A critical appraisal of amyloid-β-targeting therapies for Alzheimer disease

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Abstract | Brain accumulation of the amyloid-β (Aβ) peptide is believed to be the initial event in the Alzheimer disease (AD) process. Aβ accumulation begins 15–20 years before clinical symptoms occur, mainly owing to defective brain clearance of the peptide. Over the past 20 years, we have seen intensive efforts to decrease the levels of Aβ monomers, oligomers, aggregates and plaques using compounds that decrease production, antagonize aggregation or increase brain clearance of Aβ. Unfortunately, these approaches have failed to show clinical benefit in large clinical trials involving patients with mild to moderate AD. Clinical trials in patients at earlier stages of the disease are ongoing, but the initial results have not been clinically impressive. Efforts are now being directed against Aβ oligomers, the most neurotoxic molecular species, and monoclonal antibodies directed against these oligomers are producing encouraging results. However, Aβ oligomers are in equilibrium with both monomeric and aggregated species; thus, previous drugs that efficiently removed monomeric Aβ or Aβ plaques should have produced clinical benefits. In patients with sporadic AD, Aβ accumulation could be a reactive compensatory response to neuronal damage of unknown cause, and alternative strategies, including interference with modifiable risk factors, might be needed to defeat this devastating disease.

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Alzheimer disease (AD) is an incurable, progressive neurodegenerative disorder with a long presymptomatic period that is clinically characterized by cognitive and behavioural impairment, social and occupational dysfunction and, ultimately, death. In the USA, AD was the sixth most common cause of death in 2015 and showed the largest age-adjusted increase (16%) relative to 2014 (REF.[1]). According to current estimates, 17% of people aged 75–84 years in the USA have AD, and the disease costs the country US$236 billion per year. The prevalence is projected to triple by 2050 to >15 million, with annual costs of >$700 billion[2].

Diagnosis of AD is usually based on medical history and clinical findings, sometimes corroborated by brain imaging. Therapies are symptomatic and do not affect disease progression; currently, cholinesterase inhibitors and the *N*-methyl-D-aspartate receptor antagonist memantine are the only available options. Despite extensive research into the pathophysiology of AD, the large number of drugs entering clinical development and the enormous expenditure on large and complex trials, no new drug has been approved since memantine in 2003. Many reasons have been proposed to explain this failure, including inappropriate patient selection, variable rates of progression, suboptimal dosing, drug exposure and/or target engagement, inappropriate time of intervention, inappropriate outcome measures and low sensitivity of clinical scales. In addition, an incomplete understanding of AD pathophysiology might have led to selection of the wrong targets.

Most of the drugs tested for AD in the past 20 years have targeted the accumulation of the amyloid-β (Aβ) peptide. In this article, we consider the current anti-Aβ drugs and the possible reasons for their failure to provide meaningful clinical benefits. Anti-Aβ therapies have been extensively tested in sporadic late-onset forms of AD, which will be the main focus of our discussion; no therapeutic studies have yet been conducted in individuals with the less common familial forms.

The amyloid cascade hypothesis

The Aβ peptide is generated by metabolism of amyloid precursor protein (APP), a type I transmembrane glycoprotein of 695–770 amino acids. APP is cleaved close to the membrane by an extracellular protease known as α-secretase. This cleavage liberates a soluble extracellular fragment, sAPPα. APP is also cleaved by an aspartyl protease known as β-secretase 1 (BACE1),

Key points

- Genetic, biochemical, histopathological, biomarker and cognitive studies have suggested that brain accumulation of the amyloid-β (Aβ) peptide is the initial event in the Alzheimer disease (AD) process.
- Over the past 15 years, several drugs that decrease Aβ production, antagonize Aβ aggregation or increase brain Aβ clearance have been tested in patients with mild to moderate AD but without success.
- Anti-Aβ drugs have also produced disappointing results in individuals at earlier stages of the disease who have biomarker evidence of Aβ brain deposition.
- This series of clinical failures has raised the possibility that Aβ accumulation represents an epiphenomenon rather than a cause of AD, casting doubt on the prevailing amyloid cascade hypothesis of AD.
- Aducanumab, a potent monoclonal antibody specifically directed against Aβ oligomers, produced encouraging preliminary results in patients with prodromal or mild AD, suggesting that oligomeric Aβ species may represent a valid biological target.
- As accumulation of Aβ in the brain starts 15–20 years before the onset of clinical symptoms, drugs are now being tested in preclinical or asymptomatic stages of AD and in cognitively healthy individuals at risk of AD.
- Other promising approaches directed against key elements of the disease, such as CNS inflammation, brain insulin resistance and tau aggregation, must be more intensively pursued to avoid a therapeutic vacuum should the present anti-Aβ therapies fail even in asymptomatic individuals.

generating a soluble extracellular fragment, sAPPβ, and a cell-membrane-bound fragment (C99). C99 is cleaved within the membrane by an enzymatic complex of four proteins (presenilin, nicastrin, anterior pharynx-defective 1 and presenilin enhancer 2), collectively termed γ-secretase. Presenilin is the catalytic subunit of γ-secretase and can be encoded by either the *PSEN1* or the *PSEN2* gene. The γ-secretase cleavage releases Aβ and an intracellular peptide known as amyloid intracellular domain. Aβ exists in various lengths, including the most abundant form, which consists of 40 amino acids ($Aβ_{1–40}$), and a less soluble 42-amino-acid form ($Aβ_{1–42}$). Aβ aggregates to form oligomers, protofibrils, fibrils and, ultimately, plaques, which are one of the hallmarks of AD pathology (FIG 1).

Brain accumulation of Aβ is believed to be the initial event of the AD process. Aβ accumulation starts in the hippocampus and entorhinal cortex. In addition, intracellular deposition of hyperphosphorylated tau protein in neurofibrillary tangles (NFTs) leads to progressive cytoskeletal changes and disrupts axonal transport. The concept that Aβ accumulation is the central event in the pathogenesis of AD was initially proposed in 1991 by three independent groups[3–5]. The 'amyloid cascade hypothesis' was formally proposed by Hardy and Higgins 1 year later[6]. Initially, this hypothesis stated that Aβ deposition in the brain drives tau phosphorylation, NFT formation, synapse loss, neuron death and cognitive impairment. The hypothesis was supported by the discovery that AD could result from autosomal dominant mutations in the *APP* gene, and that autosomal dominant mutations in *PSEN1* and *PSEN2* increased Aβ production, thereby promoting Aβ aggregation and deposition. Transgenic mice that expressed forms of APP or presenilin protein containing mutations associated with human familial AD progressively developed brain Aβ plaques and memory deficits, further reinforcing the idea that Aβ accumulation can cause AD[7].

In 2012, the APP amino acid substitution Ala673Thr was found to protect against AD onset and cognitive decline in cognitively healthy elderly individuals. The mutant amino acid is adjacent to the BACE cleavage site, and its presence resulted in an ~40% reduction in the formation of Aβ peptides in vitro[8].

In late-onset sporadic AD, accumulation of brain Aβ has been attributed to defective clearance[9] and increased BACE activity[10]. Aβ accumulation has also been linked to the strongest genetic risk factor for late-onset sporadic AD, the apolipoprotein E ε4 (*APOE*ε4*) allele. In the CNS, the fat-binding protein APOE is produced mainly by astrocytes and transports cholesterol to neurons via APOE receptors. The *APOE* gene is polymorphic, with three major alleles: *APOE*ε2*, *APOE*ε3* and *APOE*ε4*. The risk of AD is up to 15 times higher in people with two *APOE*ε4* alleles than in *APOE*ε3* carriers, and the *APOE*ε2* allele might confer protection against AD. The *APOE*ε3* allele seems to be neutral with respect to AD risk. The different APOE proteins show differing abilities to mediate Aβ clearance, with APOE2 being the most effective and APOE4 the least effective[11]. The *APOE*ε4* allele is consistently linked to abnormal Aβ aggregation and predicts longitudinal Aβ accumulation in plaque-free elderly individuals without dementia. Conversely, *APOE*ε2* carriers are protected against longitudinal Aβ accumulation[12].

