were associated with ARIA, although rates vary widely between agents; ARIA-E also associates to some extent with ventricular volume change (Figure 1C,D). There are notable differences between agents that remain unexplained.

Other amyloid targeting therapies

Excess volume changes have also been seen with other amyloid-targeting therapies, principally small molecule inhibitors of enzymes involved in amyloid-beta production. With beta-site amyloid precursor protein cleaving enzyme 1 (BACE) inibition (e.g. lanabecestat, verubecestat, atabecestat), excess whole brain and hippocampal volume reduction was seen compared with placebo, with relatively little change in ventricular volume.[11,30,31] With verubecestat, there was an excess brain volume reduction of 4·8mL (0·5% of baseline), excess hippocampal volume reduction of 0·015mL (0·6% of baseline) and minimal change in ventricular volume (0·39mL excess) and very little change in amyloid burden (approx. 3·7CL difference less with verubecestat).[32] These changes were non-progressive after 13 weeks.[32] With atabecestat, excess whole brain volume reduction was observed, and treatment at a group level was associated with worse cognitive outcomes, which reversed after cessation.[30] Semagacestat, a gamma-secretase inhibitor, was associated with increased ventricular volume and a signal to increased hippocampal volume reduction, although this trial was discontinued early so there is significant uncertainty around these outcomes.[33] The distinct temporal and spatial patterns observed in these therapies compared with the volume changes observed in amyloid-beta targeting immunotherapy suggests that different dominant mechanisms underly these observations, - these

enzymes have numerous non-amyloid beta substrates that could mediate these volume changes under inhbition.[34,35]

Possible mechanisms for volume loss with treatment

We now consider possible mechanistic explanations for the changes observed following amyloid-beta targeting immunotherapy. We address initially whether these volume changes could be explained by bulk clearance of Aβ plaques and associated cellular responses, before considering alternative proposed mechanisms including neurodegeneration and fluid shifts.

Amyloid removal?

Given that therapies that induce the most amyloid clearance are associated with the greatest change in cerebral and ventricular volume, could the excess volume loss be explained by removal of Aβ pathology? While the total mass of Aβ peptide in the AD brain has been estimated to be far less than is necessary to account for these volume changes,[36] it is important to note that amyloid plaques occupy a volume much greater than that simply due to the Aβ protein itself. Each plaque also contains a host of other proteins, dystrophic neurites, and is associated with reactive glia and fluid, all of which occupy volume (Figure 2). The dry weight of Aβ in the brain is therefore unlikely to be a good guide to the volume changes one might expect with extensive plaque removal.

Post-mortem estimates of the area fraction (and corresponding cortical volume) occupied by Aβ plaques vary depending on technique. Some studies have examined one cortical region while others have assessed multiple lobes. Estimates of Aβ plaque-related volume include: 5-8% of a range of cortical/subcortical regions;[37] 1% of neocortex;[38] 6·9% of frontal and 10·1% of entorhinal cortex;[39] 6·7% of frontal and visual cortex;[40] 6·7% of supramarginal gyrus;[41] 11% of temporal cortex;[42] 6% of temporal, frontal, parietal and cingulate cortices;[43] and 8·7% of frontal, 6·5% of temporal and 4·5% of caudate.[44] Together these studies suggest that a reasonable estimate of the proportion of cortical grey matter occupied by amyloid-beta plaques in post-mortem AD brain is ~6-8%, i.e. ~2-3% of total brain volume. This is much higher than, and more than enough to account for, the excess volume losses (<1%) seen in the clinical trials of immunotherapies, noting that while the trial population comprises individuals with MCI and mild dementia, all have significant amyloid-beta pathology.

There are relatively few autopsy estimates of the Aβ plaque reduction of patients treated with immunotherapies. A patient previously treated with aducanumab was shown to have markedly reduced temporal neocortical Aβ plaque compared to untreated AD case-controls (area fraction – 0·17% vs 2·5-12%).[45] A subset of patients immunized with AN1792,[46] showed dramatically lower plaque burden even some years after treatment compared to untreated AD case-controls (inferior parietal lobule mean Aβ area fraction – 1·7% vs 7·2%).[47]

A key area that requires explanation is the apparent temporal disconnect between the amyloid PET changes and the volumetric MRI changes, with amyloid removal occurring early at a group level and then plateauing, whereas the volume changes continue throughout the trials.[12,13] This suggests that amyloid removal is not the sole explanatory factor: complete removal of plaques (including dystrophic neurites etc.) and resolution of the associated inflammatory cell response, discussed below, may both be important and both may lag behind reductions on amyloid PET.

Changes in the cellular response to the presence of amyloid?

The cellular response to Aβ deposition is highly complex and includes, amongst other processes, reactive astrogliosis and microglial activation.[48] In addition to the volume changes that might be explained by direct plaque removal another contributing factor could be attenuation of the cellular response to aggregated Aβ. There is some evidence that immunotherapy-induced clearance of plaques may reduce some elements of this cellular response – donanemab and lecanemab reduce plasma GFAP, a marker of astrocytosis;[12,49] at post-mortem increased microglial plaque engagement was seen after treatment with aducanumab, although the total burden of microglia was not reported.[45]

With active immunotherapy, an initial increase in microglial activity is a proposed key mechanism of plaque clearance, which is followed by dispersal and downregulation after amyloid-beta clearance.[50,51] Histological studies in patients who received AN1792 showed the percentage area of cerebral cortex occupied by microglia was halved compared with untreated AD (CD64 microglial marker: AN1792 treated AD 0·4% vs untreated AD 1·1%);[51] these changes could contribute either directly or indirectly to the volume reduction observed. Qualitative observations indicate that plaque-associated astrocytes also become less activated and they too reduce in size, and although astrocyte changes were not quantified in a similar manner to microglia, it seems likely that changes in astrocytes could also contribute to the volume changes observed.[52] There is also pathologic evidence that this astrocytic response is not attenuated until there is complete plaque removal – which could be a factor accounting for the temporal disconnect described above.[53]

Excess cerebral volume loss has been observed in trials of anti-inflammatory agents in AD such as resveratrol.[54] Analogies have also been drawn between the excess volume loss in AD immunotherapy with the volume loss observed in highly active DMTs for multiple sclerosis (e.g. natalizumab), where there is an initial accelerated volume loss with treatment (referred to as "pseudoatrophy"), presumed due to a reduction in inflammation and/or fluid shifts, followed by a slowing of brain volume loss with treatment, presumed due to disease modification.[15,55,56] Longer follow-up is required to see whether similar patterns are seen in patients with AD treated with effective immunotherapy.

If Aβ removal and/or the attenuation of the cellular response does account for the excess brain volume losses seen in these trials it is reasonable to ask whether amyloid-beta deposition (albeit over a much longer time frame) is associated with volume increases. There is some evidence in support of this, with increased cortical thickness reported in the early stages of the Alzheimer's continuum, before subsequent atrophy rates increase and likely obscure any volume effects of continuing amyloid accumulation.[57-61] These changes are also associated with markers of cellular response, including MRI, PET and CSF markers of both reactive astrogliosis and microglial activation.[61-63]

Neuronal changes, accelerated neurodegeneration?

The possibility that the excess volume loss seen with immunotherapies might reflect accelerated neurodegeneration (i.e. an increased rate of neuronal loss) is of course the greatest concern. Possible mechanisms for this could include deletrious effects of Aβ oligomer release following plaque clearance, as a consequence of ARIA, or unknown off-target effects.[11]

From a clinical perspective and acknowledging that follow-up is to date limited, it is notable that in the lecanemab and donanemab phase 3 trials, patients on treatment had, at a group level, less clinical decline despite showing increased brain volume reductions.[12,13] In a comparison of results across multiple different drug targets in AD trials, Aβ removing antibodies consistently show a dissociation between (excess) volume changes and (improved) cognitive outcomes (Figure 1E,F), in contrast with other therapies where excess volume losses were associated with poorer outcomes.[64] It is conceivable that any clinical detriment associated with excess volume loss could be delayed, but based on the limited longer term data available there is no evidence for this – in the lecanemab phase 2 open-label extension, where treatment was interrupted prior to the open label extension for an average of 24 months (range 9-59 months), there was no delayed worsening in the treated group, although this should be interpreted cautiously due to possible selective attrition.[65]

Arguing against the volume changes associated with Aβ immunotherapy being due to accelerated AD neurodegeneration is that, as highlighted above, the hippocampi – brain regions typically associated with some of the most pronounced neurodegeneration and volume loss in AD – are spared.

