The amyloid hypothesis in Alzheimer disease: new insights from new therapeutics

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Abstract
Many drugs targeting amyloid-β (Aβ) in Alzheimer disease (AD) have failed to demonstrate clinical efficacy. However, four anti-Aβ antibodies have been shown to mediate the removal of amyloid plaque from brains of patients with AD, and the US FDA has recently granted accelerated approval to one of these, aducanumab, using reduction of amyloid plaque as a surrogate endpoint. The rationale for approval and the extent of the clinical benefit from these antibodies are under intense debate. With the aim of informing this debate, we review clinical trial data for drugs targeting Aβ from the perspective of the temporal interplay between the two pathognomonic protein aggregates in AD — Aβ plaques and tau neurofibrillary tangles — and their relationship to cognitive impairment, highlighting differences in drug properties that could affect their clinical performance. Based on this, we propose that Aβ pathology drives tau pathology, that amyloid plaque would need to be reduced to a low level (~20 centiloids) to reveal significant clinical benefit and that there will be a lag between the removal of amyloid and the potential to observe a clinical benefit. We conclude that the speed of amyloid removal from the brain by a potential therapy will be important in demonstrating clinical benefit in the context of a clinical trial.

Introduction

The amyloid cascade hypothesis of Alzheimer disease (AD), which proposes that deposition of the amyloid-β (Aβ) peptide in the brain is a central event in disease pathology (Figure 1), is strongly supported by neuropathological and human genetic evidence [1,2-4,5]. Consequently, it has long been the primary focus of efforts to develop drugs that might slow or delay the progression of AD — a field that we reviewed a decade ago [1]. Since then, fifteen potential therapeutics intended to target the role of Aβ in AD in various ways, including inhibition of enzymes involved in Aβ production and removal of Aβ from the brain using antibodies, have been tested in phase III trials (Table 1). Yet so far, eleven of these have clearly failed to demonstrate clinical efficacy.

The exception to this list is the Aβ-targeted monoclonal antibody (mAb) aducanumab, developed by Biogen and Eisai. Two identically designed, randomized, placebo-controlled phase III trials of aducanumab – Engage (NCT02477800) and Emerge (NCT02484547) – in which the primary endpoint measure was the Clinical Dementia Rating-Sum of Boxes (CDR-SB) [6] were both terminated following futility analyses in March 2019. However, in October 2019, Biogen announced that its analysis of the set of trials conducted with aducanumab, including additional data from the Engage and Emerge trials, indicated that treatment provided benefits on measures of cognition and function, and subsequently filed an application for regulatory approval by the US FDA (see the Biogen press release in Further information).

When asked to consider whether the combined evidence in this application (also including data from Study 103, an earlier and smaller dose-finding study) supported the effectiveness of aducanumab for AD, the FDA's Peripheral and Central Nervous System Drugs Advisory Committee voted 0 for and 10 against, with 1 member uncertain (see [7] for a detailed critique). Moreover, the FDA's own statistical analysis concluded there was insufficient evidence for efficacy. Despite this, the FDA controversially granted accelerated approval for aducanumab (now marketed as Aduhelm) for the treatment of AD in June 2021 [8]. This decision was based on the effects of aducanumab on the surrogate endpoint of reduction of amyloid plaque in the brain of patients with AD, which at that point was not validated as being predictive of clinical benefit [9]. Biogen is required to conduct a further appropriately controlled clinical study to verify the efficacy of aducanumab and submit the final report by February 2030.

In the view of the authors, this outcome does not provide unambiguous clinical proof for the amyloid cascade hypothesis, although we believe that targeting Aβ is a viable therapeutic approach. Importantly, following the accelerated approval of aducanumab, two further mAbs that target amyloid, lecanemab and donanemab, have been granted breakthrough therapy designation by the FDA. Given these developments and the controversy around the approval of aducanumab, here we analyse the characteristics and clinical data for agents targeted at amyloid pathology, with a focus on the anti-Aβ antibodies in late-stage clinical development, in the hope that this could inform the ongoing discussion about these agents. We discuss three key questions: what is the therapeutic hypothesis for approaches that target amyloid pathology; to what extent have recent agents based on this approach actually tested this hypothesis, and what might the temporal relationship be between targeting amyloid pathology and beneficial effects on cognition?

The amyloid therapeutic hypothesis

In the last decade or so, there has been significant progress in our understanding of the temporal interplay of tau and amyloid pathology in AD, due to the development and use of positron emission tomography (PET) imaging agents that can reveal neurofibrillary tau tangles (NFT) and amyloid plaques [10-13] in the brains of living humans.

Post-mortem neuropathology [14], as well as cross-sectional [15] and longitudinal [16] amyloid PET imaging in humans, reveal that Aβ deposition occurs in a step-wise manner, starting in the temporobasal and frontomedial areas, then spreading to the remaining neocortex, the primary sensory-motor cortex and finally the striatum. Substantial amyloid pathology is already present in the neocortex decades prior to overt AD symptomatology [17]. Tau pathology is initiated well before amyloid pathology [18] in the medial temporal lobe, basal forebrain, brainstem, and olfactory areas (bulb and cortex) in a clinically benign manner sometimes called primary-age related tauopathy [19], before spreading to limbic regions and finally the neocortex. Thus, there is both a spatial and temporal disconnect between the cardinal pathologies of AD. From both cross-sectional [20] and longitudinal studies [21], it has become evident that it is the spread of NFT pathology that correlates best, and possibly causes, substantial cognitive impairment in AD (Box 1).

Given the lack of a direct and significant effect of amyloid on cognitive impairment, a central question for an anti-Aβ/amyloid therapy is how would a therapeutic benefit be mediated? Put simply, what is the therapeutic hypothesis for such an agent? While the detailed cellular pathology [22] that links Aβ/amyloid and tau remains to be elucidated, in aggregate the data strongly support the concept that amyloid provokes the spread of tau pathology that ultimately causes neuronal loss. We previously posited three broad categories by which amyloid might exert this pathological effect: as a *trigger*, with immediate effect; by achieving some *threshold* amount in the brain parenchyma; or as a continuous, load-dependent, *driver* for downstream neurodegenerative events [1].

While it is still uncertain which (if any) of these scenarios describes the real situation, the *driver* scenario seems unlikely because multiple studies show that amyloid pathology is reaching a plateau in the brain prior to the onset of significant cognitive impairment [23]. The *trigger* scenario can be discounted as the onset of amyloid deposition may occur ~20 years prior to cognitive alterations [17]. Recent evidence suggests that the *threshold* hypothesis is concordant with our current understanding. Studies demonstrate that robust tau pathology is more commonly seen in patients with amyloid pathology [24], and yet the pattern of tau pathology can differ significantly from person to person [25]. It is likely that individuals have a variable response to amyloid, but that once a threshold is surpassed, the spread of tau pathology is accelerated.

Indeed, this has been recently demonstrated in work by Knopman and colleagues [26], who analyzed the increase in tau PET flortaucipir signal in 4 regions of interest (entorhinal, inferior temporal, lateral parietal and a meta-region) in 167 cognitively normal participants that were placed into four groups according to amyloid status: low, < 8 centiloids (CL); subthreshold, 9–21 CL; suprathreshold, 22–67 CL; and high >68 CL (see Box 2 for discussion of the centiloid scale). They demonstrated that only in the >68 CL group was there a significant increase in annualized change in tau PET standard uptake value ratio (SUVR). Interestingly, the annualized rate in tau PET SUVR increase reached a plateau at approximately 100 CL, with the exception of the entorhinal cortex, where the rate continued to increase over the study period. Sanchez et al. [27] also performed longitudinal tau imaging in 443 participants with diverse ages, cognitive status and amyloid loads. They demonstrated that the initial site of tau pathology was in the transentorhinal cortex and that further spreading into neocortex occurred at amyloid burdens of >40 CL.

There is an alternative school of thought that proposes that amyloid plaque is not the most relevant pathological Aβ species: instead, oligomers composed of small numbers of Aβ peptides are the culprit, with amyloid plaques perhaps serving as a reservoir for such species [28,29]. In this scenario, disaggregation of plaques might release oligomer species that would be damaging; however, to our knowledge there is no direct clinical or preclinical in vivo evidence that this occurs [30].

To summarize, a thorough understanding of the cellular or physiological events by which any anti-Aβ/amyloid approach would mediate a therapeutic benefit is lacking. However, there is a poorly defined therapeutic hypothesis that reducing either soluble (oligomeric) Aβ, or amyloid plaques, or both, in the brain to non-pathological levels — that is, below the level that provokes tau pathology spread — will mediate a therapeutic benefit. A critically important aspect of this hypothesis is that the action of an anti-Aβ/amyloid therapeutic is distant from the mechanisms by which potential clinical benefit may be derived.