Amyloid pathology can be assessed by PET tracers or, indirectly, by a reduction in $Aβ_{1–42}$ levels in the cerebrospinal fluid (CSF), a supposed indicator of Aβ aggregation in the brain. In terms of biomarker dynamics, the Aβ cascade hypothesis proposes that initial changes in Aβ concentrations are evident in the CSF, followed in sequence by Aβ accumulation in the brain, increases in CSF tau, hippocampus and grey matter volume losses, decreased glucose metabolism, memory impairment and, finally, dementia[13]. Post-mortem studies have shown that cognitively healthy elderly people can have extensive amyloid pathology[14]. Brain imaging studies indicated that Aβ pathology was present in up to 44% of cognitively healthy older individuals[15], and those with amyloid deposits at baseline experienced a faster decline in cognitive performance, brain volume and brain glucose metabolism than those without amyloid pathology[16–20]. Aβ deposition has been estimated to precede the clinical symptoms of AD by 15–20 years[17].

Amyloid-β and tau crosstalk

Neuropathological, genetic and molecular data indicate that the tau protein mediates AD pathophysiology. Tau pathology correlates with neuronal loss[21] and AD duration and severity[22,23], mediates the association between brain Aβ load and AD occurrence[24] and is visible in the entorhinal cortex in people with subjective memory complaints[25]. In the absence of Aβ, hippocampal tau deposition might be insufficient to trigger the neurodegenerative process that leads to AD[26]. Interestingly, longitudinal studies in people with sporadic[27] or dominantly inherited AD[28] have shown that CSF tau levels rise in the early stages but decline once the symptoms manifest. The results of a stable isotope labelling kinetics study suggested that Aβ pathology enhanced the production

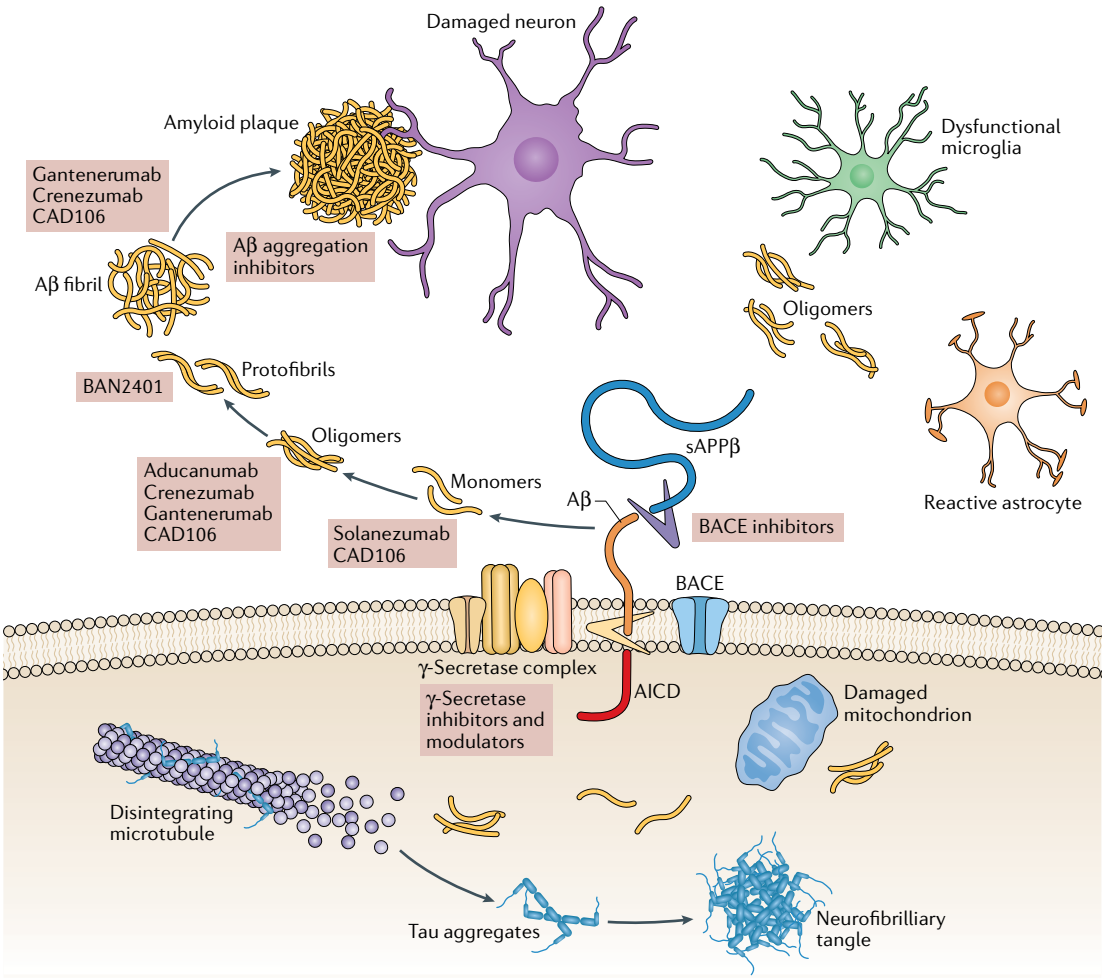

Fig. 1 | **Targets of anti-Aβ drugs.** Mechanisms of action of the main anti-amyloid-β (Aβ) drugs that are currently in phase III clinical development for the treatment of Alzheimer disease. AICD, amyloid precursor protein intracellular domain; BACE, β-secretase; sAPPβ, soluble amyloid precursor protein-β.

of tau protein[29]. These findings conflict with the idea that elevated CSF tau levels in people with AD arise primarily from dead and dying neurons. Neuritic Aβ plaques trigger the formation of a specific type of tau aggregate, which in turn fuels the formation and spreading of NFTs and neuropil threads[30]. High tau and low $A\beta_{1-42}$ levels in the CSF are associated with progression to mild cognitive impairment (MCI) in cognitively healthy individuals[16] and progression to AD in patients with MCI[31].

In view of the fact that Aβ and tau aggregates show different spatial and temporal patterns of progression within vulnerable brain regions[32], three distinct temporal phases in AD have been proposed. First, in clinically unaffected individuals, tau-associated network disruption occurs in specific brain regions. Second, this disruption can trigger Aβ-associated compensatory brain network changes. Last, Aβ deposition marks the saturation of functional compensation and heralds acceleration of the inciting phenotype-specific, tau-associated network failure[33].

Putative disease-modifying therapies

FIGURE 2 summarizes the stage of clinical development of current and abandoned anti-Aβ drugs for AD. In this section, we review the anti-Aβ drugs that are in phase III trials at present and have the potential to modify AD progression. These drugs include anti-Aβ immunotherapies, which stimulate Aβ clearance, and BACE inhibitors, which decrease Aβ production.

Active anti-amyloid-β immunotherapies

Active immunization involves administration of an Aβ antigen that can elicit an immunological response against Aβ. Elan Pharmaceuticals pursued this approach in 2002 by administering pre-aggregated $A\beta_{1-42}$ with the immunological adjuvant QS-21. This vaccine, known as AN-1792, significantly reduced Aβ deposits in the brains of patients with AD but produced no cognitive or clinical benefits[34]. In addition, AN-1792 elicited a T cell-mediated immunological response; thus, ~6% of the treated patients developed meningoencephalitis[35]. Safer Aβ antigens and adjuvants were later developed but did not produce clinical benefits (TABLE 1). Vanutide is a conjugate of multiple short Aβ fragments linked to a carrier of inactivated diphtheria toxin and was designed to avoid the safety concerns associated with AN-1792. Vanutide, with or without QS-21 adjuvant, was tested in ascending doses in two placebo-controlled phase IIa studies involving 245 patients with mild to moderate