Another argument against the hypothesis of treatment-accelerated neurodegeneration as the principal explanation for brain volume loss is that CSF and plasma neurofilament light (NfL) and t-tau concentrations typically remained stable or decreased during treatment.[66] These markers predict brain volume loss due to neurodegeneration measured by imaging,[67] are more sensitive than imaging measures to detect neuroaxonal injury in mild brain trauma,[68] and can be used to detect drug-related neurotoxicity in trials and clinical practice in other fields of neurology.[69-71] More specifically, treatment with lecanemab demonstrated a reduction in CSF t-tau, a small reduction in plasma NfL, and stable CSF NfL concentration.[12] In the phase 3

trial of donanemab, plasma NfL was increased relative to placebo at week 24 but subsequently reduced relative to placebo in weeks 52 and 76.[18] In an analysis of phase 2 trial data of donanemab, increasing plasma NfL was correlated with a reduction in brain volume but this did not separate excess volume change attributable to donanemab treatment with volume loss due to disease progression.[49] With gantenerumab, treatment was associated with lower CSF NfL and t-tau.[9]

Post-mortem studies of AN1792-immunized patients did suggest some increased neuronal loss and cortical spongiotic change (compared to AD-controls), but also raised the possibility of improved health of residual neurons with less neuritic curvature and the presence of fewer pro-apoptotic neurons in the immunized brains, interpreted as due to the removal of "sick" neurons.[53,72,73] This was consistent with the reduction in other Aβ plaque-associated components such as dystrophic neurites, intraneuronal hyperphosphorylated tau, apo-E proteins and an overall reduction in pro-apoptotic proteins,[46,72,74,75] i.e. consistent with the "changes in the cellular response to the presence of amyloid" hypothesis, above.

The role of ARIA?

ARIA has been proposed as a cause for excess volume loss.[11] While ARIA can cause acute clinical manifestations, and rarely death, to date there has been no link between ARIA and long-term adverse cognitive outcomes. *APOE*-ε4 carriers have higher rates of ARIA, however they appear to derive similar clinical benefits from immunotherapy,[76] although the benefits for *APOE*-ε4 homozygotes are less clear than in heterozygotes or non-carriers (with a negative point estimate for lecanemab and positive for donanemab). This may be mediated by ARIA, or could be due to the relatively small number of *APOE*-ε4 homozygotes – there were wide confidence intervals for these point estimates, and warrants further evaluation.[12,13] There is a correlation between ARIA-E incidence and treatment-related increases in ventricular volumes (Figure 1), although as discussed above, this may be confounded by more pronounced amyloid removal.[11] In a post-hoc analysis of the bapineuzumab trials, participants with ARIA-E had more amyloid removal on PET, a greater increase in ventricular volume, and greater hippocampal volume reduction; however higher *APOE*-ε4 carrier frequency in the ARIA group or other factors may have confounded these observations.[77] ARIA may lead to focal reductions in amyloid-PET but whether this translates to regional volume loss has, to our knowledge, not to date been evaluated.[78,79]

Fluid shifts?

The apparent disproportionate ventricular enlargement relative to brain volume reduction raises the possibility that Aβ immunotherapy may result in alteration in CSF dynamics, e.g. impaired CSF resorption, leading to ventriculomegaly.[11,15] Immunotherapy related solubilization and mobilization of Aβ to the vessel wall with associated inflammation could be a common pathway: altered glymphatic function and/or leakage of intravascular fluid into the parenchymal interstitial space manifests as parenchymal ARIA-E, involvement of leptomeningeal vessels leading to leakage of proteinaceous fluid into the subarachnoid space manifests as sulcal ARIA-E,[16] and each of these in turn could impede CSF resorption resulting in ventricular enlargement. In many other areas of neurology, therapies cause brain volume changes unrelated to neurodegeneration and are instead due to reduced inflammation or fluid shifts, such as with acute corticosteroid treatment, mannitol administration or hemodialysis.[80-82]

Conclusion

The explanation for the observed brain volume changes in anti-Aβ immunotherapy trials is incompletely understood and likely multifactorial. There are many unanswered questions (Panel 2), including the longer term trajectory of volume changes and, critically, whether excess volume change after amyloid-beta removal adversely influences longer term outcomes. Given these medications are entering clinical practice and undergoing regulatory evaluation, urgent examination and reporting of patient level data from the existing large datasets from the published trials is needed. Scrutiny of the available data does, however, allow for a number of conclusions. (1) Excess volume loss is only seen with immunotherapies that achieve amyloid removal, and the magnitude of excess volume loss appears to be related to the extent of amyloid removal. (2) This excess volume loss spares the hippocampi, and is not associated with worse cognitive outcomes (at a group level), arguing against this being substantially due to neurodegeneration. (3) The volume occupied by Aβ plaques in the brains of people with AD is not trivial (~6% of cortex at post-mortem). The extent of excess volume change seen in treated patients is considerably lower than this and, even allowing for the fact

that immunotherapy trials involve people at much earlier stages of the disease with lower plaque burdens, the highly effective removal of Aβ plaques could reasonably explain the changes, through plaque clearance and plaque-associated glial changes, likely accompanied by fluid shifts. We suggest that available evidence suggests that this phenomena is neither "paradoxical" nor due to accelerated neurodegeneration, and pending longer term outcome data and further mechanistic insights, could now be referred to as "amyloid-removal related pseudo-atrophy (ARPA)". With this we do not aim to diminish its significance, but rather to facilitate the use of a common term for research and clinical trials. Analysis of existing patient-level clinical trial data is urgently needed, and longer term follow up will be important to clarify whether these volume changes are an indicator of efficacy rather than a cause for concern – or neither. For future trials, MRI volume outcomes should be clearly and transparently reported as key safety measures alongside ARIA. We predict that effective therapies that slow neurodegeneration enough and for long enough will ultimately also slow rates of atrophy – the hypothesis with which incorporating serial MRI measurments in trials began.

Panel 1: Volume loss in Alzheimer's disease - natural history

Cerebral volume loss in AD is closely associated with cognitive loss, both temporally and spatially, in natural history studies.[2] Typical, amnestic, AD has a characteristic pattern of atrophy, thought to relate to tau pathology and neuronal loss, with disproportionate hippocampal atrophy; over time atrophy becomes more generalized and rates increase as individuals become symptomatic.[2-6] For example, in healthy individuals in their 70s, whole brain atrophy rates are on average around 0·5%/year increasing to 1%/year in mild cognitive impairment and to 1·5%/year in mild AD dementia, for hippocampus the rates are 1%/year in controls, 2·6%/year in MCI and 4·4%/year in AD, and ventricular volumes increase by 1.4mL/year in controls, 2.8mL/year in MCI and 4.5mL/year in AD.[7] It was these differences in atrophy rates between AD and healthy aging, the precision with which they could be measured, and their association with cognitive decline that led to the widespread adoption of atrophy rates as outcome measures in AD trials.[8] These rates hold for the early AD populations included in current amyloid immunotherapy trials, for example, in the placebo arms of the GRADUATE trials of gantenerumab, there was an annual brain volume loss of 1·2%, cortical grey matter loss of 1·5% and hippocampal volume loss of 4%.[9]

Panel 2: Gaps in current evidence – key areas for further evaluation

- On an individual patient level, does the excess volume reduction with amyloid immunotherapy maintain the same negative clinical and biomarker associations with volume loss in the natural history of AD or do these associations loosen, as has been noted at a group level?[64]
- What happens to cerebral volumes beyond the duration reported in current trials – do these observations represent a consistently increased rate of volume loss with ongoing treatment, or does the excess volume change plateau (or decrease) once optimal removal of amyloid is achieved? How do these volume changes relate to longer term clinical outcomes?
- What brain regions are driving these volume changes, as the ventricular and whole brain volumes most commonly reported are not region specific?
- At the invidividual patient level, how related (both in extent and topography) are these excess brain volume changes to the amount of amyloid removed (as measured by PET) and the presence of ARIA?
- Do markers of glymphatic function and CSF dynamics influence volume changes in the presence of amyloid-removing immunotherapy (or the converse)? Is the increase in ventricular volume associated with an adverse change in CSF dynamics?