A therapeutic hypothesis that is poorly defined is not readily falsified. This accounts, in part, for the field's response to the failure of anti-Aβ drugs, which is not to accept the null hypothesis but to proffer alternatives: for example, that the treatment has been administered too late in the disease course. While there may be genuine merit in such an alternative hypothesis, it exemplifies the difficulties that emerge from our incomplete understanding of the disease. By comparison, let us hypothesize that antagonizing a neurotransmitter receptor X will mediate cognitive benefit. With a potent and selective X antagonist, plus a PET ligand to the receptor, it is not difficult to use receptor occupancy to select a clinical dose that provides robust X antagonism and test this hypothesis in a clinical trial. If the trial does not provide evidence for efficacy, then the null hypothesis can be accepted. Such a clear experiment has been very difficult to conduct with anti-Aβ approaches: the target is not clearly defined (amyloid plaques, Aβ monomers or oligomers), and the mechanism by which Aβ/amyloid affects cognition is unknown (direct or indirect synaptic toxicity, or induction of tau pathology, or neuroinflammation, or a combination of all and other effects). However, we can learn from previous clinical trials with anti-Aβ/amyloid drugs to inform future studies.

Clinical trials of anti-Aβ drugs

Approaches targeting soluble Aβ that have been tested in phase III trials.

Approaches that have directly targeted soluble Aβ (Table 1) include γ-secretase inhibitors, β-site amyloid precursor protein cleaving enzyme 1 (BACE1) inhibitors and anti-Aβ antibodies. Here, we discuss four such agents that have reached phase III trials: semagecestat, verubecestat; solanezumab and crenezumab. We do not discuss three other agents in Table 1 — tarenflurbil, tramiprosate and gammagard — for which we consider that their proposed primary pharmacology and mechanism of action are not strongly supported by available data, and/or target engagement in humans was never established; for a review of these agents, see [31].

Semagecestat, a γ-secretase inhibitor, was tested in patients with mild to moderate AD at two doses: 100 mg and 140 mg administered daily [32]. Semagecestat reduced Aβ production in the brain modestly [33,34], but was dose limited due largely to side effects mediated by inhibition of Notch processing, which is involved in many different developmental and homeostatic processes [35]. The trial was terminated early on the recommendation of the data and safety monitoring board: cognitive decline worsened with therapy [32,36], probably due to the deleterious inhibition of other substrates of gamma secretase, resulting, among other adverse effects, in an increased incidence of skin cancer. The once daily (QD)

administration of a drug with a half life of only a few hours probably resulted in peak doses that efficiently blocked Notch cleavage. There was no reduction in brain Aβ PET signal with treatment. Further therapeutic development on this target has largely halted, although avenues that might lead to more selective γ-secretase inhibitors exist [34].

Verubecestat, a BACE1 inhibitor, was tested in two phase III trials in patients with prodromal [38] and mild to moderate [39] AD that were terminated early following futility analyses. At the highest dose of 40 mg, verubecestat inhibited brain Aβ production by up to 75% at steady state. Verubecestat was shown to significantly increase the rate of cognitive decline and was also associated with a range of adverse events, including suicidal ideation, falls, weight loss, sleep disturbance, hair color change and rash. Verubecestat inhibited both BACE1 and its homologue BACE2, and as with semagecestat it seems probable that the inhibition of other substrates [40] was responsible for the majority of the adverse effects. At the top dose of 40 mg, the amyloid PET signal was reduced by 20% from baseline (~20 CL). This interesting finding revealed that some resolution of amyloid plaques is possible when Aβ production is significantly reduced. While this study is confounded by the early iatrogenic cognitive impairment, it is reasonable to conclude that a modest reduction in amyloid is insufficient for therapeutic benefit. Subsequently, other BACE inhibitors in clinical development were withdrawn.

Solanezumab is a mAb that targets the mid-domain of Aβ and does not bind to amyloid plaques [41] (see Figure 2 for anti-Aβ antibody epitopes). Solanezumab has been tested in four phase III trials: two in mild to moderate AD (Expedition and Expedition 2; solanezumab administered intravenously (i.v.) at 400 mg/4 weeks (Q4W)), one in mild AD (Expedition 3; solanezumab administered at 400 mg/Q4W), and one in dominantly inherited AD (DIAD; solanezumab administered initially at 400 mg/Q4W increasing to 1,600 mg/Q4W). While solanezumab was able to lower cerebrospinal fluid (CSF) levels of free Aβ40 significantly, there was no significant effect on free Aβ42, on brain amyloid PET signal or on cognitive decline in any of the Expedition trials [42-44]. In the DIAD study, an initial 2 year biomarker study in asymptomatic and symptomatic patients was transitioned to a 4-year treatment trial [45]. Patients' doses were escalated (400, 800, 1,600 mg/Q4W i.v.), with 75% of patients receiving escalated doses for an average duration of 1.44 years out of the 4 years. The primary cognitive outcome measure was the Dominantly Inherited Alzheimer Network Multivariate Cognitive End Point (DIAN-MCE) [46], which measures cognitive domains that are affected in early AD, including episodic memory, executive functioning, processing speed, and mental status. In this study, solanezumab failed to reduce brain Aβ and was without efficacy [47].

Another anti-Aβ mAb, crenezumab, targets a very similar epitope to solanezumab, although the X-ray structures of the binding modes of the antibodies are different [48] and there are data demonstrating that crenezumab binds to fibrillar and other forms of Aβ. Like solanezumab [49], however, crenezumab treatment results in a rapid increase in peripheral Aβ, as monomeric Aβ is captured by the antibody [50]. Crenezumab does not bind avidly to amyloid plaques when administered to transgenic PS2APP mice [51], and therefore we consider that crenezumab's primary target is monomeric Aβ. Crenezumab was tested in two identical phase III trials (CREAD 1 and 2) at 60 mg/kg i.v. Q4W (~4,200 mg/patient) in patients with prodromal or mild AD with MMSE score >22 and a CDR of 0.5 or 1.0. The planned duration was 2 years with the primary outcome measure being the change in CDR. Following interim analyses for futility, both studies were halted. In a phase II study, crenezumab was administered for 69 weeks at 15 mg/kg Q4W i.v. and there was no significant difference in florbetapir amyloid PET between the placebo

and dosed groups [52], and given the similarity of crenezumab to solanezumab in overall profile, it seems likely that there was no significant reduction in brain amyloid in the larger phase III studies.

From these clinical data, we conclude that reductions in monomeric Aβ, and by extension, oligomeric forms of Aβ [53], are not efficacious in the patient populations tested and also do not mediate significant removal of plaque. Strong suppression of Aβ production, via BACE inhibition, reduced amyloid deposition by about 20% and resulted in a modest increase in cognitive impairment, probably due to unwanted on-target effects mediated via inhibiting other BACE substrates [39], although the possibility that soluble Aβ has important physiological functions cannot be excluded [54]. To summarize, anti-Aβ mAbs that predominantly bind to monomeric Aβ do not mediate substantial removal of plaque and lack clinical efficacy [42,43].

Approaches targeting amyloid plaques. Several mAbs that target Aβ plaques via binding to various Aβ epitopes (Figure 2) have been tested in clinical trials: bapineuzumab, gantenerumab, lecanemab, aducanumab and donanemab.

Bapineuzumab targeted the N-terminal region of Aβ and did not distinguish between monomeric Aβ and plaque [55,56]. Dose levels were limited due to the high incidence of vasogenic edema (amyloid-related imaging abnormalities-edema (ARIA-E)) [57] experienced by patients, especially apolipoprotein *E4* gene (*APOE4*) carriers. Bapineuzumab was tested in two phase III trials (Study 301 and Study 302) in mild to moderate AD. Bapineuzumab was administered at 0.5 and 1.0 mg/kg i.v. every 13 weeks (Q13W) in the *APOE4* non-carriers and at 0.5 mg/kg Q13W in the *APOE4* carriers. The trial duration was 78 weeks and the co-primary outcomes were the AD assessment score (ADAS Cog11) [58] and Disability Assessment for Dementia [59]. Bapineuzumab failed its primary outcome measures [60]. Amyloid PET measurements were conducted in *APOE4* carriers and non-carriers. While the amyloid PET data were somewhat variable, in pooled data, there was a (nominally) statistically significant difference between the placebo group and both the 0.5 mg/kg and the 1.0mg/kg bapineuzumab groups: however, this difference was largely driven by the increase in the amyloid PET signal in the placebo arm [61]. The decrease in amyloid (~5 CL) from baseline in the 1.0 mg/kg group was very modest and therapeutically irrelevant.