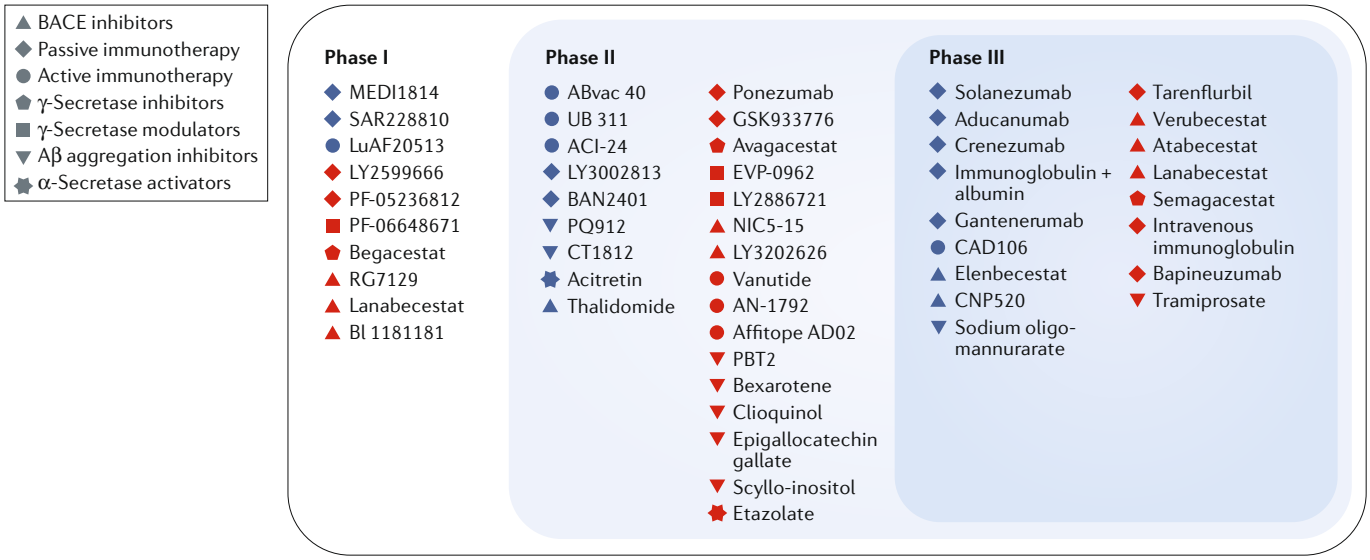

Fig. 2 | **Stage of clinical development of anti-Aβ drugs to treat Alzheimer disease.** The figure does not include drugs that indirectly interfere with amyloid-β (Aβ) or do not have fully proven anti-Aβ mechanisms of action. Trials of drugs shown in red have been discontinued or are inactive. BACE, β-secretase.

AD[36]. The drug was well tolerated, but no differences in cognitive measures, brain volumes or CSF biomarkers were observed between the vanutide and placebo groups.

Only one active anti-Aβ vaccine, CAD106, is currently in phase III trials. CAD106 is an Aβ antigen that consists of multiple copies of the $Aβ_{1–6}$ fragment, coupled to an adjuvant carrier (Qβ virus-like particle). In two *APP* transgenic mouse lines, CAD106 reduced brain amyloid accumulation without evidence of increased microhaemorrhages or inflammatory reactions[37]. Antibodies elicited by CAD106 reacted with Aβ monomers and oligomers and blocked Aβ toxicity in cell cultures[38]. However, no studies are available on the behavioural or cognitive effects of CAD106 in animal models of AD.

A 1-year, double-blind, placebo-controlled phase I trial of two subcutaneous doses of CAD106 in 58 people with mild to moderate AD found dose-related Aβ antibody titre responses but no significant changes on cognitive or clinical scales[38]. In a 66-week open-label extension study and a phase II study, prolonged increases in Aβ-specific antibody titres were observed, and no unexpected adverse events were reported[39].

A 90-week, double-blind, placebo-controlled phase IIb study assessed the safety, tolerability and immunogenicity of CAD106 in 121 patients with mild AD[40]. Patients received up to seven doses of CAD106 (150 μg or 450 μg) or placebo, with or without alum adjuvant, over 60 weeks. Serious adverse events (SAEs) were reported in 7% of the placebo-treated patients and in 25% of the CAD106-treated patients. Amyloid-related imaging abnormalities (ARIAs) occurred in six patients treated with CAD106. The drug induced strong antibody responses in 55% of patients on the 150 μg dose and in 81% of patients on the 450 μg dose. Serum Aβ–immunoglobulin G (IgG) titres correlated with the reduction in brain amyloid burden, as assessed by PET, from baseline to week 78. Unexpectedly, cognitive decline over 78 weeks, measured on the Mini-Mental State Examination (MMSE) scale, was greater in CAD106-treated patients who had a strong serological response than in the control group, although the difference failed to reach statistical significance[40].

CAD106 is being evaluated in a preventive paradigm in a 5-year, double-blind, placebo-controlled phase II/III study (Generation S1) involving 1,340 homozygous *APOE*ε4* carriers who are cognitively healthy. Half of the participants will be randomly assigned to receive intramuscular CAD106 or a matching placebo, whereas the other half will be randomly assigned to receive oral CNP520 (a BACE inhibitor) or a matching placebo. The trial will measure the ability of the treatment to delay diagnosis to MCI or AD dementia and will also assess the change in the Alzheimer's Prevention Initiative Composite Cognitive (APCC) test score[41]. The study is expected to be completed by May 2024.

Passive anti-amyloid-β immunotherapies

Passive anti-Aβ immunotherapies consist of monoclonal or polyclonal humanized anti-Aβ antibodies. Several such antibodies were found to be ineffective in patients mild to moderate or mild AD (TABLE 1). Five antibodies — solanezumab, gantenerumab, crenezumab, aducanumab and BAN2401 — are now being developed in patients with early AD, people at a preclinical stage of familial AD and asymptomatic individuals at high risk of AD.

Solanezumab. Solanezumab is a humanized IgG1 monoclonal antibody that targets the central region of Aβ ($Aβ_{13–28}$). Studies in transgenic mice and humans indirectly suggest that solanezumab recognizes soluble monomeric Aβ because it causes a substantial rise in total plasma Aβ levels. However, in vitro studies in human brain tissue indicate that this antibody also binds to Aβ plaques[42]. A single injection of m266, the mouse version

Table 1 | **Principal failed clinical studies on anti-Aβ therapies in AD and related disorders**

Drug	Company	Mechanism of action	Patient population	Trial phase	Main reasons for failure	Remarks
2002						
AN-1792 (REF.[35])	Elan	Aβ antigen	Mild to moderate AD	II	Toxicity and lack of efficacy	–
2007						
Tramiprosate[204]	Neurochem	Aβ aggregation inhibitor	Mild to moderate AD	III	Lack of efficacy	–
2009						
Tarenflurbil[205]	Myriad Genetics/ Lundbeck	γ-Secretase modulator	Mild AD	III	Lack of efficacy	Worsens global status
Scyllo-inositol[206]	Transition Therapeutics/Elan	Aβ aggregation inhibitor	Mild to moderate AD	II	Toxicity and lack of efficacy	• Increases mortality • Inactivates Aβ oligomers
2010						
Begacestat[207]	Wyeth	γ-Secretase inhibitor	Mild to moderate AD	II	Toxicity and lack of efficacy	–
2011						
Ponezumab[208]	Pfizer	Anti-Aβ monoclonal antibody	Mild to moderate AD	II	Lack of efficacy	–
Semagacestat[209]	Eli Lilly	γ-Secretase inhibitor	Mild to moderate AD	III	Toxicity and lack of efficacy	Worsens cognition
2012						
Bapineuzumab[210]	Elan/Wyeth	Anti-Aβ monoclonal antibody	Mild to moderate AD	III	Lack of efficacy	–
Avagacestat[211]	Bristol-Myers Squibb	γ-Secretase inhibitor	Mild to moderate AD	II	Toxicity and lack of efficacy	Worsens cognition
Avagacestat[212]	Bristol-Myers Squibb	γ-Secretase inhibitor	Prodromal AD	II	Toxicity and lack of efficacy	Worsens cognition
2013						
Solanezumab[49]	Eli Lilly	Anti-Aβ monoclonal antibody	Mild to moderate AD	III	Lack of efficacy	–
Vanutide[36]	Janssen	Aβ antigen	Mild to moderate AD	II	Lack of efficacy	–
Immunoglobulin[213]	Baxter	Anti-Aβ polyclonal antibody	Mild to moderate AD	III	Lack of efficacy	–
LY2886721 (REF.[214])	Eli Lilly	β-Secretase inhibitor	Mild to moderate AD	II	Toxicity	–
AZD3839 (REF.[215])	AstraZeneca	β-Secretase inhibitor	Healthy volunteers	I	Toxicity	–
2014						
Affitope AD02 (REF.[216])	Affiris/ GlaxoSmithKline	Aβ antigen	Early AD	II	Lack of efficacy	Worsens cognition
CAD106 (REF.[40])	Novartis	Aβ antigen	Mild AD	II	Lack of efficacy	Worsens cognition
PBT2 (REF.[217])	Prana Biotechnology	Aβ aggregation inhibitor	Prodromal AD	II	Lack of efficacy	–
Crenezumab[65]	Genentech/Roche	Anti-Aβ monoclonal antibody	Mild to moderate AD	II	Lack of efficacy	Binds oligomeric Aβ
Gantenerumab[57]	Chugai/Roche	Anti-Aβ monoclonal antibody	Prodromal AD	II	Lack of efficacy	Binds oligomeric Aβ
Gantenerumab[59]	Chugai/Roche	Anti-Aβ monoclonal antibody	Mild AD	II	Lack of efficacy	Binds oligomeric Aβ
2016						
Solanezumab[50]	Eli Lilly	Anti-Aβ monoclonal antibody	Mild AD	III	Lack of efficacy	–
Solanezumab[218]	Eli Lilly	Anti-Aβ monoclonal antibody	Prodromal AD	III	Strategic	–
Verubecestat[80]	Merck	BACE inhibitor	Mild to moderate AD	III	Lack of efficacy	• Increases mortality • Worsens cognition
2018						
Verubecestat[82]	Merck	BACE inhibitor	Prodromal AD	III	Lack of efficacy	Worsens cognition
Atabecestat[96]	Janssen	BACE inhibitor	Asymptomatic at risk of AD	III	Toxicity	Worsens cognition
Lanabecestat[88]	• Astra • Eli Lilly	BACE inhibitor	Early AD	III	Lack of efficacy	Worsens cognition
Lanabecestat[88]	• Astra • Eli Lilly	BACE inhibitor	Mild AD	III	Lack of efficacy	Worsens cognition