Figure 1

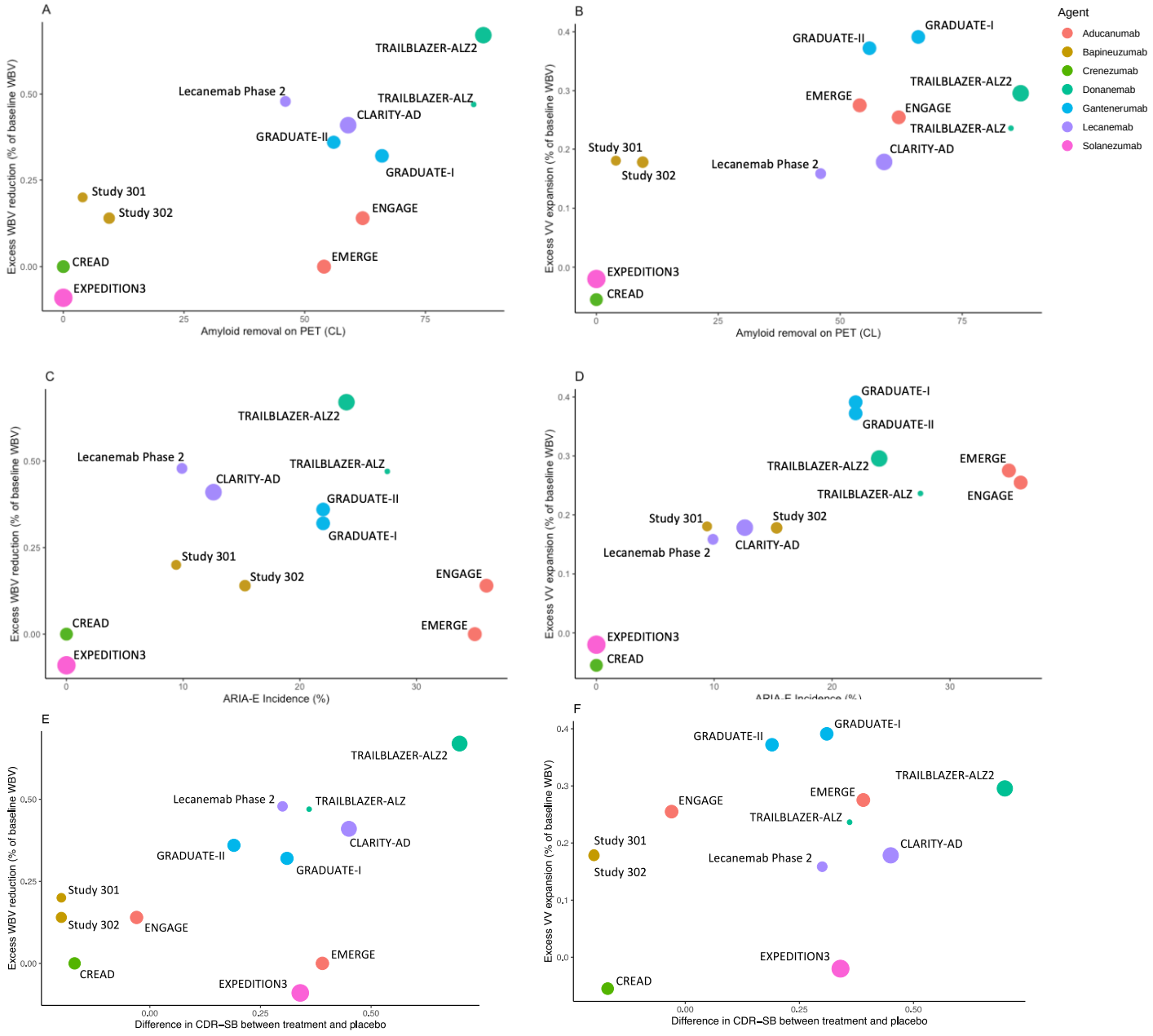

Figure 1 A-F illustrates whole brain volume (WBV) and ventricular volume (VV) outcomes in key trials, each represented as treatment group – placebo group. A) WBV excess reduction expressed as a percentage of baseline WBV plotted against amyloid removal on PET in centiloids (CL). B) VV excess expansion expressed as a percentage of baseline WBV plotted against amyloid removal on PET in CL. C) WBV excess reduction expressed as a percentage of baseline WBV plotted against ARIA-E incidence D) VV excess expansion expressed as a percentage of baseline WBV against ARIA-E incidence. E) WBV excess reduction expressed as a percentage of baseline WBV plotted against the mean difference in CDR-SB. F) VV excess expansion expressed as a percentage of baseline WBV against the mean difference in CDR-SB; the bapineuzumab trials (Study 301 and 302) are overplotted due to an identical point estimate. Mean difference in CDR-SB presented as it was the primary outcome for 6/12 depicted studies and reported as a secondary endpoint for the remainder; for consistency this is presented so a positive value represents benefit in treatment group relative to controls. Points are coloured by agent and their area scaled by number included in the imaging analysis of the respective trial. In each trial, if multiple doses were used the highest dose arm was included, excepting the lecanemab phase 2 data which is reported as a weighted mean of the 10mg/kg 2-weekly and monthly treatment arms due to changes in randomization of APOE-e4 carriers during the trial.

Figure 2

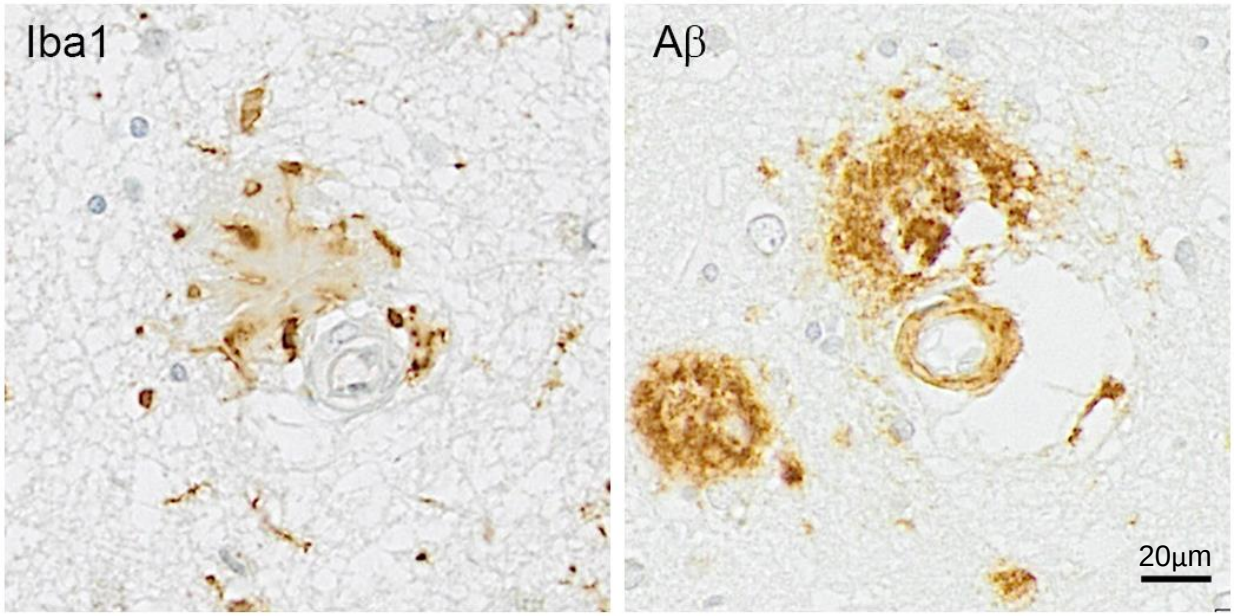

Figure 2 - Microglia clustering around Aβ plaques in the cortex of inferior parietal lobule from an 84-year old women diagnosed with Alzheimer's disease. Antibodies employed: Aβ (pan-Ab 4G8, Covance), Iba1 (microglia, Wako). Slides counterstained with H&E. Slides digitized on a Olympus VS110 slide scanner (Olympus America Inc.). Scale bar = 20 µm. Tissue sourced from South West Dementia Brain Bank (NRES Committee South West Central Bristol, REC reference: 08/H0106/28 + 5).

Search strategy

References were identified using PubMed search terms "Alzheimer's disease" AND "amyloid" AND "immun*" AND "trial". ClinicalTrials.gov and AlzForum.org were also searched for immunotherapies (active and passive) targeting amyloid-beta in Alzheimer's disease and publications covering clinical or biomarker endpoints were sought. An initial search was performed for papers published January 2000 - March 2023 by CRSB, with contributions from NCF. It was repeated after the subsequent publications of additional phase 3 trials key to the subject matter (donanemab and gantenerumab), with the final paper considering publications through to May 2024. Conference presentations reporting relevant biomarker endpoints were also sought if not included in primary publications. Papers were included based on relevance of intervention and reported outcomes to the content of this review. The reference lists of papers generated in this way were also examined for relevance to the discussion and additional papers were included from this.