Gantenerumab has a conformational epitope that targets both N-terminal and mid domain Aβ epitopes on amyloid plaque: the antibody does not bind avidly to soluble Aβ [62]. Gantenerumab was investigated in an amyloid PET study where 60 and 200 mg doses were administered i.v. Q4W up to a maximum of 7 doses to mild-to-moderate AD patients. Brain amyloid was assessed using PiB amyloid PET at baseline and at the end of treatment. In this small exploratory study, there was evidence for the removal of amyloid compared to baseline (~20 CL) at the 200 mg dose [63]. Subsequently, gantenerumab was tested in two phase III trials, known as Scarlet Road and Marguerite Road. In Scarlet Road, gantenerumab was administered subcutaneously (s.c.) at 105 mg and 225 mg Q4W in patients with prodromal AD and evidence of amyloid deposition concluded from low CSF Aβ42 levels [64]. The study was planned to run for 104 weeks with the primary endpoint being the change from baseline in CDR-SB. The study was halted for futility when 50% of the patients had reached the 104-week time point. In a florbetapir amyloid PET sub-study, at the top dose of 225 mg, there was only a 4.8% reduction in levels of Aβ from baseline (~3 CL), although the authors also concluded from an analysis of other reference regions that increasing the dose would remove more amyloid.

As a consequence of the termination of Scarlet Road, Marguerite Road, a parallel study being conducted in patients with mild AD, was halted and converted to an open-label extension study that investigated

higher doses of the drug. Gantenerumab doses were increased Q8W and starting doses depended on whether patients had previously received gantenerumab and whether they were *APOE4* carriers. For *APOE4* carriers, the dose titration was 225 mg; 450mg; 900 mg; 1,200 mg, and for *APOE4* non-carriers it was 300 mg; 600 mg; 1,200 mg. These dosing regimens were employed to minimize the incidence of ARIA-E. The incidence of ARIA-E in the various groups ranged from 24–39%, with *APOE4* carriers having a higher incidence than non-carriers. At the 2-year time point, approximately 50% of the patients were deemed to be amyloid-negative (defined as <24 CL by the sponsor) and in those patients with mild AD who started receiving drug for the first time during the open-label extension, amyloid had been reduced from baseline by 78 CL. Based on these studies, two phase III studies, Graduate I (NCT03444870) and II (NCT03443973) have been launched in patients with prodromal to mild AD. Gantenerumab will be titrated up to 1,020 mg s.c. Q4W in a 2-year study, with the CDR-SB as the primary outcome measure.

Gantenerumab has also been tested in a cohort of asymptomatic and symptomatic patients with DIAD using the DIAN-MCE as the primary outcome [47]. In this 4-year study, the dose of gantenerumab was escalated (225, 450, 675, 900, 1,200 mg s.c. Q4W), with 75% of patients receiving escalated doses for an average duration of 2.41 years out of the 4 years. In the asymptomatic cohort, gantenerumab lowered amyloid from a baseline of 38 CL to 22 CL; and in the symptomatic cohort, from a baseline of 102 CL to 80 CL. In this study, the asymptomatic patients did not demonstrate cognitive decline, and hence there was no possibility of the drug demonstrating efficacy. In the symptomatic cohort, gantenerumab was without efficacy.

Lecanemab (BAN 2401) is the humanized version of murine mab158, which was raised to Aβ1–42 protofibrils harbouring the mid-Aβ domain Arctic APP mutation (E22G) [65]. As Aβ (E22G) monomers rapidly form protofibrils in vitro, it is posited that targeting these species, viewed by some as being particularly important in propelling downstream pathological changes, may confer superior therapeutic properties. Following phase I studies [66], lecanemab was assessed in a phase II study in patients with mild cognitive impairment (MCI)–mild AD using a Bayesian design [67] involving multiple cognitive assessments using the AD Composite Score (ADCOMS) scale [68] every 3 months to help select the optimal clinical dose. The lecanemab doses were 2.5mg/kg Q2W; 5mg/kg Q4W; 5 mg/kg Q2W; 10 mg/kg Q4W and 10mg/kg Q2W for 18 months. More patients were assigned to receive the top doses of 10mg/Q4W and 10mg/kg Q2W consequent to the Bayesian analysis, although *APOE4* carriers were excluded from the high dose following advice from health regulatory bodies, thus creating an imbalance in the study. Whether APOE4 genotype affects disease progression is still a matter of debate, but it will be important in future trials to address this aspect [69,70]. At the top dose of 10mg/kg Q2W, amyloid was reduced from 75 CL to 5 CL over the 18-month duration of the study [71,72]. The ARIA-E rate was modest, at about 10% overall, with 60% occurring within the first three months of treatment. In follow-up analysis and modelling, it transpired that ARIA-E was probably driven by C_{max} drug levels [73].

These promising studies led to the phase III Clarity trial (NCT03887455), which will investigate a lecanemab dose of 10 mg/kg Q2W in an 18-month study in 1,566 patients with early AD, using CDR-SB as the primary outcome measure. Lecanemab is also being tested in the AHEAD 3-45 study, which consists of two trials within a single protocol, participant screening and assessment regime (NCT04468659). The phase II, AHEAD 3 trial will recruit 400 cognitively normal participants who have amyloid levels of between 20–40 CL, who will be randomized to placebo or initially receive lecanemab at a dose of 5 mg/kg i.v. Q4W, titrating up to 10 mg/kg Q4W for 216 weeks. The primary outcome measure will be amyloid PET assessment. The phase III, AHEAD 45 trial will enroll 1,000 cognitively normal participants with amyloid levels of >40 CL who will receive placebo or lecanemab at a dose of 5 mg/kg Q2W for 8 weeks, then 10 mg/kg Q2W for 96 weeks. From week 96–216, participants will receive

placebo or maintenance dosing of lecanemab at 10 mg/kg Q4W to sustain amyloid-negative status. The primary outcome is change from baseline on the Preclinical Alzheimer Cognitive Composite 5 (PACC5) Score at week 216. The AHEAD 3 study is close to a primary prevention protocol, with the AHEAD 45 study seeking to bring down amyloid levels in a patient population at greater risk of developing cognitive impairment.

The therapeutic hypothesis for lecanemab has changed during clinical development. As stated earlier, this mAb was designed to target Aβ protofibrils, rather than amyloid plaque [74]. However, the robust removal of amyloid plaque in patients mediated by lecanemab is not currently considered to be an irrelevant off-target effect. Lecanumab has been granted breakthrough therapy designation by the FDA.

Aducanumab is a human IgG1 mAb that targets amino acids 3–7 of the Aβ peptide and is specific for amyloid plaque [75]. In phase Ib studies (NCT01677572), aducanumab demonstrated robust time- and dose-dependent removal of amyloid plaque at doses up to 10mg/kg Q4W: this top dose removed 44 CL at 26 weeks and 57 CL at 54 weeks, bringing patients to a level of 27 CL [76]. The incidence of ARIA-E at the 10 mg/kg Q4W dose was 47%, compared with a placebo incidence of 5%. These studies led to two identically designed phase III studies, Emerge and Engage, of 78 weeks' duration in patients with early and mild AD, using change in the CDR scale as the primary outcome measure. These studies were halted for futility after 50% of patients had been enrolled for 78 weeks.

Following further collection and analysis of data, together with data from a phase Ib dose-escalation study, the sponsors determined that there was sufficient evidence to seek regulatory approval. Emerge showed statistically significant benefit using the CDR assessment scale at the 10 mg/kg QW4 dose of aducanumab, while the other study, Engage, did not. In Emerge, 64 CL of amyloid were removed at the 10mg/kg QW4 dose, bringing levels down to 21 CL by the end of the 78-week study [77]. In Engage, rather less amyloid was removed, 54 CL, bringing amyloid levels to 37 CL at 78 weeks. There were some hints that tau PET signal was reduced in a medial temporal composite region of interest analysis, but the group sizes reported are very small and include data from both studies, thus making any conclusions challenging. The incidence of ARIA-E was 35% at the 10mg/kg Q4W dose. In the protocol, about one third of the duration of the trials was used in titrating up to the 10mg/kg dose to minimize the incidence of ARIA-E, such that patients were only approaching amyloid negativity towards the end of the 78-week trial in Emerge and were not at amyloid negativity in Engage. We believe this is an important factor, as we will discuss later.

Donanemab targets N-terminally truncated 3-x Aβ peptide in which the N-terminal glutamate is cyclized to form the pyroglutamate (pE3Aβ). This epitope was specifically targeted because, due to its high insolubility, pE3Aβ is found almost exclusively in Aβ plaque, hence this antibody can be considered as plaque-specific [56,78,79]. In phase Ib studies (NCT02624778) [80] , donanemab was given in single or multiple doses to patients with MCI–mild AD and a positive florbetapir amyloid PET scan. Donanemab demonstrated a high rate of anti-drug antibodies, at the 10 mg/kg i.v. QW4 dose and had a plasma half-life of ~10 days compared to a typical IgG1 half-life of 23 days [81]. Donanemab was very effective in reducing the florbetapir amyloid PET signal: at 20mg/kg Q4W there was a 68 CL reduction from baseline levels at 24 weeks [82]. In these studies, the ARIA-E incidence in patients treated with donanemab was 25%.