Studies are grouped by year of publication of the main results. Aβ, amyloid-β; AD, Alzheimer disease; BACE, β-secretase.

of solanezumab, reversed memory deficits without affecting brain amyloid plaques in the PDAPP transgenic mouse model of AD[43], raising the possibility of targeting the soluble pool of Aβ[44]. However, m266 did not attenuate the cognitive deficits in J20 transgenic mice, another model of AD[45].

A phase I study evaluated the safety and tolerability of single, escalating intravenous doses of solanezumab in patients with AD[46]. A dose-dependent increase in total Aβ levels was seen in plasma and CSF. In a study of multiple 400 mg doses in 33 patients with AD, a marked dose-dependent increase in total Aβ plasma levels was observed[47]. In a 12-week, double-blind, placebo-controlled phase II study in 52 patients with AD, which used 4 different dose regimens of solanezumab, increases in total $Aβ_{1–40}$ and $Aβ_{1–42}$ levels were observed in both plasma and CSF, but no significant changes in cognitive scores were reported[48].

In two 80-week, double-blind, placebo-controlled phase III studies involving 2,052 patients with mild to moderate AD (EXPEDITION 1 and EXPEDITION 2), monthly 400 mg intravenous solanezumab infusions failed to show efficacy with regard to cognitive and functional outcomes[49]. A subgroup analysis of EXPEDITION 1, but not of EXPEDITION 2, indicated reduced cognitive decline in mildly affected individuals who were receiving the drug. In both studies, increases in total plasma $Aβ_{1–40}$ and $Aβ_{1–42}$ levels were observed in solanezumab-treated individuals. CSF analysis revealed a decrease in total $Aβ_{1–40}$ and an increase in total $Aβ_{1-42}$ levels. In both studies, the incidence of ARIAs was similar in the solanezumab and placebo groups[49].

A third phase III study, EXPEDITION 3, was conducted in 2,129 patients with mild AD (MMSE score 20–26) who had biomarker evidence of Aβ deposition in the brain[50]. The participants received intravenous solanezumab (400 mg) or placebo every 4 weeks for 76 weeks. The drug did not produce a significant improvement on the primary outcome measure of efficacy, the 14-item Alzheimer's Disease Assessment Scale–Cognitive subscale (ADAS-Cog14). Patients on solanezumab performed slightly, though statistically significantly, better than those on placebo on most of the secondary variables, including MMSE and Clinical Dementia Rating scale–Sum of Boxes (CDR-SB) scores. Solanezumab did not reduce brain amyloid deposits, as assessed by florbetapir PET. A further 2-year phase III study (ExpeditionPRO), which planned to enrol 2,450 individuals with prodromal AD or MCI due to AD and positive Aβ PET, was started but was later discontinued for strategic reasons.

Solanezumab is also being evaluated in preventive paradigms. A 3-year, double-blind, placebo-controlled study, A4, is testing solanezumab in 1,150 asymptomatic or mildly symptomatic elderly individuals with biomarker evidence of brain amyloid deposition[51]. The primary efficacy variable is performance on a cognitive composite scale[52], the Alzheimer's Disease Cooperative Study–Preclinical Alzheimer Cognitive Composite (ADCS–PACC). In June 2017, the intravenous dose of solanezumab was increased from 400 mg to 1,600 mg, administered every 4 weeks, and the duration of the study increased to 4.5 years. The trial is expected to be completed by 2022.

Another double-blind, placebo-controlled study, DIAN-TU (Dominantly Inherited Alzheimer Network–Trials Unit), is being conducted in 438 asymptomatic or mildly symptomatic carriers of autosomal dominant mutations in *APP*, *PSEN1* or *PSEN2* to test the efficacy of solanezumab, gantenerumab and atabecestat in arresting or delaying the onset of cognitive deficit. A biomarker evaluation will be carried out after 2 years of treatment (DIAN-TU Biomarker Trial). Drugs that show promising effects on AD biomarkers will advance to a 4-year study (DIAN-TU Adaptive Trial) in 266 mutation carriers, the primary end point of which is a composite battery of cognitive tests (DIAN-TU cognitive composite score).

Gantenerumab. Gantenerumab is a fully human recombinant monoclonal IgG1 antibody that binds to both amino-terminal and central regions of Aβ. Gantenerumab shows higher affinities for Aβ oligomers and fibrils than for Aβ monomers[53]. The low affinity of gantenerumab for monomeric Aβ is supported by the observation that the drug does not alter plasma Aβ levels in vivo. Weekly intravenous injections of gantenerumab were shown to reduce brain Aβ plaques both in hAPP[54] and double hAPP/PS2 (REF.[53]) transgenic mouse models of AD. However, no studies are available on the behavioural or cognitive effects of gantenerumab in mouse models. In cynomolgus monkeys, the CSF–plasma ratio of intravenous gantenerumab was shown to be very low[55] (0.006–0.010%), indicating that the drug failed to cross the blood–brain barrier.

In a multiple ascending dose phase I study in 18 patients with mild to moderate AD, 2 patients who were receiving 200 mg intravenous gantenerumab developed vasogenic oedema. Compared with placebo, the 60 mg and 200 mg doses were associated with 16% and 36% reductions in cortical brain amyloid loads, respectively[56].

A 2-year, double-blind, placebo-controlled, phase II/III study, SCarlet RoAD, investigated subcutaneous gantenerumab at 105 mg or 225 mg every 4 weeks in 797 patients with prodromal AD who had biomarker evidence of brain Aβ deposition. The study was prematurely discontinued for futility and an increase in ARIAs[57], although the gantenerumab groups showed a dose-dependent reduction in brain amyloid on PET as well as reduced levels of total and phosphorylated tau in the CSF[58]. The trial was converted into an open-label extension study to evaluate the safety and biological effects of higher doses of gantenerumab (up to 1,500 mg administered subcutaneously).

Another double-blind, placebo-controlled, phase II/III study, Marguerite RoAD, evaluated monthly subcutaneous injections of gantenerumab in patients with mild AD[59]. Recruitment was halted at 389 patients and the study was converted to an open-label extension trial with higher doses of gantenerumab (up to 1,200 mg administered subcutaneously).

Preliminary results from these open-label extension studies indicate that gantenerumab has an acceptable safety profile at higher doses[60]. Two 2-year, double-blind,

placebo-controlled phase III studies (GRADUATE 1 and GRADUATE 2), employing undisclosed high doses, were recently started in patients with early AD and biomarker evidence of brain Aβ deposition. Gantenerumab is also being investigated in the aforementioned DIAN-TU phase II/III trial.