Author's Contributions

CRSB - conceptualisation, literature review, writing - original draft, review & editing, preparation of figures; NCF - conceptualisation, literature review, writing – reviewing & editing. DB – conceptualisation, writing – review and editing, preparation of figure; JARN – conceptualisation, writing – review and editing; ZJ – writing, review and editing; HZ – writing, review and editing; JMS - data interpretation, writing - reviewing and editing; FB – conceptualisation, literature review, writing – review and editing.

Declaration of Interests

DB has been a consultant/advisor relating to Alzheimer immunization programmes for: Elan Pharmaceuticals (travel and accommodation) and Biogen (consultancy fees). JARN has been a consultant/advisor relating to Alzheimer immunization programmes for: Elan Pharmaceuticals (travel and accommodation), GlaxoSmithKline (consultancy fees), Novartis, Roche (consultancy fees), Janssen (consultancy fees), Pfizer, Biogen (consultancy fees, travel and accommodation), and Eisai. HZ has served at scientific advisory boards and/or as a consultant for Abbvie, Acumen, Alector, Alzinova, ALZPath, Amylyx,

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Brain volume change following amyloid-beta immunotherapy for Alzheimer's disease: amyloid-removal related pseudo-atrophy

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Summary

Progressive cerebral volume loss on MRI is a hallmark of Alzheimer's disease and has been widely used as an outcome in Alzheimer trials, with the prediction that disease-modifying treatments would slow loss. However, multiple anti-amyloid immunotherapy trials reported *excess* volume loss with treatment. Explanations for this range from reduced amyloid-beta plaque burden and related inflammatory changes, through to treatment-induced toxicity. We review these hypotheses and their compatibility with data arising from amyloid immunotherapy trials and histopathological findings. We conclude that these excess volume changes are characteristic of *only* those immunotherapies that achieve amyloid-beta lowering; *are* compatible with plaque removal; and that evidence to date does *not* suggest an association with harm. Understanding the causes, and consequences, of these changes is important to enable informed decisions about treatments. Patient-level analyses of trials is urgently needed along with longitudinal follow-up and imaging to determine the longer-term trajectory of volume changes and clinical correlates. Post-mortem examination of cerebral tissue from treated patients and correlation with antemortem imaging is a priority. Based on current evidence, we propose the provisional term "amyloid-removal related pseudo-atrophy (ARPA)" to describe this phenomenon.

Introduction

Progressive cerebral volume loss, often referred to as atrophy, is a characteristic and diagnostic feature of Alzheimer's disease (AD) and an accepted biomarker of neurodegeneration (Panel 1).[1] Measurement of global and regional brain volume changes from serial magnetic resonance imaging (MRI) has been widely used in trials of candidate disease-modifying treatments (DMTs), the presumption being that effective treatment would, in time, slow neurodegeneration and lead to a reduction in rates of brain volume loss.[7,8]

However, in the first trial of immunization against amyloid-beta (Aβ) using the agent AN1792, *excess* volume loss was observed in patients on active drug – considered "paradoxical" at the time.[10] A similar phenomenon was seen subsequently in several other immunotherapies directed at Aβ,[11] including recently reported phase 3 trials of gantenerumab, lecanemab and donanemab.[9,12,13] The cause of this "paradoxical" volume loss is not well understood, but has led to concerns that it might represent accelerated neurodegeneration and so lead to deleterious long term outcomes.[11,14] Other proposed explanations include that the excess volume loss is due to removal of Aβ plaques, reduction in plaque-associated inflammatory changes, or alterations in cerebrospinal fluid (CSF) dynamics.[15]

One of the difficulties in disentangling causation is that therapies that are effective in removing Aβ also cause the potentially serious side effect of amyloid-related imaging abnormalities with oedema/effusions (ARIA-E) or microhaemorrhages (ARIA-H),[16] which in turn might influence brain volume.

Given that some of these treatments are now in clinical use and others are in or entering clinical trials, it is vital to understand whether these volume changes are a signal of harm, efficacy, or neither. In this review, we examine the potential explanations, their plausibility and fit with available data, and propose areas for further evaluation.

Summary of current evidence

Volume loss in anti-amyloid-beta immunotherapy

Immunotherapies designed to stimulate the removal of Aβ from the brain and so to slow AD progression have been a major focus of therapeutic development over the last 25 years. These efforts started with the AN1792 trial of active immunization against full length Aβ1-42 peptide, the phase II trial of which was stopped after 6% of those on active treatment developed meningoencephalitis.[17] Despite early termination, strong antibody titre-dependent excess brain volume reduction and ventricular enlargement was seen compared with placebo over ~11 months follow-up.[10] Notably, individuals in the highest titre group did *better* cognitively despite having greatest brain volume reductions; this group also had disproportionately greater ventricular volume increase relative to brain loss – a deviation from the normal balance of volume changes seen in AD.[7,10]

Excess brain volume reduction has been observed in many, but not all, subsequent amyloid-beta immunotherapy trials, dependent largely on their abilities to remove amyloid-beta (Table 1 and Figure 1). Notably, despite influencing plasma and CSF amyloid-beta, solanezumab and crenezumab neither achieved significant amyloid reduction on amyloid-PET nor were they associated with excess volume changes.[21-24]

Bapineuzumab was the first amyloid-beta antibody tested in a phase 3 trial. *APOE*-ε4 carriers and non-carriers were enrolled in separate phase 3 trials, with different maximal doses,[25] all of which were relatively low compared to those used in more recent studies. Within each of the trials, relative to placebo, those on treatment showed small or equivocal effects on amyloid burden, accompanied by significant increases in ventricular enlargement, and small (1-2mL) but not statistically significant increases in brain volume loss (Table 1 and [26]). *APOE*-ε4 non-carriers receiving 1mg/kg bapineuzumab (the highest dose) had greater brain and hippocampal volume declines and ventricular enlargement compared with pooled carriers and non-carriers on placebo.[26]

In the ENGAGE and EMERGE phase 3 trials of aducanumab, both of which showed pronounced amyloid removal (54-62 centiloids (CL) from a baseline of 76-77 CL in high dose groups), a dose-dependent increase in ventricular volume was seen in all active treatment arms compared to placebo, with an excess of ~2·6mL

at 78 weeks in the high dose groups; no significant differences in brain or hippocampal volumes were observed.[27]

In the GRADUATE I and II phase 3 trials of gantenerumab, treatment was evaluated out to 116 weeks; in GRADUATE I, treated patients had greater brain volume reduction (3·0% of baseline vs. 2·7% in placebo; an excess of 0·32% or 4·2mL) with proportionally greater reduction in cortical volumes (0·64% of baseline, 3·3mL).[9] A greater expansion in ventricular volume compared to placebo (5mL) was also observed. Very similar changes were seen in GRADUATE II. Gantenerumab did not demonstrate statistically significant clinical benefit in its primary endpoint, though there was robust amyloid removal (56-66 CL reduction relative to placebo from a baseline of 94-96 CL).[9]

The phase 3 studies of lecanemab[12] and donanemab[13] were both positive, achieving their primary outcomes as well as showing robust amyloid removal. Lecanemab treatment reduced amyloid burden from a mean baseline of 78 CL to 23 CL. The MRI outcomes were not initially published,[12] but were presented at Clinical Trials on Alzheimer's Disease conference (CTAD) in 2022.[28] At a dose of 10mg/kg fortnightly, after 79 weeks, there was greater brain volume reduction compared with placebo (21·8mL vs 17·7mL; a difference of 4·1mL, equivalent to 0·4% of baseline brain volume);[12,28] there was also a greater increase (1·8mL) in ventricular volume. Hippocampal volume, however, declined 0·02mL (0·3% of baseline) *less* in the treated compared to the placebo group.