Based on these data, the TRAILBLAZER-ALZ (NCT03367403) phase II study was initiated in patients with early AD and positive amyloid and tau PET scans. The primary outcome was the change from baseline in the iADRS score, which is a combined cognitive and functional assessment [83]. Donanemab was given at

10 mg/kg i.v. Q4W for three doses, prior to increasing the dose to 20 mg/kg for the rest of the 18-month study [84]. By 24 weeks, donanemab treatment resulted in a 68 CL reduction in amyloid from a starting baseline of 108 CL, and 40% of patients were amyloid-negative (defined by the sponsor as <24 CL). By 78 weeks, 68% of patients were amyloid-negative. The ARIA-E incidence in patients treated with donanemab was 27%, of which one fifth were symptomatic. Anti-drug antibodies were present in about 90% patients. Donanemab met the primary outcome measure, although the clinical meaningfulness of the iADRS has not yet been established [84]. Of note, while the composite flortaucipir tau PET signal was not statistically significantly lowered in donanemab-treated patients, individual regions of interest did show significant reductions.

Donanemab has been granted breakthrough therapy designation by the FDA and the sponsors, Lilly, have made clear their intention to submit a Biological Licence Application (BLA) for accelerated approval based on the TRAILBLAZER-ALZ study. TRAILBLAZER-ALZ 2, a phase III study in prodromal and mild dementia due to AD with confirmed amyloid PET and tau PET pathology, is a 76 week study with a planned 78 week extension period will recruit 1800 participants. The primary outcome will be iADRS, and effectiveness will be judged using a disease-progression model. TRAILBLAZER-ALZ3 will enroll 3,300 cognitively normal people with amyloid pathology based on elevated plasma ptau217. In this study, participants on active therapy will receive 3 doses 10mg/kg Q4W, followed by 6 doses 20mg/kg Q4W. Participants will be followed until 434 primary clinical events have been registered, defined as an increase in global CDR to > 0 at two consecutive visits.
Thus far we can conclude that anti-Aβ antibodies that predominantly target amyloid plaque and not monomeric Aβ are able to remove amyloid plaque. Such antibodies cause ARIA-E to a varying degree as an adverse event. There are signs of clinical efficacy with these antibodies, but we must still be cautious in our interpretation of these. Those clinical studies that demonstrate robust removal of amyloid PET signal (and presumably amyloid plaque) prompt some questions. Foremost, is what constitutes an abnormal amount (amyloid positive), and/or a pathological amount, of amyloid in the human brain, and following from this, to what level must amyloid be lowered to mediate a therapeutic benefit.

Hypotheses and questions

Defining a threshold for amyloid lowering drugs. The use of amyloid PET imaging and the calibration of the PET signal to the gold standard of post-mortem neuropathology is central to these assessments. A number of studies have investigated the amyloid positivity/negativity threshold values for amyloid PET ligands by imaging living patients and comparing SUVr values with subsequent post-mortem histological assessment of amyloid plaques [85-87] [88] (Box 2). From this work, ≤20 CL is an accepted threshold for amyloid negativity. But is this threshold value also the target for mediating a clinical benefit?

Setting aside the existing clinical data on the effects of cognition, as these are currently from phase II studies or in the case of aducanumab, the subject of considerable controversy, there are other data that support the 20 CL level as being clinically benign. Knopman et al. [26] have shown that there is no increase in annualized tau PET signal in cognitively normal subjects aged 65-85 years below a threshold of 21 CL, but that above 68 CL, tau PET signal significantly increases. Jack et al. [89] have shown there is no meaningful increase in tau PET signal in cognitively unimpaired subjects below 22 CL, although an increase amyloid PET SUVr signal of 20% (up to ~35 CL) was associated with a statistically significant increase in annualized tau PET signal. In the DIAD trial of gantenerumab, 60% of the patients enrolled were cognitively normal (CDR=0) with a baseline amyloid level in the placebo group of 39 CL that increased to 51 CL over the course of the 4-year study. These patients failed to demonstrate cognitive

decline. Those patients in the placebo arm that were symptomatic (CDR ≥ 0.5) at baseline further declined during the course of the study: their baseline amyloid was 97 CL and increased to 104 CL. While the cohort sizes were small, these data further support the notion that a threshold amount of brain amyloid is required to drive cognitive impairment.

The acceleration of tau pathology progression mediated by the increase in amyloid burden may be different in magnitude to its deceleration consequent to a therapeutically mediated reduction in amyloid, because in the latter case tau pathology will be more extensive with a greater opportunity to propagate further. Nevertheless, we conclude from these longitudinal studies that amyloid should be reduced to below 20 CL, or thereabouts, to give the best possible opportunity for demonstrating clinical benefit.

***A therapeutic hypothesis for anti-Aβ antibodies that clear amyloid plaque*.** The field now has 4 anti-Aβ/amyloid antibodies that have demonstrated the ability to clear amyloid plaque, and clinical trials of some have shown signs of clinical improvement that remain to be confirmed by appropriately powered studies. However, the antibodies differ in key features that could be relevant to their clinical performance, as shown in Table 2. Using data either published or presented at meetings, we have expressed antibody-mediated amyloid clearance as a normalized rate (CL removed/4 weeks/10 mg antibody). We have also calculated an index that captures the incidence of ARIA-E divided by the rate of Aβ removal for each antibody. These data should be treated cautiously because in some cases they are based on a small sample size. There is also evidence that amyloid removal is faster in those patients with high baseline levels, further confounding a direct comparison of anti-Aβ antibodies [90]. Nevertheless, there are some tantalizing observations. The antibodies have quite different propensities to cause ARIA-E, which will limit the dose administered and determine whether dose titration is required. There does not appear to be a correlation between rate of amyloid removal and the incidence of ARIA-E, in contrast to what has previously been postulated [91] and as we will discuss below.

Clinical trialists have to balance rapid amyloid removal versus the incidence of ARIA-E at a given dose to assure equipoise in the study. As the reduction in brain amyloid represents the major therapeutic hypothesis for all four antibodies (with the potential exception of lecanemab), then the faster this is achieved, the more rapidly patients would manifest an amelioration of tau pathology propagation and consequent cognitive decline. The relationship between the removal of plaque and demonstrating a clinical benefit, if that is possible, is likely to be complex, as we have discussed. It is unlikely that the amelioration of cognitive decline will temporally closely follow the therapy-mediated reduction in amyloid load, given that the therapeutic mechanism acts at several (currently unknown) biological steps removed from the clinical effect.

Therefore, we propose a 'lead and lag' scenario: amyloid removal will *lead* and the clinical benefit will *lag* (Figure 3). We have previously highlighted that AD is a complex interplay of cellular pathology probably involving multiple cell types and cellular processes [22], and it seems reasonable to believe that a period of low/no amyloid status would be required to enable the brain to restore homeostasis. Clinical efficacy will be demonstrated after TΔA - the time taken to reduce amyloid to a low level, for example, ≤ 20 CL. Thereafter, the full clinical benefit of amyloid negativity will be manifested and at TΔE a sufficient separation between the placebo and treated groups will be evidenced.

If this model reflects the clinical situation, it has some important implications for clinical development. Removing amyloid rapidly affords a greater opportunity to reveal clinical efficacy within a given time. Thus, while all 4 anti-Aβ antibodies under consideration might be able to render patients 'amyloid

negative' over time, they may not be equivalent in their ability to provide clinical efficacy over the same time duration. In the phase III trials with aducanumab, amyloid negativity was only being reached towards the end of the study period, assuming a linear removal of amyloid between timepoints 6 and 18 months. At the conclusion of the aducanumab phase III studies there is a difference of 17 CL between a failed study (Engage) and a successful study (Emerge). It seems unlikely to the authors that this very modest difference can be reconciled biologically to the different clinical outcomes. Further, we hypothesize that the rate of amyloid removal was not fast enough to provide convincing clinical benefit within the duration of the study. This concept is exemplified in Table 3. In the donanemab Trailblazer trial, it is notable that clinical improvement begins to manifest at week 36, at which time amyloid levels have already been profoundly reduced [84], although this needs to be replicated in much larger studies. The amyloid removal profiles for aducanumab, donanemab and lecanemab are shown in Figure 4.

Anti-Aβ antibodies that reduce amyloid levels slowly to an amyloid-negative status may also eventually demonstrate an amelioration in cognitive decline, but this will be challenging to demonstrate for a variety of reasons. Firstly, given the normal patient attrition rate from clinical trials, the longer the trial takes to conduct, the greater the group sizes need to be to ensure sufficient statistical power at the trial's conclusion. Secondly, conducting such trials when alternative anti-Aβ antibodies are available that are superior with respect to rate of amyloid removal will make patient recruitment difficult, assuming other properties of the antibodies – such as propensity to cause ARIA – are equivalent. Finally, the efficacy of anti-Aβ antibodies may well be dependent on the stage of AD. If the removal of amyloid takes several years, there will be a risk that the disease will have progressed to a stage where tau pathology is well-established and its propagation self-sustaining.