Crenezumab. Crenezumab is a humanized anti-Aβ monoclonal IgG4 that binds to multiple species of Aβ, with particular affinity for pentameric oligomeric and fibrillary 16mer assemblies of aggregated Aβ[61,62]. Crenezumab also blocks aggregation and promotes disaggregation of Aβ[63]. In the Tg2576 mouse model of AD, intracerebral injection of crenezumab did not cause notable inflammatory changes[64]. No published studies are available on the chronic effects of crenezumab on brain Aβ deposition and memory deficits in animal models of AD.

In single and multiple ascending dose phase I studies in 56 patients with AD, crenezumab treatment was not associated with vasogenic oedema or cerebral microhaemorrhage[61].

ABBY, a double-blind, placebo-controlled phase II study in 433 patients with mild to moderate AD, evaluated intravenous and subcutaneous doses of crenezumab given for 68 weeks, with final measurements being performed at 73 weeks. The drug failed to show significant differences compared with placebo on the primary outcome variables of efficacy[65] (12-item ADAS-Cog and CDR-SB scores). A smaller phase II brain imaging study (BLAZE) in 91 patients also failed to demonstrate cognitive or clinical benefit[66]. No significant effects on amyloid PET were observed, although an effect on CSF Aβ levels was detected. Further analysis of the ABBY and BLAZE data suggested a possible efficacy signal in patients with mild AD[65,66]. In the ABBY study, patients with MMSE scores between 22 and 26 who received the higher dose showed a significant reduction in ADAS-Cog decline compared with the placebo group. However, the groups showed no statistically significant differences in scores on clinical global (CDR-SB) or functional (ADCS–Activities of Daily Living (ADCS-ADL)) scales between groups. In the BLAZE study, a favourable cognitive trend for crenezumab was also observed in the more mildly affected subgroup, but the analysis was based on a very limited sample size, and *P* values were not corrected for multiple comparisons.

A further double-blind, placebo-controlled phase Ib study in 75 individuals with mild to moderate AD and positive amyloid PET evaluated the safety of higher intravenous doses of crenezumab over 13 weeks and into an open-label extension. Nine SAEs and two cases of brain microhaemorrhage were reported[67].

A 100-week, double-blind, placebo-controlled phase III study (CREAD) is evaluating the efficacy of 60 mg/kg intravenous crenezumab, delivered every 4 weeks, in 813 patients with prodromal to mild AD and evidence of cerebral amyloid pathology. The primary end point of this study is the change in the CDR-SB score at 2 years[68], and it is expected to be completed in September 2020. Another double-blind, placebo-controlled phase III study of the same dose in 750 patients with prodromal to mild AD (CREAD2) is also underway; this trial will be completed in November 2021.

In addition, crenezumab is being tested as a preventive treatment in a double-blind, placebo-controlled study (API ADAD) in 252 presymptomatic carriers of the autosomal dominant *PSEN1* Glu280Ala mutation who will receive bimonthly, subcutaneous injections of crenezumab or placebo over 5 years[69]. This trial should be completed by March 2022.

Aducanumab. Aducanumab is a recombinant human IgG1 antibody that binds to soluble Aβ aggregates and insoluble fibrils with > 10,000-fold selectivity over monomers. It recognizes amino-terminal residues 3–7 of the Aβ sequence[70]. Preclinical studies in Tg2576 mice showed 1.3% brain penetration after a single intraperitoneal administration[71]. The drug reduced Aβ plaque size in a dose-dependent manner in young (9-month-old)[71] but not in aged (22-month-old)[72] Tg2576 mice. No cognitive or behavioural effects were reported in animal models of AD.

A phase Ia study evaluated single ascending intravenous doses of aducanumab in 53 individuals with mild to moderate AD[73]. Three patients who received 60 mg/kg aducanumab developed SAEs of symptomatic ARIAs, which completely resolved in 8–15 weeks. At this dose, plasma levels of $A\beta_{1-40}$ and $A\beta_{1-42}$ increased for ~3 weeks, suggesting that at high doses, aducanumab binds to soluble monomeric Aβ. At 24 weeks, no significant differences in mean changes on the 13-item ADAS-Cog were observed between the aducanumab-treated and placebo groups[73].

A 12-month, double-blind, placebo-controlled, multiple ascending dose phase Ib study evaluated the safety and tolerability of intravenous aducanumab in 165 patients with prodromal to mild AD who had positive ^{18}F-florbetapir PET amyloid scans[71]. Forty patients (24%) discontinued treatment, mostly owing to SAEs (20 patients) or withdrawal of consent (14 patients). Treatment discontinuation rates for SAEs were considerably higher in the 10 mg/kg aducanumab group than in patients who received lower doses or placebo. ARIA-vasogenic oedema (ARIA-E) occurred in 3–31% of aducanumab recipients in a dose-dependent manner. ARIA-E was more common in *APOE*ε4* carriers, reaching 55% at the highest dose. The occurrence of ARIAs might have contributed to unblinding of the study.

Aducanumab dose-dependently and time-dependently decreased brain amyloid burden at 12 months[71]. At 52 weeks, aducanumab treatment was associated with attenuated decline in both MMSE and CDR-SB scores. The effects of the 10 mg/kg dose reached statistical significance on both variables, although no *P* value adjustments were made for multiple comparisons. These data are encouraging because they provide the first demonstration of a lowering effect on the brain Aβ load coupled with a positive effect on cognition and clinical global status, with dose-dependent trends. However, in the patients with mild AD, the dropout rate was higher in the 10 mg/kg group than in the placebo group (53% versus 29%), which might have contributed to this apparent effect. After study completion, a further arm was

added consisting of 31 *APOE*ε4* carriers, in whom the drug was titrated from 1 mg/kg up to 10 mg/kg. In this group, the occurrence of ARIA-E was 35%. Around 80% of patients who completed the double-blind phase entered a long-term open extension period, with all participants receiving aducanumab.

Two 18-month, double-blind, placebo-controlled phase III studies (ENGAGE and EMERGE) are each currently enrolling 1,350 patients with prodromal AD and positive amyloid PET scans[74]. Participants are randomly assigned in a 1:1:1 ratio to receive low-dose aducanumab (3 mg/kg in *APOE*ε4* carriers and 6 mg/kg in non-carriers), high-dose aducanumab (10 mg/kg) or placebo for 18 months, with the possibility of enrolment in a 24-month long-term extension. The primary end point will be change in CDR-SB scores from baseline to week 78, and the study is expected to be completed in December 2022. Recently, Biogen decided to increase the sample size of the two studies by 510 patients owing to unexpected increased variability in CDR-SB interim data.

β-Secretase inhibitors

BACE1-mediated cleavage of APP is the first step in the generation of Aβ. BACE inhibition represents upstream interference with the amyloid cascade, regardless of which species or aggregation state of Aβ exerts toxicity. Studies in BACE1-deficient and BACE2-deficient mice demonstrated that these proteases affect many physiological substrates and functions within and outside the nervous system. BACE1 influences axon guidance, neurogenesis, muscle spindle formation, neuronal network functions and myelination, whereas BACE2 is involved in pigmentation and pancreatic β-cell function[75]. Prolonged treatment with BACE1 inhibitors seems to negatively affect spine formation and density, hippocampal long-term potentiation (LTP) and cognition in wild-type mice[76,77]. Few BACE inhibitors are currently in phase III clinical development (TABLE 2), because several were abandoned because of toxicity in humans (FIG. 2; (TABLE 1).

Verubecestat. Verubecestat is an oral BACE1 inhibitor that displays nanomolar affinity for BACE1 but not for BACE2 (REF.[78]). In aged Tg2576 mice (18–22 months old), 12 weeks of treatment with verubecestat (110 mg/kg per day) reduced CSF $A\beta_{1-40}$ and $A\beta_{1-42}$ concentrations by 62% and 68%, respectively; attenuated accumulation of brain $A\beta_{1-40}$ and $A\beta_{1-42}$; and reduced the thioflavin-S-positive plaque load without inducing microhaemorrhages[79]. No data are available on the effects of this drug on behaviour or cognition in murine models of AD.