In the donanemab phase 3 trial, participants were stratified by baseline tau-PET and a prespecified analysis was performed examining those with low-medium levels of tau deposition ("low-medium tau population") as well as the full study population ("combined" population). A~~a~~ very similar pattern to lecanemab was seen, with donanemab-treated patients showing a very significant reduction in amyloid burden (a mean of 87 CL, from 103 CL to 16 CL) accompanied by excess brain volume reduction (27·5mL vs 20·8mL; 6·7mL difference, equivalent to ~0·7% of baseline) and ventricular enlargement (3mL).[13] As with lecanemab, there was *less* hippocampal volume loss in treated patients (0·02mL over 76 weeks; $p<0·01$ in the full combined population), although in the low-medium tau population this was not statistically significant. Additional imaging outcome measures for the phase 2 trial of donanemab reported on clinicaltrials.gov shows that there were similar excess volume changes observed in that population with again proportionally greater loss in cortex than in whole brain.[29]

In summary, trials of anti-amyloid monoclonal antibodies that have achieved successful amyloid removal have consistently shown excess brain volume changes (Table 1 and Figure 1A,B) – of a magnitude less than 1% of brain volume. A reasonably consistent pattern of volume change emerges, with proportionally greater excess volume change in the ventricular system than the brain, and in the cortex compared to the brain as a whole.[9,29] Importantly, there is no consistent evidence for excess hippocampal volume loss – indeed in trials showing slowing of cognitive decline there was if anything slight attenuation of hippocampal volume loss.[13,28] All amyloid removing antibodies were associated with ARIA, although rates vary widely between agents; ARIA-E also associates to some extent with ventricular volume change (Figure 1C,D). There are notable differences between agents that remain unexplained.

Other amyloid targeting therapies

Excess volume changes have also been seen with other amyloid-targeting therapies, principally small molecule inhibitors of enzymes involved in amyloid-beta production. With beta-site amyloid precursor protein cleaving enzyme 1 (BACE) inibition (e.g. lanabecestat, verubecestat, atabecestat), excess whole brain and hippocampal volume reduction was seen compared with placebo, with relatively little change in ventricular volume.[11,30,31] With verubecestat, there was an excess brain volume reduction of 4·8mL (0·5% of baseline), excess hippocampal volume reduction of 0·015mL (0·6% of baseline) and minimal change in ventricular volume (0·39mL excess) and very little change in amyloid burden (approx. 3·7CL difference less with verubecestat).[32] These changes were non-progressive after 13 weeks.[32] With atabecestat, excess whole brain volume reduction was observed, and treatment at a group level was associated with worse cognitive outcomes, which reversed after cessation.[30] Semagacestat, a gamma-secretase inhibitor, was associated with increased ventricular volume and a signal to increased hippocampal volume reduction, although this trial was discontinued early so there is significant uncertainty around these outcomes.[33] The distinct temporal and spatial patterns observed in these therapies compared with the volume changes observed in amyloid-beta targeting immunotherapy suggests that different dominant mechanisms underly these observations, - these

enzymes have numerous non-amyloid beta substrates that could mediate these volume changes under inhbition.[34,35]

Possible mechanisms for volume loss with treatment

We now consider possible mechanistic explanations for the changes observed following amyloid-beta targeting immunotherapy. We address initially whether these volume changes could be explained by bulk clearance of Aβ plaques and associated cellular responses, before considering alternative proposed mechanisms including neurodegeneration and fluid shifts.

Amyloid removal?

Given that therapies that induce the most amyloid clearance are associated with the greatest change in cerebral and ventricular volume, could the excess volume loss be explained by removal of Aβ pathology? While the total mass of Aβ peptide in the AD brain has been estimated to be far less than is necessary to account for these volume changes,[36] it is important to note that amyloid plaques occupy a volume much greater than that simply due to the Aβ protein itself. Each plaque also contains a host of other proteins, dystrophic neurites, and is associated with reactive glia and fluid, all of which occupy volume (Figure 2). The dry weight of Aβ in the brain is therefore unlikely to be a good guide to the volume changes one might expect with extensive plaque removal.

Post-mortem estimates of the area fraction (and corresponding cortical volume) occupied by Aβ plaques vary depending on technique. Some studies have examined one cortical region while others have assessed multiple lobes. Estimates of Aβ plaque-related volume include: 5-8% of a range of cortical/subcortical regions;[37] 1% of neocortex;[38] 6·9% of frontal and 10·1% of entorhinal cortex;[39] 6·7% of frontal and visual cortex;[40] 6·7% of supramarginal gyrus;[41] 11% of temporal cortex;[42] 6% of temporal, frontal, parietal and cingulate cortices;[43] and 8·7% of frontal, 6·5% of temporal and 4·5% of caudate.[44] Together these studies suggest that a reasonable estimate of the proportion of cortical grey matter occupied by amyloid-beta plaques in post-mortem AD brain is ~6-8%, i.e. ~2-3% of total brain volume. This is much higher than, and more than enough to account for, the excess volume losses (<1%) seen in the clinical trials of immunotherapies, noting that while the trial population comprises individuals with MCI and mild dementia, all have significant amyloid-beta pathology.

There are relatively few autopsy estimates of the Aβ plaque reduction of patients treated with immunotherapies. A patient previously treated with aducanumab was shown to have markedly reduced temporal neocortical Aβ plaque compared to untreated AD case-controls (area fraction – 0·17% vs 2·5-12%).[45] A subset of patients immunized with AN1792,[46] showed dramatically lower plaque burden even some years after treatment compared to untreated AD case-controls (inferior parietal lobule mean Aβ area fraction – 1·7% vs 7·2%).[47]

A key area that requires explanation is the apparent temporal disconnect between the amyloid PET changes and the volumetric MRI changes, with amyloid removal occurring early at a group level and then plateauing, whereas the volume changes continue throughout the trials.[12,13] This suggests that amyloid removal is not the sole explanatory factor: complete removal of plaques (including dystrophic neurites etc.) and resolution of the associated inflammatory cell response, discussed below, may both be important and both may lag behind reductions on amyloid PET.

Changes in the cellular response to the presence of amyloid?

The cellular response to Aβ deposition is highly complex and includes, amongst other processes, reactive astrogliosis and microglial activation.[48] In addition to the volume changes that might be explained by direct plaque removal another contributing factor could be attenuation of the cellular response to aggregated Aβ. There is some evidence that immunotherapy-induced clearance of plaques may reduce some elements of this cellular response – donanemab and lecanemab reduce plasma GFAP, a marker of astrocytosis;[12,49] at post-mortem increased microglial plaque engagement was seen after treatment with aducanumab, although the total burden of microglia was not reported.[45]

With active immunotherapy, an initial increase in microglial activity is a proposed key mechanism of plaque clearance, which is followed by dispersal and downregulation after amyloid-beta clearance.[50,51] Histological studies in patients who received AN1792 showed the percentage area of cerebral cortex occupied by microglia was halved compared with untreated AD (CD64 microglial marker: AN1792 treated AD 0·4% vs untreated AD 1·1%);[51] these changes could contribute either directly or indirectly to the volume reduction observed. Qualitative observations indicate that plaque-associated astrocytes also become less activated and they too reduce in size, and although astrocyte changes were not quantified in a similar manner to microglia, it seems likely that changes in astrocytes could also contribute to the volume changes observed.[52] There is also pathologic evidence that this astrocytic response is not attenuated until there is complete plaque removal – which could be a factor accounting for the temporal disconnect described above.[53]

Excess cerebral volume loss has been observed in trials of anti-inflammatory agents in AD such as resveratrol.[54] Analogies have also been drawn between the excess volume loss in AD immunotherapy with the volume loss observed in highly active DMTs for multiple sclerosis (e.g. natalizumab), where there is an initial accelerated volume loss with treatment (referred to as "pseudoatrophy"), presumed due to a reduction in inflammation and/or fluid shifts, followed by a slowing of brain volume loss with treatment, presumed due to disease modification.[15,55,56] Longer follow-up is required to see whether similar patterns are seen in patients with AD treated with effective immunotherapy.

If Aβ removal and/or the attenuation of the cellular response does account for the excess brain volume losses seen in these trials it is reasonable to ask whether amyloid-beta deposition (albeit over a much longer time frame) is associated with volume increases. There is some evidence in support of this, with increased cortical thickness reported in the early stages of the Alzheimer's continuum, before subsequent atrophy rates increase and likely obscure any volume effects of continuing amyloid accumulation.[57-61] These changes are also associated with markers of cellular response, including MRI, PET and CSF markers of both reactive astrogliosis and microglial activation.[61-63]

Neuronal changes, accelerated neurodegeneration?