All of the anti-Aβ antibodies discussed here are the same IgG1 isotype. However, they have other discriminating properties: the Aβ epitope targeted by the antibody, and whether the epitope is present in CAA, parenchymal plaque and soluble Aβ; the affinity of the antibody for its epitope; the antibody exposure in the brain; extent of non-specific binding; half-life in the circulation; and dose-limiting side effects (for example, ARIA-E).

The Aβ epitope targeted by the antibody is important and current anti-Aβ antibodies are effectively plaque-specific. One clear differentiating factor is that different antibodies seem to induce different rates of amyloid clearance and ARIA-E. While some literature suggests that ARIA-E is a feature of the speed of amyloid removal, in fact this is not well supported by the clinical experience overall. For example, bapinezumab caused significant ARIA-E but removed very little amyloid [61]. ARIA-E is probably mediated by a response between the antibody and vascular amyloid, and interestingly vascular amyloid is somewhat different in its composition [79] and potentially, its conformation from the parenchymal Aβ plaques [92]. These two factors probably explain the differences in ARIA-E and amyloid removal. There is currently very little mechanistic understanding of ARIA. It is clear from all the studies with the plaque removing mAbs considered here that the incidence of ARIA is elevated in ApoE4 carriers. However, we believe that it is too soon to conclude that the risk of ARIA-E will always be present with this therapeutic mechanism, which is still in its infancy in terms of clinical exploration.

It is notable that donanemab, targeting pE3Aβ, mediates rapid amyloid removal. The pE3Aβ is present at low concentrations, but often associated with dense core plaques [56,78]. One caveat with respect to interpreting the amyloid lowering effects of donanemab concerns the pE3Aβ species targeted. There are data that show that the amyloid PET ligand PiB may bind preferentially to amyloid plaques containing the pE3Aβ species [93,94] and although it is not known whether this is the case for other amyloid PET ligands, such preferential binding might over-estimate the extent of amyloid clearance. However, this

phase II trial also met its primary endpoint regarding slowing decline measured using the iADRS indicating that the amyloid load threshold to see clinical benefit was reached in this study. Interestingly, donanemab has a high incidence of ARIA (27%), although this was not evident in preclinical studies [56]. Donanemab also induces a 90% anti-drug antibody response, and it might be that this, in some way, is connected to the incidence of ARIA.

***Selection of patients for anti-Aβ therapeutic trials*.** Clinical trials are large and complex experiments. They are constrained in a number of ways, including cost, the ability to enroll and keep patients in clinical studies and the group sizes required to demonstrate meaningful effect sizes. Paramount is ensuring patient safety. It might be that the most cogent clinical scientific experiment to test a therapeutic hypothesis in AD is not operationally feasible or affordable. For example, Insel and colleagues [95] examined the cognitive decline in cognitively unimpaired individuals who are amyloid-positive as defined by amyloid PET or via CSF biomarkers. This study revealed that a study with 2,000 participants per group in a trial lasting 4 years would provide 80% power to reveal a 25% treatment effect - a huge undertaking.

There is a conundrum faced by AD clinical trialists. While therapeutic intervention early in the disease process is more likely to demonstrate clinical benefit, demonstrating that benefit becomes increasingly more difficult and expensive. For a therapeutic removing amyloid, unless a preventative trial is being considered, then patients who are amyloid positive will have to be selected. However, the amount of amyloid present in the brain will be important as well because current data suggest that only as individuals reach a level of amyloid >~67 CL does tau pathology become accelerated. Recruiting participants below this threshold might result in slow/no cognitive decline in the placebo arm, requiring large group sizes and long duration trials as discussed above. While recruiting participants that already have cognitive impairment reduces the trial enrollment screen failure rate, as they are more likely to have pathologically advanced disease, this will, however, reduce the likelihood of slowing disease progression. Some of these themes are exemplified by the current clinical trials underway for lecanemab, discussed above. The Clarity AD study (NCT03887455) will enroll 1,566 patients with early AD and be of 1.5 years' duration with a completion date in 2022. The AHEAD 45 trial (NCT04468659) will enroll 1,000 cognitively normal participants with Aβ levels >40 CL and be of 4 years' duration – a significant logistical undertaking that will be completed in 2027.

One response to these problems has been the design of clinical instruments that purport to be able to reveal treatment effects with greater sensitivity: for example, the iADRS [83] or the preclinical Alzheimer cognitive composite [96]. While being able to demonstrate subtle clinical changes is clearly useful, how such outcomes will be viewed by organizations that are ultimately required to license and pay for therapeutics remains to be seen. Obviously, the field hopes that modest, cognitive and functional improvements demonstrated over relatively short periods of time in early disease with a therapeutic mechanism that is plausibly disease-modifying presages a profound disease-modifying effect that will be revealed over a much longer time frame.

It seems likely that with additional clinical studies of amyloid-targeted therapeutics the field will be able to refine more accurately the baseline characteristics of patients that will respond favourably to the rapid removal of brain amyloid. We conclude that, especially within the concept of a 'lead and lag', demonstration of amyloid removal is a measure of target engagement rather than of clinical efficacy. With the clear correlation and closer biological proximity of tau pathology to cognitive decline, demonstrating a reduction in tau pathology that correlates with improvement in cognitive performance and activities of daily living may well be adopted as a surrogate endpoint for multiple therapeutic

approaches. Recent breakthroughs with the detection of specific phosphorylated tau fragments in blood, some with apparent high specificity for early AD, are opening new perspectives in this regard [97]. Ultimately, these issues require a broad review with all stakeholders.

The FDA has controversially granted accelerated approval to aducanumab and has opined that the reduction of amyloid is reasonably likely to predict clinical benefit[7,9,98-107]. We consider that the relationship between the removal of amyloid and potential consequent clinical benefit may be more subtle. Surprisingly, the FDA licensed aducanumab without a requirement that patients prescribed aducanumab should first be tested to determine whether they are likely to have amyloid in their brains. As several studies have demonstrated that a significant proportion of patients that are clinically diagnosed with AD do not have amyloid in their brain, we hope that clinical practice with Aduhelm [108] evolves such that patients are screened for amyloid positivity, otherwise patients may receiving aducanumab with no likelihood of clinical benefit [109].

Optimal therapeutic characteristics. Now that an anti-Aβ antibody has received an accelerated regulatory approval, it is tempting to consider the characteristics of the ideal anti-Aβ antibody. We propose that such an anti-Aβ antibody should:
Mediate rapid removal of brain amyloid, reducing the lead and lag effect.

Have acceptable pharmacokinetic properties: e.g. half life of ~ 20-25 days in man.

Have acceptable blood brain barrier penetration into the CNS (~0.1–-0.2% of plasma levels). Future engineered therapeutics that engage transcytosis receptors such as the transferrin receptor may have radically higher blood–brain barrier penetration that may accelerate brain Aβ/amyloid removal, as being pioneered by RG6102 (NCT04023994), which is gantenuramb engineered to bind to the transferrin receptor [110,111].

Not induce anti-drug antibodies (or such antibodies are not neutralizing and do not affect antibody half life).

Have high affinity/avidity for amyloid plaque without binding soluble Aβ monomeric forms.

Not induce ARIA-E. Not require dose titration or i.v. infusion.

Require dosing intervals ≥ Q4W for patient convenience

Conclusion

Although the many failures in developing therapeutics for AD have led some large pharmaceutical companies to leave the field, others have continued and tried to learn from their experiences. We are now at a crucial moment when new insights should result in an acceleration of our efforts.

Researchers and drug developers now have a clearer idea of the evolution and interplay of the two pathognomonic aggregates in AD: amyloid and tau. In tauopathies such as frontal temporal lobar dementia, tau pathology can spread through the brain in the absence of amyloid, and so there is still the risk that removing amyloid will not halt, or sufficiently slow, tau pathology spread in AD to provide significant amelioration of cognitive decline.

It is also unclear whether removing amyloid will be clinically beneficial in those patients that are diagnosed as having AD but who have multiple pathologies, such as TDP-43 and α-synuclein pathology. Additional imaging tools or other biomarkers for these pathologies are needed urgently.

Another issue that requires careful examination is the definition of clinical meaningfulness as it relates to the effects of therapeutic intervention. This is especially important as researchers develop new clinical and functional rating schemes with which to establish evidence for the efficacy of novel therapeutics. Prior work has suggested that the minimal clinically important difference for the CDR-SB and MMSE assessments in mild AD is 1.63 and 2.3, respectively [112]. To place that into context, the Emerge study with high-dose aducanumab demonstrated a difference from placebo in the CDR-SB and MMSE assessments of 0.39 and 0.6, respectively. In fact, there is very little numerical difference in the placebo versus treatment changes in CDR-SB and MMSE in the Emerge, Expedition and Trailblazer-Alz studies [113].

One significant gap in our understanding is the paucity of gold-standard neuropathology data on clinical trial participants. It is possible that as the current anti-Aβ antibodies target different epitopes, they may remove different populations or types of amyloid plaques. Post mortem neuropathological assessment of patients where amyloid PET data are available pre- and post-treatment would provide insights into the various antibodies' mechanisms of action and also potentially enable an evaluation of the clinical consequences of having other pathologies.