Single and repeated administration of verubecestat in phase I studies in healthy adult volunteers reduced CSF Aβ levels in a sustained and dose-dependent manner[78]. In a double-blind, placebo-controlled phase IIa study in 32 patients with mild to moderate AD, 7 days of treatment with verubecestat at a daily dose of 12 mg, 40 mg or 60 mg produced CSF $A\beta_{1-40}$ reductions of 70%, 83% and 90%, respectively. No SAEs were reported.

EPOCH, a 78-week, double-blind, placebo-controlled phase II/III study in 1,958 patients with mild to moderate AD, assessed the safety and efficacy of 12 mg, 40 mg and 60 mg daily doses of verubecestat[80]. Patients were not pre-screened for the presence of brain amyloid. The study was terminated for futility 5 months before its scheduled completion. Although the drug dose-dependently and robustly lowered CSF Aβ concentrations, patients did not benefit in terms of either cognition (ADAS-Cog scale) or functionality (ADCS-ADL scale). Adverse events that resulted in discontinuation of the assigned trial regimen were observed in 5.8%, 8.3% and 9.4% of patients in the placebo, 12 mg and 40 mg groups, respectively. There were 5 deaths in the placebo group (0.8%), 9 deaths in the 12 mg group (1.4%) and 12 deaths in the 40 mg group (1.8%).

At the end of the double-blind, placebo-controlled phase, patients had the opportunity to enter an open-label extension study, in which 360 patients from the original placebo group switched to 40 mg verubecestat and 346 patients in the 12 mg group and 333 patients in the 40 mg group continued on their existing doses. Because the trial was prematurely discontinued, the open-label extension phase lasted for only a short period, but four deaths in the 12 mg group and ten deaths in the 40 mg group were reported. The death rate in the original placebo group was 0.8% (5 of 653) during the double-blind phase and rose to 2.2% (8 of 360) in the open-label extension phase. In addition, BACE inhibition by verubecestat produced a rapid, non-progressive reduction in whole-brain and hippocampal volumes[81].

Verubecestat was also tested at doses of 12 mg and 40 mg per day in a 104-week, double-blind, placebo-controlled study (APECS) in 1,500 individuals with prodromal AD or amnestic MCI due to AD who had a positive flutemetamol PET scan[82]. This trial was prematurely discontinued owing to an unfavourable risk–benefit ratio. A worsening of cognition in verubecestat-treated patients was observed.

Lanabecestat. Lanabecestat is an oral, long-acting BACE1 inhibitor that has subnanomolar affinity for BACE1 but not BACE2. The drug has 80% oral bioavailability in dogs and ~10% brain penetration in mice. In mice, guinea pigs and dogs, lanabecestat produced significant dose-dependent and time-dependent reductions in $A\beta_{1-40}$ and $A\beta_{1-42}$ levels in the CSF and brain[83]. Chronic treatment with lanabecestat in dogs and rats caused macroscopic and microscopic hypopigmentation of skin, hair and mucosa[84]. No studies are available on the cognitive effects of the drug in animal models of AD.

Single and multiple ascending dose phase I studies of lanabecestat were performed in healthy volunteers. In a phase IIa study in which 16 patients with AD received repeated doses over 13 days[85], lanabecestat produced prolonged and dose-dependent reductions in CSF $A\beta_{1-42}$. The drug produced similar findings in two Japanese studies[86].

A 2-year, double-blind, placebo-controlled phase II/III study (AMARANTH) that was evaluating lanabecestat in 2,202 patients with mild AD or MCI due to AD, and biomarker evidence of Aβ brain deposition[87], was discontinued for futility on the recommendation of an independent data monitoring committee, which said the study was unlikely to meet its primary end points on completion[88]. A delayed-start extension study (AMARANTH-EXTENSION) for patients who had completed the AMARANTH study was also terminated. In addition,

Table 2 | **Ongoing double-blind, placebo-controlled phase III studies of anti-Aβ therapies for AD and related disorders**

Study and sponsor	Drug(s)	Mechanism of action	Study cohort and treatment duration	Primary outcomes	Expected completion
• AMBAR (NCT01561053) • Grifols Biologicals	Immunoglobulin + albumin	Polyclonal antibodies	• 496 patients with mild to moderate AD • 14 months	• ADAS-Cog • ADCS-ADL	Completed[219]
• NCT02293915 • Shanghai Green Valley	Sodium oligo-mannurarate (GV-971)	Aβ aggregation inhibitor	• 818 patients with mild to moderate AD • 72 weeks	ADAS-Cog	Completed[220]
• NCT02051608 • Roche–Genentech	Gantenerumab	Monoclonal antibody	• 1,000 patients with mild AD • 100 weeks	• ADAS-Cog13 • ADCS-ADL	July 2020
• MissionAD1 (NCT02956486) • Eisai–Biogen	Elenbecestat	BACE inhibitor	• 1,330 patients with early AD • 24 months	CDR-SB	December 2020
• MissionAD2 (NCT03036280) • Eisai–Biogen	Elenbecestat	BACE inhibitor	• 1,330 patients with early AD • 24 months	CDR-SB	December 2020
• API ADAD (NCT01998841) • Roche–Genentech	Crenezumab	Monoclonal antibody	• 252 asymptomatic *PSEN1* Glu280Ala carriers • 60 months	APCC	February 2022
• EMERGE (NCT02477800) • Biogen	Aducanumab	Monoclonal antibody	• 1,350 patients with early AD • 78 weeks	CDR-SB	March 2022
• ENGAGE (NCT02484547) • Biogen	Aducanumab	Monoclonal antibody	• 1,350 patients with early AD • 78 weeks	CDR-SB	April 2022
• ADCS A4 (NCT02008357) • Eli Lilly	Solanezumab	Monoclonal antibody	• 1,150 asymptomatic individuals at risk of AD • 240 weeks	ADCS–PACC	July 2022
• GRADUATE 1 (NCT03444870) • Roche–Genentech	Gantenerumab	Monoclonal antibody	• 750 patients with early AD • 104 weeks	CDR-SB	June 2023
• GRADUATE 2 (NCT03443973) • Roche–Genentech	Gantenerumab	Monoclonal antibody	• 750 patients with early AD • 104 weeks	CDR-SB	June 2023
• DIAN-TU (NCT01760005) • Eli Lilly, Roche–Genentech and Janssen	• Solanezumab • Gantenerumab • Atabecestat	• Monoclonal antibody • Monoclonal antibody • BACE inhibitor	• 438 asymptomatic *APP* or *PSEN* mutation carriers • 208 weeks	DIAN-TU composite score	December 2023
• API Generation S1 (NCT02565511) • Novartis	• CAD106 • CNP520	• Aβ antigen • BACE inhibitor	• 1,340 asymptomatic homozygous *APOE*ε4* carriers • 60 months	• MCI diagnosis • APCC	May 2024
• API Generation S2 (NCT03131453) • Novartis	CNP520	BACE inhibitor	• 2,000 asymptomatic homozygous *APOE*ε4* carriers and heterozygous *APOE*ε4* carriers with brain amyloid accumulation • 60 months	• MCI diagnosis • APCC	July 2024

The table shows the status of studies on 10 July 2018, as reported in ClinicalTrials.gov. Aβ, amyloid-β; AD, Alzheimer disease; ADAS-Cog, Alzheimer's Disease Assessment Scale–Cognitive subscale; ADAS-Cog13, 13-item ADAS-Cog; ADCS-ADL, AD Cooperative Study–Activities of Daily Living; ADCS–PACC, Alzheimer's Disease Cooperative Study–Preclinical Alzheimer Cognitive Composite; APCC, Alzheimer disease Prevention Initiative Composite Cognitive; *APOE*, apolipoprotein E gene; *APP*, amyloid precursor protein gene; BACE, β-secretase; CDR-SB, Clinical Dementia Rating scale–Sum of Boxes; DIAN-TU, Dominantly Inherited Alzheimer Network–Trials Unit; MCI, mild cognitive impairment; *PSEN*, presenilin gene.

a 3-year, double-blind, placebo-controlled, delayed-start study (DAYBREAK-ALZ) that was evaluating two undisclosed once-daily doses of lanabecestat in 1,899 patients with mild AD dementia and biomarker-proven brain amyloid accumulation was discontinued[88].