The possibility that the excess volume loss seen with immunotherapies might reflect accelerated neurodegeneration (i.e. an increased rate of neuronal loss) is of course the greatest concern. Possible mechanisms for this could include deletrious effects of Aβ oligomer release following plaque clearance, as a consequence of ARIA, or unknown off-target effects.[11]

From a clinical perspective and acknowledging that follow-up is to date limited, it is notable that in the lecanemab and donanemab phase 3 trials, patients on treatment had, at a group level, less clinical decline despite showing increased brain volume reductions.[12,13] In a comparison of results across multiple different drug targets in AD trials, Aβ removing antibodies consistently show a dissociation between (excess) volume changes and (improved) cognitive outcomes (Figure 1E,F), in contrast with other therapies where excess volume losses were associated with poorer outcomes.[64] It is conceivable that any clinical detriment associated with excess volume loss could be delayed, but based on the limited longer term data available there is no evidence for this – in the lecanemab phase 2 open-label extension, where treatment was interrupted prior to the open label extension for an average of 24 months (range 9-59 months), there was no delayed worsening in the treated group, although this should be interpreted cautiously due to possible selective attrition.[65]

Arguing against the volume changes associated with Aβ immunotherapy being due to accelerated AD neurodegeneration is that, as highlighted above, the hippocampi – brain regions typically associated with some of the most pronounced neurodegeneration and volume loss in AD – are spared.

Another argument against the hypothesis of treatment-accelerated neurodegeneration as the principal explanation for brain volume loss is that CSF and plasma neurofilament light (NfL) and t-tau concentrations typically remained stable or decreased during treatment.[66] These markers predict brain volume loss due to neurodegeneration measured by imaging,[67] are more sensitive than imaging measures to detect neuroaxonal injury in mild brain trauma,[68] and can be used to detect drug-related neurotoxicity in trials and clinical practice in other fields of neurology.[69-71] More specifically, treatment with lecanemab demonstrated a reduction in CSF t-tau, a small reduction in plasma NfL, and stable CSF NfL concentration.[12] In the phase 3

trial of donanemab, plasma NfL was increased relative to placebo at week 24 but subsequently reduced relative to placebo in weeks 52 and 76.[18] In an analysis of phase 2 trial data of donanemab, increasing plasma NfL was correlated with a reduction in brain volume but this did not separate excess volume change attributable to donanemab treatment with volume loss due to disease progression.[49] With gantenerumab, treatment was associated with lower CSF NfL and t-tau.[9]

Post-mortem studies of AN1792-immunized patients did suggest some increased neuronal loss and cortical spongiotic change (compared to AD-controls), but also raised the possibility of improved health of residual neurons with less neuritic curvature and the presence of fewer pro-apoptotic neurons in the immunized brains, interpreted as due to the removal of "sick" neurons.[53,72,73] This was consistent with the reduction in other Aβ plaque-associated components such as dystrophic neurites, intraneuronal hyperphosphorylated tau, apo-E proteins and an overall reduction in pro-apoptotic proteins,[46,72,74,75] i.e. consistent with the "changes in the cellular response to the presence of amyloid" hypothesis, above.

The role of ARIA?

ARIA has been proposed as a cause for excess volume loss.[11] While ARIA can cause acute clinical manifestations, and rarely death, to date there has been no link between ARIA and long-term adverse cognitive outcomes. *APOE*-ε4 carriers have higher rates of ARIA, however they appear to derive similar clinical benefits from immunotherapy,[76] although the benefits for *APOE*-ε4 homozygotes are less clear than in heterozygotes or non-carriers (with a negative point estimate for lecanemab and positive for donanemab). This may be mediated by ARIA, or could be due to the relatively small number of *APOE*-ε4 homozygotes – there were wide confidence intervals for these point estimates, and warrants further evaluation.[12,13] There is a correlation between ARIA-E incidence and treatment-related increases in ventricular volumes (Figure 1), although as discussed above, this may be confounded by more pronounced amyloid removal.[11] In a post-hoc analysis of the bapineuzumab trials, participants with ARIA-E had more amyloid removal on PET, a greater increase in ventricular volume, and greater hippocampal volume reduction; however higher *APOE*-ε4 carrier frequency in the ARIA group or other factors may have confounded these observations.[77] ARIA may lead to focal reductions in amyloid-PET but whether this translates to regional volume loss has, to our knowledge, not to date been evaluated.[78,79]

Fluid shifts?

The apparent disproportionate ventricular enlargement relative to brain volume reduction raises the possibility that Aβ immunotherapy may result in alteration in CSF dynamics, e.g. impaired CSF resorption, leading to ventriculomegaly.[11,15] Immunotherapy related solubilization and mobilization of Aβ to the vessel wall with associated inflammation could be a common pathway: altered glymphatic function and/or leakage of intravascular fluid into the parenchymal interstitial space manifests as parenchymal ARIA-E, involvement of leptomeningeal vessels leading to leakage of proteinaceous fluid into the subarachnoid space manifests as sulcal ARIA-E,[16] and each of these in turn could impede CSF resorption resulting in ventricular enlargement. In many other areas of neurology, therapies cause brain volume changes unrelated to neurodegeneration and are instead due to reduced inflammation or fluid shifts, such as with acute corticosteroid treatment, mannitol administration or hemodialysis.[80-82]

Conclusion

The explanation for the observed brain volume changes in anti-Aβ immunotherapy trials is incompletely understood and likely multifactorial. There are many unanswered questions (Panel 2), including the longer term trajectory of volume changes and, critically, whether excess volume change after amyloid-beta removal adversely influences longer term outcomes. Given these medications are entering clinical practice and undergoing regulatory evaluation, urgent examination and reporting of patient level data from the existing large datasets from the published trials is needed. Scrutiny of the available data does, however, allow for a number of conclusions. (1) Excess volume loss is only seen with immunotherapies that achieve amyloid removal, and the magnitude of excess volume loss appears to be related to the extent of amyloid removal. (2) This excess volume loss spares the hippocampi, and is not associated with worse cognitive outcomes (at a group level), arguing against this being substantially due to neurodegeneration. (3) The volume occupied by Aβ plaques in the brains of people with AD is not trivial (~6% of cortex at post-mortem). The extent of excess volume change seen in treated patients is considerably lower than this and, even allowing for the fact

that immunotherapy trials involve people at much earlier stages of the disease with lower plaque burdens, the highly effective removal of Aβ plaques could reasonably explain the changes, through plaque clearance and plaque-associated glial changes, likely accompanied by fluid shifts. We suggest that available evidence suggests that this phenomena is neither "paradoxical" nor due to accelerated neurodegeneration, and pending longer term outcome data and further mechanistic insights, could now be referred to as "amyloid-removal related pseudo-atrophy (ARPA)". With this we do not aim to diminish its significance, but rather to facilitate the use of a common term for research and clinical trials. Analysis of existing patient-level clinical trial data is urgently needed, and longer term follow up will be important to clarify whether these volume changes are an indicator of efficacy rather than a cause for concern – or neither. For future trials, MRI volume outcomes should be clearly and transparently reported as key safety measures alongside ARIA. We predict that effective therapies that slow neurodegeneration enough and for long enough will ultimately also slow rates of atrophy – the hypothesis with which incorporating serial MRI measurments in trials began.

Panel 1: Volume loss in Alzheimer's disease - natural history

Cerebral volume loss in AD is closely associated with cognitive loss, both temporally and spatially, in natural history studies.[2] Typical, amnestic, AD has a characteristic pattern of atrophy, thought to relate to tau pathology and neuronal loss, with disproportionate hippocampal atrophy; over time atrophy becomes more generalized and rates increase as individuals become symptomatic.[2-6] For example, in healthy individuals in their 70s, whole brain atrophy rates are on average around 0·5%/year increasing to 1%/year in mild cognitive impairment and to 1·5%/year in mild AD dementia, for hippocampus the rates are 1%/year in controls, 2·6%/year in MCI and 4·4%/year in AD, and ventricular volumes increase by 1.4mL/year in controls, 2.8mL/year in MCI and 4.5mL/year in AD.[7] It was these differences in atrophy rates between AD and healthy aging, the precision with which they could be measured, and their association with cognitive decline that led to the widespread adoption of atrophy rates as outcome measures in AD trials.[8] These rates hold for the early AD populations included in current amyloid immunotherapy trials, for example, in the placebo arms of the GRADUATE trials of gantenerumab, there was an annual brain volume ~~WBV~~ loss of 1·2%, cortical grey matter loss of 1·5% and hippocampal volume ~~HCV~~ loss of 4%.[9]

Panel 2: Gaps in current evidence – key areas for further evaluation

- On an individual patient level, does the excess volume reduction with amyloid immunotherapy maintain the same negative clinical and biomarker associations with volume loss in the natural history of AD or do these associations loosen, as has been noted at a group level?[64]
- What happens to cerebral volumes beyond the duration reported in current trials – do these observations represent a consistently increased rate of volume loss with ongoing treatment, or does the excess volume change plateau (or decrease) once optimal removal of amyloid is achieved? How do these volume changes relate to longer term clinical outcomes?
- What brain regions are driving these volume changes, as the ventricular and whole brain volumes most commonly reported are not region specific?
- At the invidividual patient level, how related (both in extent and topography) are these excess brain volume changes to the amount of amyloid removed (as measured by PET) and the presence of ARIA?
- Do markers of glymphatic function and CSF dynamics influence volume changes in the presence of amyloid-removing immunotherapy (or the converse)? Is the increase in ventricular volume associated with an adverse change in CSF dynamics?