The recent accelerated approval of aducanumab for the treatment of AD is hugely significant for the field but has attracted much controversy. Over the coming months and years, the effects of this decision will be manifested as other Aβ-directed agents are assessed by regulatory agencies and other therapeutic approaches continue to be developed. We sincerely hope that well-powered phase III and IV clinical studies of Aβ-directed drugs are able to run to completion for a robust and unambiguous assessment of their clinical efficacy, and that patient recruitment to clinical studies testing other therapeutic targets will not be adversely affected while ensuring that patients have access to effective medicines. Other critical downstream processes that warrant clinical investigation using a range of therapeutic approaches include the propagation of tau pathology, neuroinflammation and granulovacuolar degeneration. In our view, an area of investigation of particular interest is in linking the microglial response to the deposition of amyloid to downstream sequelae [22]. Targets may be discovered that are more proximate than amyloid to cognitive impairment. The challenge for researchers is determining those elements of the disease-associated microglial response that propel the disease process, versus those that are merely responses to it. This will require sophisticated in vitro and in vivo models that enable researchers to study the nexus of glia, amyloid, and tau pathology propagation. The field of neurodegenerative disease is entering an exciting new phase.

Table 1 | Completed or terminated phase III trials of agents targeting Aβ in Alzheimer disease

Year	Drug	Company	Mechanism of Action	Target	Patient Population	Outcome	Observations
2007	Tramiprosate	Neurochem	Unclear-may interact with Aβ oligomers	Soluble Aβ/ Aβ oligomers	Mild to moderate AD	Lack of efficacy	–
2009	Tarenflurbil	Myriad Genetics/Lundbeck	γ- Secretase modulator	Soluble Aβ	Mild AD	Lack of efficacy	Unlikely to have a adequate target e the brain
2011	Semagacestat	Eli Lilly	γ- Secretase inhibitor	Soluble Aβ	Mild to moderate AD	Toxicity and lack of efficacy	Increases cognitiv lowering of brain
2012	Bapineuzumab	Elan/Pfizer/ Johnson and Johnson	Anti- Aβ monoclonal antibody	Soluble Aβ + plaque	Mild to moderate AD	Lack of efficacy	No significant ren amyloid
2013	Gammagard	Baxter	Unclear – IvIg may bind soluble Aβ	Soluble Aβ	Mild to moderate AD	Lack of efficacy	–
2013	Solanezumab	Eli Lilly	Anti- Aβ monoclonal antibody	Soluble Aβ	Mild to moderate AD	Lack of efficacy	No removal of am
2016	Gantenerumab	Hoffman-LaRoche	Anti- Aβ monoclonal antibody	plaque	Mild AD	Lack of efficacy	Converted to an o study
2016	Solanezumab	Eli Lilly	Anti- Aβ monoclonal antibody	Soluble Aβ	Mild AD	Lack of efficacy	No removal of am
2016	Solanezumab	Eli Lilly	Anti- Aβ monoclonal antibody	Soluble Aβ	Prodromal AD	Trial halted	–
2016	Verubecestat	Merck	BACE inhibitor	Soluble Aβ	Mild to moderate AD	Lack of efficacy	Increases cognitiv modest lowering amyloid (~20CL)
2018	Verubecestat	Merck	BACE inhibitor	Soluble Aβ	Prodromal AD	Lack of efficacy	Increases cognitiv
2018	Atabecestat	Janssen	BACE inhibitor	Soluble Aβ	Asymptomatic at risk of AD	Toxicity	Increases cognitiv
2018	Lanabecestat	Astra Eli Lilly	BACE inhibitor	Soluble Aβ	Early AD	Lack of efficacy	Increases cognitiv
2018	Lanabecestat	Astra/ Eli Lilly	BACE inhibitor	Soluble Aβ	Mild AD	Lack of efficacy	Increases cognitiv
2019	Crenezumab	AC Immune/ Hoffman-LaRoche	Anti- Aβ monoclonal antibody	Soluble Aβ	Prodromal to mild AD	Lack of efficacy	–
2019	Elenbecestat	Biogen/Eisai	BACE inhibitor	Soluble Aβ	Prodromal to MCI due to AD	Lack of efficacy	Increases cognitiv
2019	Umibecestat	Amgen/Novartis	BACE inhibitor	Soluble Aβ	Asymptomatic at risk of AD	Lack of efficacy	Increases cognitiv
2019	Amilomotide	Novartis	Vaccine	Aβ	Asymptomatic at risk of AD	Trial halted	–
2020	Aducanumab	Biogen/Eisai	Anti- Aβ monoclonal antibody	plaque	MCI to Early Dementia	Evidence for efficacy	BLA given accelen by the FDA but re CHMP of the EMA

Table 2 | Comparison of properties of anti-Aβ antibodies

Drug	**Normalized dose (mg/kg/4 wk)**	**Amyloid removal (centiloids/number of patients)**	**% ApoE4 carriers**	**Duration of administration (weeks)**	**Normalized rate of amyloid removal (CL/4 wk/10mg/kg) (A)**	**Incidence of ARIA-E (%) (B)**	**ARIA-E/amyloid removal rate. Index (B/A)**
Bapineuzumab [60,61]	0.3	~7 [a]/12	0	71	0	9	-
Gantenerumab [90]	13[1]	42 [b]/27	67	52	2.5	48[b]	19.2
Lecanemab [71,114]	20	62 [c]/43	30[2]	52	2.4	10	4.2
Aducanumab [115]	6[3]	64 [d]/109	67[2]	78	5.5	35	6.4
Donanemab [84]	15[1]	67/115	72[2]	24	7.5	27	3.6

[1]Assumes average 70 kg patient weight. [2]% ApoE4 carriers in the cohort from which subjects were chosen for amyloid PET imaging. [3]Mean cumulative dose = 118 mg/kg at week 78 in Emerge study.
[a] Centiloids estimated assuming Δ1.258 SUVr = 100CL [116].
[b] Estimated from baseline to 52 weeks in group MR-DBP [90]
[c] Estimated from baseline to 52 weeks using CL=230xSUVr-240.6
[d] High-dose aducanumab in Emerge study

To explore the relationship between amyloid removal and ARIA-E selected clinical experiments where both amyloid removal and ARIA were measured have been analysed. To assess the intrinsic propensity of each antibody to mediate amyloid removal the rate of amyloid removal per mg of antibody administered per unit time was calculated and then converted to a standard dose that can be related to clinical practice (10mg/kg Q4W). The incidence of ARIA-E was divided by the normalized rate of amyloid removal to express the ARIA-E liability for amyloid removal for each antibody. Of significant importance is that the incidence of ARIA-E in patients treated with anti-Aβ antibodies is higher in ApoE4 carriers versus non-carriers. Thus, for lecanumab, while the incidence of ARIA-E was 10% overall, it was 14% in ApoE4 carriers and 8% in non-carriers.

Table 3 | Comparison of hypothetical anti-Aβ antibodies*

Normalized rate of amyloid removal (CL/4 wk/10mg/kg)	Weeks taken to reach 20 CL from a baseline of 100 CL	Weeks remaining post amyloid negativity
10	16	88
5	32	72
2.5	64	40
1.0	160	0

*The table illustrates the potential impact of four hypothetical anti-Aβ antibodies with different amyloid clearance potencies administered at 20 mg/kg every 4 weeks in a 104-week clinical trial.

Figure legends

Figure 1| **The amyloid hypothesis of Alzheimer disease**.

Schematic representation of the amyloid cascade hypothesis modified from Karran et al, [1]. $A\beta_{42}$ and other long Aβ peptides aggregate either as a consequence of abnormal production or decreased clearance. Soluble oligomeric Aβ and deposited Aβ in amyloid plaques interact with microglia, astroglia, blood vessels and neurons to induce different, damaging cellular responses that ultimately leads to neuronal dysfunction and death. Accumulation of paired helical tau filaments within neurons correlates best with the cognitive decline that characterizes the end stage of the disease. For simplicity the hypothesis is presented as a linear cascade, but there are likely feed-back and feed-forward signaling mechanisms [22]

Figure 2 | **Aβ epitopes of monoclonal antibodies tested in clinical trials for Alzheimer disease.**

Aβ amino acid sequence is indicated in one letter code and amino acids participating in the epitope recognized by the antibodies are stained in red (key amino acid) and orange [48,56,62,75,117,118]. *The pE3Aβ peptide is highly insoluble and consequently donanemab is effectively plaque-specific.

Figure 3 | **The relationship between amyloid removal and clinical response.**

The graph illustrates the hypothetical relationship between amyloid plaque removal and consequent amelioration of cognitive decline. Central to this hypothesis is that there are several unknown biological steps between amyloid deposition and cognitive decline, and thus some period of time will be required for the damaging cellular reactions to subside before amyloid removal exerts a beneficial effect. By extrapolating from the levels of amyloid required to propel tau pathology, which is the most proximate biomarker to cognitive impairment, we postulate that as anti-Aβ mAb therapy reduces brain amyloid to low levels (~ 20 CL) so cognitive decline will start to ameliorate. In the context of a clinical trial, the shorter the duration required to reach low amyloid levels (TΔA) so the longer the period available to reveal a statistically significant clinical efficacy between treatment and placebo groups (TΔE).