Elenbecestat. Elenbecestat is a BACE1 inhibitor that has been shown to lower Aβ concentrations in the brain and CSF of rats, guinea pigs and non-human primates. In phase I studies, dose-dependent reductions in plasma and CSF Aβ levels were observed[89,90].

A phase IIa study has evaluated the effects of 7 different single doses of elenbecestat on CSF Aβ levels in 71 Aβ-positive individuals with MCI, but no data have yet been disclosed. An 18-month, double-blind, placebo-controlled phase IIb study of elenbecestat in 70 Aβ-PET-positive patients with MCI, prodromal AD or mild AD showed dose-dependent decreases in CSF Aβ levels[91]. The highest dose (50 mg per day) had a lowering effect on the brain Aβ load, as measured by PET, but no significant improvements in the AD Composite Score (ADCOMS)[92] or CDR-SB score were reported.

Two 24-month, double-blind, placebo-controlled phase III studies (MISSION AD1 and MISSION AD2) are ongoing. Both studies are enrolling 1,330 individuals with MCI or mild AD who have biomarker evidence of brain amyloid pathology. Patients are receiving 50 mg elenbecestat or placebo daily, and the primary outcome is the CDR-SB score. The results are expected by March 2021.

Atabecestat. Atabecestat is a non-selective oral BACE1 inhibitor that dose-dependently reduces CSF Aβ levels in rats and monkeys. In the PS/APP mouse model of AD, chronic treatment with atabecestat reduced the brain Aβ load[93]. However, no studies are available on the cognitive or behavioural effects of atabecestat in animal models of AD.

In two phase I studies that used single and multiple ascending atabecestat doses in healthy volunteers, dose-dependent reductions in CSF $Aβ_{1–40}$ levels were observed[94]. A 4-week, double-blind, placebo-controlled phase Ib study in 45 individuals who had prodromal AD or were cognitively healthy but had biomarker evidence of brain Aβ deposition also showed dose-dependent CSF Aβ reductions but no positive effects on cognitive scores[95]. A 6-month, double-blind, placebo-controlled phase II study evaluated the biomarker effects of atabecestat in 100 individuals with asymptomatic or predementia AD and biomarker evidence of amyloid pathology. The results of this study have not yet been released.

A 54-month, double-blind, placebo-controlled, phase II/III study (EARLY) in 1,650 asymptomatic people at risk of developing AD (family history of dementia, *APOE*ε4* genotype or biomarker evidence of amyloid deposition) was halted after serious elevations of liver enzymes were seen in some patients who received the drug[96]. The primary end point of this trial was slowing of cognitive decline, as measured by change on the ADCS–PACC composite scale. Atabecestat was also being evaluated in the aforementioned DIAN-TU study, and it is unclear whether the drug has been withdrawn from this study.

CNP520. CNP520 is an oral, long-acting and selective BACE1 inhibitor that has excellent brain penetration and was shown to reduce Aβ levels in the rat brain by > 80%. A single dose in dogs reduced CSF Aβ levels for 72 h. In transgenic mouse models of AD, CNP520 dose-dependently reduced the levels of soluble and insoluble Aβ in the brain[97]. No data are available on the effects of the drug on cognitive deficits in mouse models.

Phase I studies have evaluated single and multiple (administered over 2–4 weeks) ascending doses of CNP520 in elderly healthy volunteers. After multiple dosing, a dose-dependent and long-lasting reduction in CSF $Aβ_{1–40}$ levels was observed, but no data on CSF $Aβ_{1–42}$ were reported[97,98]. A 13-week, double-blind, placebo-controlled, phase II study has evaluated various doses of CNP520 versus placebo in 125 healthy elderly individuals, but the data have not been published.

The aforementioned phase II/III Generation S1 study will test the ability of CNP520 (50 mg per day) to delay diagnosis to MCI and improve the APCC test score[99]. The results are expected by September 2024. A similar 5-year, double-blind, placebo-controlled, phase II/III study known as Generation S2 is enrolling 2,000 cognitively healthy, homozygous or heterozygous *APOE*ε4* carriers aged 60–75 years[99]. The heterozygous participants must have evidence of elevated brain amyloid. CNP520 will be tested at doses of 15 mg and 50 mg per day, and the primary outcomes are a diagnosis of MCI and the APCC test score. The study should be completed by August 2024.

Updating the amyloid cascade hypothesis

The amyloid cascade hypothesis has evolved over the past 15 years[100]. Insoluble, fibrillar aggregates of Aβ are the core neuropathological hallmark of AD and are required for definitive diagnosis, yet they correlate poorly with AD severity[22] and cognitive dysfunction[101]. Patients with AD in whom brain Aβ plaques were virtually cleared by anti-Aβ immunotherapy did not show any cognitive benefit[34].

These findings have prompted the investigation of other Aβ aggregates in patients with AD[102,103]. Numerous types of Aβ aggregates have been identified, including Aβ fibrils and Aβ oligomers. Aβ oligomers include small oligomers (dimers and trimers), medium-sized oligomers (9mers, 12mers, Aβ*52 and Aβ-derived diffusible ligands) and high-molecular-mass oligomers (protofibrils). Soluble oligomers seem to be in a complex equilibrium with the 8 nm fibrils of Aβ that are present in amyloid plaques[104]. They bind to the plasma membranes of neurons, microglia and astrocytes, triggering transmembrane signalling and abnormal intracellular changes[105]. Aβ oligomers might impair neuronal function by causing synaptic dysfunction, inducing mitochondrial dysregulation and affecting microglia[106]. Neurons exposed to Aβ oligomers are unable to form new synapses, resulting in learning deficits[107]. Thus, the updated amyloid cascade hypothesis suggests that neurotoxicity of Aβ is mediated by soluble oligomeric forms rather than insoluble aggregates. However, the dynamic nature of Aβ oligomers makes them difficult to measure reliably in body fluids and tissues[108]. In addition, opinions differ regarding the type and size of oligomers that have disease-relevant activity. According to some authors, large soluble oligomers represent the vast majority of soluble Aβ aggregates in the AD brain but have minimal cytotoxic power, whereas soluble dimers are much more toxic[104]. Other work indicates that the medium-sized oligomer Aβ*56 induces specific changes in neuronal signalling, leading to tau phosphorylation and aggregation, but dimers and trimers do not share this property[109].

Over the past 20 years, several drugs that decrease the production of Aβ (mainly γ-secretase and BACE inhibitors) or increase Aβ brain clearance (anti-Aβ monoclonal or polyclonal antibodies and Aβ antigens) have been identified. Unfortunately, in all clinical trials to date, these treatments have failed to improve cognitive outcomes despite reducing brain Aβ (FIG. 2; (TABLE 1). Interestingly, some drugs, including tarenflurbil, scyllo-inositol, semagacestat, avagacestat, AD02, CAD106,

verubecestat, atabecestat and lanabecestat tended to worsen cognitive or clinical status compared with placebo; it remains to be determined whether these worsening effects are attributable to nonspecific adverse events that outweigh the putative positive effects on cognition. Indeed, in both plaque-bearing and plaque-free transgenic mice that overexpressed human APP, administration of anti-Aβ monoclonal antibodies worsened neuronal dysfunction by increasing the frequency of Ca^{2+} transients and the proportion of hyperactive neurons, and inducing unusual neuronal synchrony[110].

Supporters of the Aβ cascade hypothesis argue that these negative trials do not disprove the hypothesis but that treatments should be more powerful and should be given before Aβ has 'caused' neuronal and synaptic loss. In addition, they point out that most of the failed trials have included people without evidence of brain amyloid pathology[111]. However, some anti-Aβ drugs have been investigated at earlier stages of AD and in biomarker-confirmed AD, and this approach has not proved beneficial to date (TABLE 1). For example, as outlined above, the EXPEDITION 3 phase III trial of solanezumab in patients with mild AD produced disappointing outcomes, and the SCarlet RoAD phase II/III trial of gantenerumab in patients with prodromal AD was prematurely halted for futility. Similarly, the APECS phase III study of verubecestat in patients with prodromal AD and a positive flutemetamol PET scan was interrupted because of an unfavourable risk–benefit ratio and worsening of cognition.