Figure 1

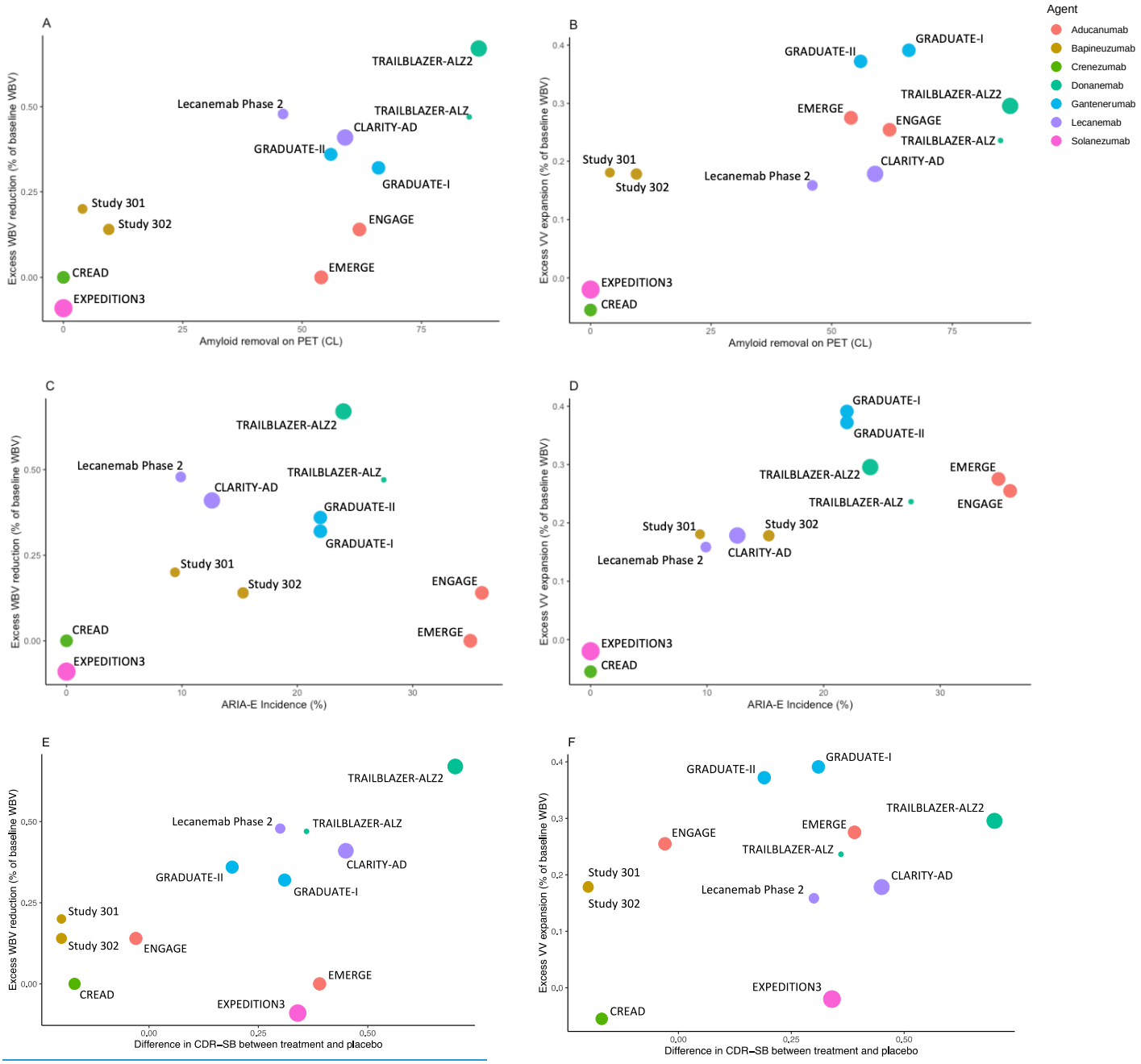

Figure 1 A-F illustrates whole brain volume (WBV) and ventricular volume (VV) outcomes in key trials, each represented as treatment group – placebo group. A) WBV excess reduction expressed as a percentage of baseline WBV plotted against amyloid removal on PET in centiloids (CL). B) VV excess expansion expressed as a percentage of baseline WBV plotted against amyloid removal on PET in CL. C) WBV excess reduction expressed as a percentage of baseline WBV plotted against ARIA-E incidence D) VV excess expansion expressed as a percentage of baseline WBV against ARIA-E incidence. E) WBV excess reduction expressed as a percentage of baseline WBV plotted against the mean difference in CDR-SB. F) VV excess expansion expressed as a percentage of baseline WBV against the mean difference in CDR-SB; the bapineuzumab trials (Study 301 and 302) are overplotted due to an identical point estimate. Mean difference in CDR-SB presented as it was the primary outcome for 6/12 depicted studies and reported as a secondary endpoint for the remainder; for consistency this is presented so a positive value represents benefit in treatment group relative to controls. Points are coloured by agent and their area scaled by number included in the imaging analysis of the respective trial. In each trial, if multiple doses were used the highest dose arm was included, excepting the lecanemab phase 2 data which is reported as a weighted mean of the 10mg/kg 2-weekly and monthly treatment arms due to changes in randomization of APOE-e4 carriers during the trial.

Figure 2

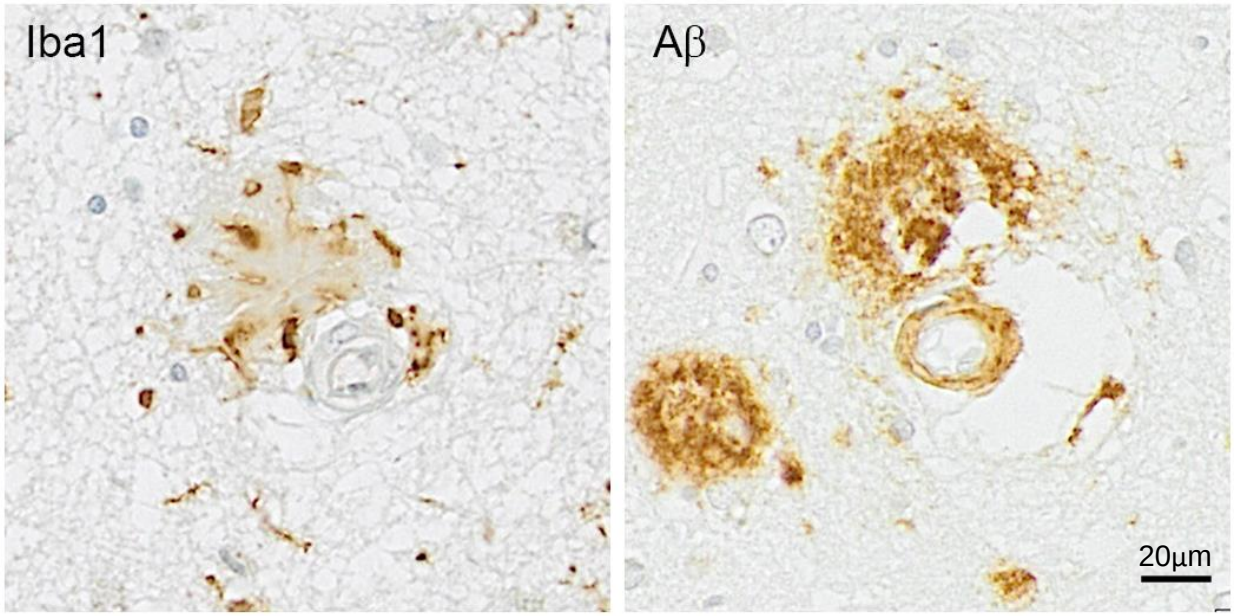

Figure 2 - Microglia clustering around Aβ plaques in the cortex of inferior parietal lobule from an 84-year old women diagnosed with Alzheimer's disease. Antibodies employed: Aβ (pan-Ab 4G8, Covance), Iba1 (microglia, Wako). Slides counterstained with H&E. Slides digitized on a Olympus VS110 slide scanner (Olympus America Inc.). Scale bar = 20 µm. Tissue sourced from South West Dementia Brain Bank (NRES Committee South West Central Bristol, REC reference: 08/H0106/28 + 5).