Figure 4 | **Aβ removal profiles for aducanumab, donanemab and lecanemab**.

The decrease in amyloid load as measured by centiloids or estimated centiloids (~) over time (see box 2 for further explanation) is indicated for four phase III trials [71,84,115].

Box 1 | Amyloid and tau pathology in Alzheimer disease: their relationship to cognitive decline

The relationship between amyloid-β (Aβ), tau and cognitive decline in Alzheimer disease (AD) is unresolved. While the autosomal dominant mutations that cause AD support the concept that Aβ deposition precedes tau pathology, two other possibilities cannot yet be excluded: that tau enhances Aβ pathology, or that both pathologies develop in parallel.

In individuals with autosomal dominant mutations in genes coding for amyloid precursor protein (APP) or Presenilin [119], alterations in Aβ generation that result in early deposition of plaque initiate the disease and patients invariably develop the full spectrum of tau pathology [120,121]. In the DIAN cohort, treatment of patients with familial (caused by autosomal dominant mutations) Alzheimer disease with gantenerumab significantly reduced amyloid plaques and cerebrospinal fluid (CSF) total tau, phospho-tau181 and neurofilament light chain, confirming an interaction between Aβ and tau. Of note, the study had insufficient power to detect cognitive benefit [47]. Autosomal dominant mutations to the MAPT (tau) gene lead to neuronal tangle pathology and frontotemporal dementia but not to amyloid plaques [122,123], suggesting that tau pathology does not induce Aβ pathology.

However, the sequence of events in sporadic AD is less clear. From large cross-sectional neuropathological studies of autopsied brain[124], it appears that tau pathology is already prevalent long before amyloid pathology appears, potentially supporting the concept that tau pathology leads to amyloid pathology [125]. In contrast, cross-sectional and longitudinal human cohort studies of biomarkers support a scenario in which amyloid pathology (Aβ42 lowering in CSF, positive amyloid PET imaging) precedes tau pathology (tau-PET and phospho-Tau elevation in CSF) and then neurodegeneration [126-128]. In a cross-sectional study, Lowe et al. [20] studied the association of neurofibrillary (NFT) tau PET and Aβ PET on cognition in 579 cognitively unimpaired adults. The participants' median age was 70 and abnormal NFT tau PET, defined as a signal in the entorhinal region, was seen in 20%, while abnormal Aβ was seen in 34% of the cohort. The NFT tau PET signal was significantly associated with impairment of various measures of cognition, on a region-of-interest basis. When the NFT tau PET signal was stratified by amyloid status, there was no significant evidence that amyloid modified the effect of the NFT tau PET signal.

Longitudinal studies in which both amyloid and tau PET imaging has been performed are particularly informative. Cho et al. [129] followed participants over 2 years who were classified at baseline into cognitively unimpaired, amyloid negative; cognitively unimpaired, amyloid positive; mild cognitive impairment (MCI), amyloid positive; and AD dementia, amyloid positive. There were global SUVr increases in tau PET signal in the MCI and AD dementia groups of 2.9% and 8.0%, respectively, that were associated with cognitive decline. There were no significant differences in tau PET signal for the cognitively unimpaired amyloid-negative and amyloid-positive groups. Jack et al. [130] studied 59 cognitively unimpaired, amyloid-negative participants; 37 cognitively unimpaired amyloid-positive participants and 30 cognitively impaired amyloid-positive participants in a 12–15 month study. This study showed that in cognitively unimpaired amyloid-negative participants, tau PET signal did not increase, while it did in cognitively unimpaired amyloid-positive and cognitively impaired amyloid-positive groups. Aschenbrenner et al. [131] performed a retrospective study of 152 participants who had received at least 1 flortaucipir tau PET scan and 1 florbetapir amyloid PET scan within a year, and ≥2 clinical and cognitive assessments. All participants entering the study had equal to or less than 0.5 on

the CDR scale; that is, they were cognitively normal or with very mild AD. The statistical analysis demonstrated that only tau PET signal significantly predicted cognitive decline, while there was also an association between high amyloid levels and tau-related cognitive decline. A 7-year longitudinal study was conducted in which 60 clinically normal elderly participants received repeated tau and amyloid PET scans and cognitive assessments [132]. This analysis revealed that increases in tau PET signal were significantly associated with cognitive decline, and while increases in amyloid PET were associated with increases in tau PET signal, it was not significantly directly associated with cognitive decline.

These and other studies [13,24,133,134] strongly support the concept of AD as being an amyloid-provoked, or facilitated, tauopathy, although there is also evidence that both pathologies act in synergy [135].

Box 2 | The centiloid scale and the meaning of amyloid negativity

The development of positron emission tomography (PET) ligands to measure amyloid plaque loads in living patients with Alzheimer disease (AD) has transformed the field , and a number of amyloid PET ligands are currently in use: ^{11}C-Pittsburgh compound-B (^{11}C-PIB), ^{18}F-flutemetamol, ^{18}F-florbetaben and ^{18}F-florbetapir tracers display 85%–98% sensitivity and 87%–100% specificity for Aβ plaques when using the postmortem truth standards of the Consortium to Establish a Registry for AD (CERAD) or the National Institute on Aging–Alzheimer's Association [85,136-138].
The accrual of amyloid in the brain is a continuum after initial deposition occurs, although the use of 'cut points' to determine 'amyloid-positive' from 'amyloid-negative' subjects has the benefit of simplicity and utility for clinical trial patient inclusion/exclusion decisions and also is relevant to establishing a reliably detected lower limit. Another subtlety is that amyloid load measurements can vary depending on the brain regions of interest used in determining the standard uptake value ratio (SUVr). Thus, comparing amyloid PET data from different research groups has proved challenging, and this prompted the Centiloid Project, which has provided guidance on how to convert SUVr information from different amyloid PET ligands into a common, 100-point scale [116]. In the centiloid (CL) scale, '0' represents the mean amyloid PET signal from healthy young adults and '100' represents the mean signal from patients with AD. Here, we have converted SUVr data into estimated CL (indicated by ~) by cross-reference to relevant published material unless CL values have been provided by the authors.

Several studies have investigated the amyloid positivity/negativity threshold values for amyloid PET ligands by imaging living patients and comparing SUVr values with subsequent post-mortem histological assessment of amyloid plaques [85-87 88]. When interpreting these studies, one should consider that the "golden" neuropathological standards like the Thal staging scheme [14] and the CERAD amyloid rating scale, measure different aspects of amyloid pathology in a qualitative way while amyloid PET ligands provide a quantitative assessment of deposited Aβ. Clarke et al [139] used florbetapir to assess amyloid in subjects spanning cognitively normal to advanced dementia who ranged from 47-103 years' of age. Those who died within 2 years had their brains assessed for amyloid burden using the CERAD scoring system for neuritic plaques and by immunohistochemistry for overall amyloid burden in six cortical regions. These data were subsequently incorporated into a study that converted SUVr data into CL [140]. The authors concluded the appropriate threshold for amyloid positivity/negativity was 24 CL (=1.1 SUVr).
La Joie et al [86] investigated 179 subjects ranging from cognitively normal to those with AD dementia who received a 11C Pib amyloid scan and who came to autopsy within 3.3 years, on average. The imaging

data were converted to CL and amyloid burden was assessed with neuropathology. A CL threshold of 12.2 provided 88% accuracy, 89% sensitivity and 86% specificity for none to sparse amyloid in the CERAD scale, and a CL threshold of 23.5 provided 89% accuracy, 86% sensitivity and 96% specificity for moderate to frequent amyloid in the CERAD scale.
Dore et al [87] studied 18F florbetaben imaging data from 52 end of life subjects who went to autopsy within 1 year, on average. 18F florbetaben SUVrs were transformed to the CL scale. An optimal cut off of 19 CL was established as separating negative from positive amyloid burden as assessed by neuropathology.
Amadoru et al [88] studied 51 subjects who had both ante mortem amyloid imaging with either Pib or Florbetaben and post mortem neuropathological assessment using the CERAD scale. The optimum threshold between low vs high amyloid plaque was 21 CL; and 10 CL for none vs. any.
Studies that have not used post-mortem data, but statistical analyses of patient cognitive status correlated with amyloid PET imaging have determined similar CL threshold values, for example, 19 CL as the value beyond which the rate of change in amyloid PET reliably increases [141].

In summary, these studies show that a CL value of ≤ 20 is a reasonable value to define 'amyloid negativity'.