Search strategy

References were identified using PubMed search terms "Alzheimer's disease" AND "amyloid" AND "immun*" AND "trial". ClinicalTrials.gov and AlzForum.org were also searched for immunotherapies (active and passive) targeting amyloid-beta in Alzheimer's disease and publications covering clinical or biomarker endpoints were sought. An initial search was performed for papers published January 2000 - March 2023 by CRSB, with contributions from NCF. It was repeated after the subsequent publications of additional phase 3 trials key to the subject matter (donanemab and gantenerumab), with the final paper considering publications through to May 2024. Conference presentations reporting relevant biomarker endpoints were also sought if not included in primary publications. Papers were included based on relevance of intervention and reported outcomes to the content of this review. The reference lists of papers generated in this way were also examined for relevance to the discussion and additional papers were included from this.

Author's Contributions

CRSB - conceptualisation, literature review, writing - original draft, review & editing, preparation of figures; NCF - conceptualisation, literature review, writing – reviewing & editing. DB – conceptualisation, writing – review and editing, preparation of figure; JARN – conceptualisation, writing – review and editing; ZJ – writing, review and editing; HZ – writing, review and editing; JMS - data interpretation, writing - reviewing and editing; FB – conceptualisation, literature review, writing – review and editing.

Declaration of Interests

DB has been a consultant/advisor relating to Alzheimer immunization programmes for: Elan Pharmaceuticals (travel and accommodation) and Biogen (consultancy fees). JARN has been a consultant/advisor relating to Alzheimer immunization programmes for: Elan Pharmaceuticals (travel and accommodation), GlaxoSmithKline (consultancy fees), Novartis, Roche (consultancy fees), Janssen (consultancy fees), Pfizer, Biogen (consultancy fees, travel and accommodation), and Eisai. HZ has served at scientific advisory boards and/or as a consultant for Abbvie, Acumen, Alector, Alzinova, ALZPath, Amylyx,

Annexon, Apellis, Artery Therapeutics, AZTherapies, Cognito Therapeutics, CogRx, Denali, Eisai, Merry Life, Nervgen, Novo Nordisk, Optoceutics, Passage Bio, Pinteon Therapeutics, Prothena, Red Abbey Labs, reMYND, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave; has given lectures in symposia sponsored by Alzecure, Biogen, Cellectricon, Fujirebio, Lilly, Novo Nordisk, and Roche; is chair of the Alzheimer's Association Global Biomarker Standardization Consortium; is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program. JMS has received tracer from Avid Radiopharmaceuticals (a wholly owned subsidiary of Eli Lilly) and Alliance Medical and has consulted for Roche Pharmaceuticals, Biogen, and Eli Lilly. FB has received consulting fees from Combinostics, Roche and IXICO; has participated in data safety monitoring or advisory boards for EISAI, Biogen, Prothena and Merck; and is a co-founder of Queen Square Analytics. NCF reports consulting fees from Biogen, Eisai, Ionis, Lilly, Roche/Genentech, and Siemens – paid to UCL; he has served on a Data Safety Monitoring Board for Biogen. The other authors declare no conflicts of interest.

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Figure

Tables for "Brain volume change following amyloid-beta immunotherapy for Alzheimer's disease: amyloid-removal related pseudo-atrophy" by Belder et al.

Table 1

Agent **[Trial; number of participants]**	**Dose**	**Duration (imaging final time-point)**	**Excess whole brain volume change (% of baseline volume)**	**Excess cortical volume change (% of baseline)**	**Excess ventricular volume change**	**Excess hippocampal volume change**	**Baseline amyloid burden on PET**	**Amyloid removal on PET**	**ARIA-E incidence (%)**
Solanezumab [EXPEDITION 3; n=2129]	400mg 4 weekly	80 weeks	-0·9mL (0·09% less loss than placebo)	-	-0·2mL (lesser increase than placebo)	-0·01mL (less loss than placebo)	-	Nil	No increase
Crenezumab [CREAD; n=813][#]	60mg/kg 4 weekly	105 weeks	Nil	-	-0·55mL (lesser increase than placebo)	-0·02mL (less loss than placebo)	72 CL[a]	Nil	No increase
Bapineuzumab [Study 302; n=1121][b]	0·5mg/kg 13 weekly	71 weeks	1·4mL (0·1%)	-	**1·8mL**	0·01mL	115 CL[c]	**-9·5 CL**[c]	15·3%
Gantenerumab [GRADUATE I; n=985]	510mg subcut. 2 weekly	116 weeks	**4·2mL (0·32%)**	**3·3mL (0·64%)**	**5·1mL**	**0·02mL in left hippocampus ,** no observed difference in right	94 CL	**-66 CL**	22%
Gantenerumab [GRADUATE II; n=980]	510mg subcut. 2 weekly	116 weeks	**4·7mL (0·36%)**	**3·3mL (0·64%)**	**4·9mL**	Nil	96 CL	**-56·4 CL**	22%
Aducanumab [ENGAGE high dose; n=1647] *(Similar results in EMERGE)*	Target 10mg/kg 4 weekly	78 weeks	1mL *(no difference in EMERGE)*	-	**2·5mL** ***(2·7mL in EMERGE)***	Nil	77 CL *(76 CL in emerge)*	**-62 CL** ***(-54 CL in EMERGE)***	36%
Lecanemab [Phase 2; n=856][#]	Various; up to 10mg/kg 2 weekly[d]	79 weeks	**4·8mL (0·48%)**	-	**1·6mL**	0·01mL	80 CL[a]	**-46 CL**[a]	9·9%
Lecanemab [Clarity-AD; n=1795][#]	10mg/kg 2 weekly	79 weeks	**4·1mL (0·41%)**	-	**1·8mL**	**-0·02mL (less loss than placebo)**	78 CL	**-59 CL**	12·6%
Donanemab [TRAILBLAZER-ALZ; n=257] (phase 2)[#]	700mg for first 3 doses then 1400mg; 4 weekly	76 weeks	**4·6mL (0·47%)**	**2·7mL (0·69%)**	**2·3mL**	0·01mL	108 CL	**-85 CL**	27·5%
Donanemab [TRAILBLAZER-ALZ 2 -	700mg for first 3 doses then	76 weeks	**6·3mL (0·65%)**	-	**2·5mL**	-0·01mL (less loss than placebo)	102 CL	**-88 CL**	24%

Low-Medium Tau population; n=1182]	1400mg; 4 weekly								
Donanemab [TRAILBLAZER-ALZ 2 – Combined population; n=1736] [#]	700mg for first 3 doses then 1400mg; 4 weekly	76 weeks	**6·7mL (0·69%)**	-	**3mL**	**-0·02mL (less loss than placebo)**	103 CL	**-87 CL**	24%
AN1792 [Phase IIa; n=372]	AN1792 225microg + QS-21 50 μg	52 weeks or early termination	**10mL (1.01%)**	-	**6mL**	0·02mL	-	-	22% of responders developed encephalitis

Table 1 – **Summary of imaging outcomes in selected trials discussed in the Review.** Mean difference between treatment and placebo presented. Volume changes reported as statistically significant in the trials **in bold**. [#]Where the trial has not reported baseline volumes, representative baseline values were imputed from those reported for TRAILBLAZER-ALZ2 (presented at Alzheimer's Association International Conference 2023)[18] to establish estimates of percentage volume change. Where exact numerical outcomes were not provided in publications or presentations, estimates have been drawn from figures. "-" – not reported. Nil – no observed difference. [a]estimated from SUVR florbetapir using the conversion [$183 \times SUVr_{Avid} - 177$] after Navitsky et al. Alzheimers Dement. 2018 Dec;14(12):1565-1571.[19] [b]converted from annualized rates presented in Novak et al J Alzheimers Dis. 2016;49(4):1123-34. [c]estimated from SUVR PiB using the conversion [100 × ((11)C-PiB SUVR - 1·009)/1·067] from Rowe et al. J Nucl Med. 2016 Aug;57(8):1233-7.[20] [d]Lecanemab phase 2 outcomes presented as weighted mean of results from the 10mg/kg 2-weekly and monthly participant groups due to changes in randomization of APOE-e4 carriers during trial.