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Related links

Biogen plans regulatory filing for aducanumab in Alzheimer's disease based on new analysis of larger dataset from phase 3 studies: https://investors.biogen.com/news-releases/news-release-details/biogen-plans-regulatory-filing-aducanumab-alzheimers-disease

Fig 1

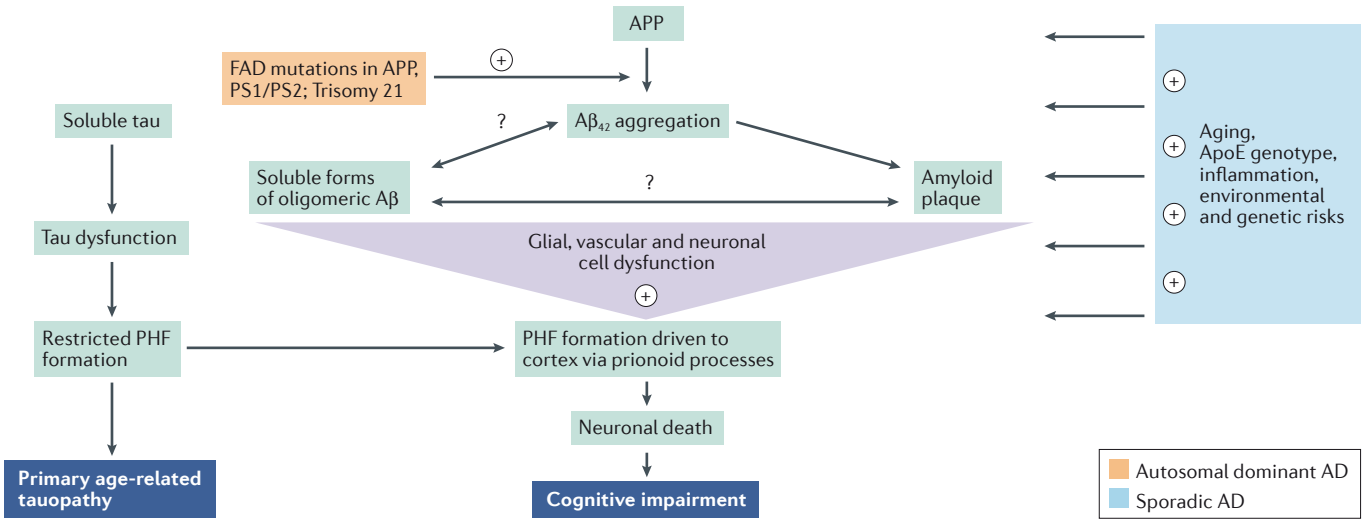

APP
FAD mutations in APP, PS1/PS2; Trisomy 21
Soluble tau
?
Aβ42 aggregation
Soluble forms of oligomeric Aβ
?
Amyloid plaque
Tau dysfunction
Glial, vascular and neuronal cell dysfunction
Aging, ApoE genotype, inflammation, environmental and genetic risks
Restricted PHF formation
PHF formation driven to cortex via prionoid processes
Neuronal death
Primary age-related tauopathy
Cognitive impairment
Autosomal dominant AD
Sporadic AD

Fig 2

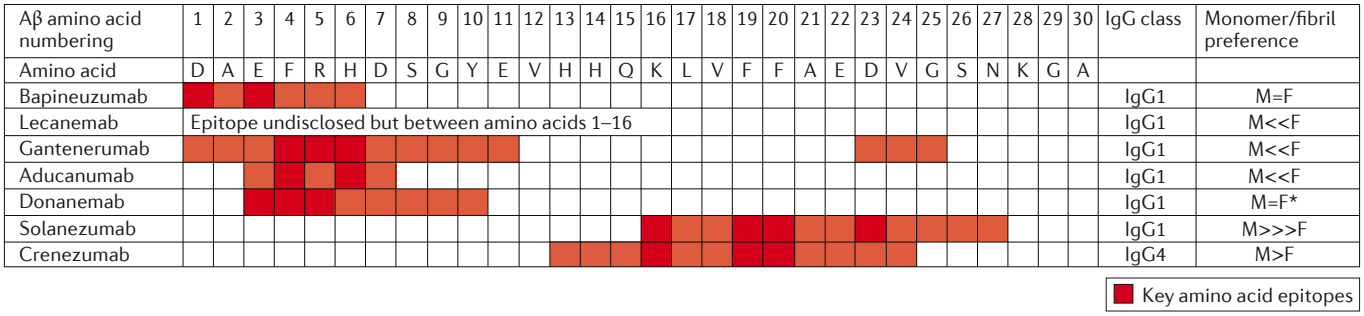

Aβ amino acid numbering
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
IgG class
Monomer/fibril preference
Amino acid
D A E F R H D S G Y E V H H Q K L V F F A E D V G S N K G A
Bapineuzumab
IgG1
M=F
Lecanemab
Epitope undisclosed but between amino acids 1–16
IgG1
M<<F
Gantenerumab
IgG1
M<<F
Aducanumab
IgG1
M<<F
Donanemab
IgG1
M=F*
Solanezumab
IgG1
M>>>F
Crenezumab
IgG4
M>F
Key amino acid epitopes

Fig 3

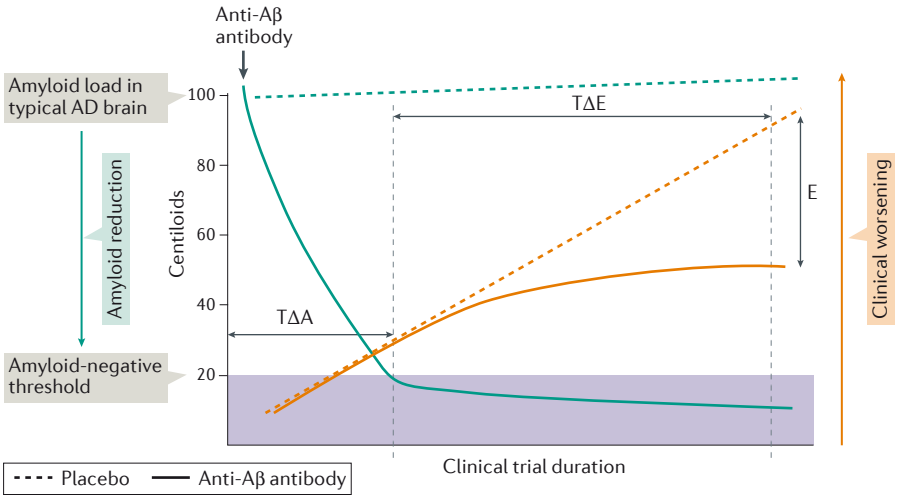
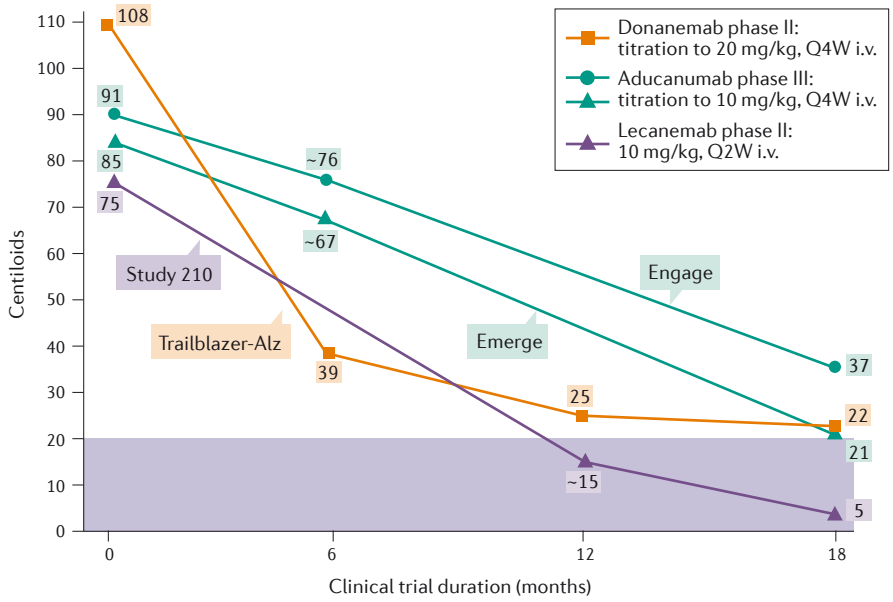

Anti-Aβ antibody
Amyloid load in typical AD brain
Amyloid reduction
Amyloid-negative threshold
Centiloids
100
80
60
40
20
TΔA
TΔE
E
Clinical worsening
Clinical trial duration
Placebo
Anti-Aβ antibody

Fig 4


Donanemab phase II:
titration to 20 mg/kg, Q4W i.v.
Aducanumab phase III:
titration to 10 mg/kg, Q4W i.v.
Lecanemab phase II:
10 mg/kg, Q2W i.v.
Centiloids
Clinical trial duration (months)
0
10
20
30
40
50
60
70
80
90
100
110
0
6
12
18
108
91
85
75
~76
~67
39
25
~15
37
22
21
5
Study 210
Trailblazer-Alz
Emerge
Engage