Clinical researchers are now trying to use agents directed against Aβ oligomers, a molecular species that is believed to be especially neurotoxic. An immunotherapy that efficiently clears Aβ monomers or Aβ fibrils would be expected, in the long term, to alter the equilibrium between different Aβ species, including Aβ oligomers. Fibrils might produce oligomers following solubilization by immunotherapy[112,113]. In rats, the Aβ aggregation inhibitor scyllo-inositol was shown to neutralize the toxic effects of Aβ oligomers, including amelioration of oligomer-induced synaptic loss, LTP inhibition and memory deficits[114]. Solanezumab might act by stabilizing Aβ monomers, thus preventing the formation of neurotoxic Aβ oligomers[115]. Crenezumab has been shown to specifically bind Aβ oligomers and fibrils with similar high affinity[116]. Gantenerumab shows 20-fold higher affinity for Aβ oligomers than for Aβ monomers[53]. Intravenous human immunoglobulin (IVIg) contains several types of anti-Aβ antibody, some of which recognize Aβ oligomers and fibrils[117]. IVIg antibodies were shown to interfere with the oligomerization and fibrillization of Aβ, thereby protecting neurons against Aβ-mediated toxicity[118,119].

None of these pharmacological approaches have shown clinical efficacy in patients with AD. However, more potent anti-Aβ oligomer monoclonal antibodies, such as aducanumab, might produce better clinical results. BAN2401, a monoclonal antibody that selectively binds soluble Aβ protofibrils[120], showed promise in an 18-month, adaptive phase IIb study in 856 individuals with prodromal or mild AD. The higher dose (10 mg biweekly) was associated with a significant slowing of both clinical decline (ADCOMS) and brain Aβ accumulation compared with placebo[121], although biases in the study design might affect these encouraging results. BAN2401 is likely to enter phase III clinical trials soon.

A methodological problem that underlies the multiple failures of anti-Aβ therapies in the clinic is that the screening and selection of clinical drug candidates to be tested in patients with sporadic AD are performed in transgenic mice bearing human genes linked to familial AD. The assumption was that the efficacy of the selected drugs would be identical in both familial and sporadic AD. Ironically, the drugs that were selected from studies in transgenic mice are only just beginning to be tested in patients with AD-associated gene mutations following 16 years of failure in sporadic AD. Some of the strongest support for the amyloid cascade hypothesis comes from rare autosomal dominant forms of AD; however, overexpression of human familial mutated forms of APP in mice produces Aβ plaques but not NFTs or extensive neurodegeneration. Transgenic mice expressing only the Aβ peptide, in the absence of APP overexpression, develop plaques but show normal cognitive performance[122].

Further unresolved issues with the amyloid cascade hypothesis include the presence of Aβ deposition in cognitively healthy individuals; the weak correlation between Aβ plaques and severity of clinical symptoms; the biochemical nature, presence and role of Aβ oligomers; and the poorly explained pathological heterogeneity and comorbidities associated with AD[123–125]. The concept that Aβ pathology, acting through tau, is the source of neurodegeneration has been the core of the amyloid hypothesis. However, in autopsy studies, tau pathology has been observed before marked Aβ deposition even in young individuals[126]. Brain imaging suggests that the initial emergence of AD-associated features, such as reductions in hippocampal volumes and glucose metabolism, in cognitively healthy individuals with preclinical AD does not depend on Aβ amyloidosis[127]. The discovery of individuals with biomarkers for neurodegeneration but no Aβ deposits (suspected non-AD pathology, or SNAP) has raised considerable interest; it is still unclear whether SNAP represents a distinct pathology from AD or whether it could represent the earliest stage of AD in some individuals. A study in cognitively healthy elderly people found that the annual rates of conversion to MCI were almost identical in individuals with SNAP (10%) and individuals with Aβ amyloidosis (11%)[128]. Other studies showed that neuronal injury independent of Aβ deposition occurs in cognitively healthy elderly *APOE*ε4* carriers[129]. Longitudinal studies in *APP* and *PSEN* mutation carriers revealed that in medial and lateral temporal areas, atrophy started before Aβ aggregation[130]. These findings question the hypothesis that Aβ biomarkers become abnormal first, followed by neurodegenerative biomarkers and cognitive symptoms[131,132], and suggest that AD is a multiparameter pathology in which tau can promote Aβ toxicity.

Longitudinal studies indicate that Aβ plaques at baseline in cognitively healthy individuals predict future cognitive impairment; however, this impairment

Box 1 | **The physiological role of amyloid-β**

Amyloid-β (Aβ) is a physiological, ubiquitously expressed peptide that is suggested to have multiple roles in brain function. Studies using 3D super-resolution microscopy showed that the 42-amino-acid form of Aβ ($Aβ_{1–42}$) was present in the presynapse of hippocampal neurons in wild-type mice[138]. Aβ has been shown to regulate neuronal electrophysiology[156,157], synaptic plasticity and memory[158,159], long-term potentiation[160], neuronal transmission[161], learning and memory[162], hippocampal memory consolidation[163], neurogenesis[164] and neuronal survival[165].

Brain Aβ levels can be increased in neurological and psychiatric conditions other than Alzheimer disease (AD), including traumatic brain injury (TBI)[166–169], chronic traumatic encephalopathy[170,171], general anaesthesia[172–176], cerebral ischaemia[177–181], major depression[182–184], and amyotrophic lateral sclerosis (ALS)[185,186]. Rapid changes in Aβ levels have been observed in cerebrospinal fluid (CSF) and plasma during cardiac arrest[187] and cardiac surgery[188,189], even in infants and children[190], probably owing to combined hypoxia and anaesthetic insult. An increase in CSF[191,192] or hippocampal[193] Aβ levels was observed in cognitively healthy adults after 1 day of sleep deprivation or slow-wave sleep disruption[194]. In both mice and humans, the effects of sleep disruption on Aβ levels have been attributed to increased production rather than decreased clearance of Aβ[192,195].

Spikes of Aβ secretion after transient acute brain injury (sleep deprivation, TBI, general anaesthesia or acute cerebral ischaemia) or steady overproduction of Aβ in chronic CNS diseases (chronic cerebral ischaemia, chronic traumatic encephalopathy, depression or ALS) might represent an attempt by the brain to mitigate or repair neuronal damage or insult[196]. Similarly, in AD, Aβ overproduction could represent an attempt to ameliorate the loss of neuronal functioning[197,198]. Possible initial causes of neuronal damage in AD include chronic inflammation[199], tau-associated network disruption[33], metabolic failure[200], abnormal microglial activation[201], oxidative stress[202] and cholesterol distress[203]. Thus, Aβ secretion could represent an adaptive response to an upstream pathophysiological process. In the absence of a reparative neuronal outcome, prolonged Aβ overproduction leads to the formation of Aβ aggregates, including Aβ oligomers, with consequent further neurotoxicity and activation of the well-known deleterious cascade.

may be minor and not clinically relevant[20,133,134]. Even if brain amyloid deposition in cognitively healthy people parallels cognitive decline over time, it might still be secondary to neuronal damage caused by unknown factors. Given that Aβ deposits result from an aggregation process that starts with progressive assembly of soluble oligomers, we can speculate that Aβ-plaque-positive individuals have been exposed to Aβ oligomers for a long period. The absence of dementia in such individuals suggests that they were not susceptible to the supposed toxic effects of Aβ oligomers[135]. Indeed, Aβ can be distributed widely through the brain and body even in cognitively healthy individuals.

Soluble Aβ has a physiological role in modulating synaptic function and facilitating neuronal growth and survival[136,137] (BOX 1). Aβ is present throughout the lifespan, it has been found in all vertebrates examined thus far and its molecular sequence shows a high degree of conservation between animal species. These characteristics are typical of a factor with an important biological role, which is supported by evidence that Aβ protects the brain from infections, repairs leaks in the blood–brain barrier, promotes recovery from injury and regulates synaptic function[138]. In vitro and in vivo studies have shown that the cellular production of Aβ rapidly increases in response to a physiological challenge and often diminishes on recovery[138]. These roles are further supported by the adverse behavioural or cognitive outcomes in some clinical trials that have attempted to deplete Aβ in order to treat AD.

Promising new avenues

An alternative option for AD therapeutics is to address the modifiable risk factors for developing AD[139]. One such factor is type 2 diabetes[140,141]. In the brain, neurons are dependent on the neurotrophic properties of insulin[142,143]. Resistance to the neurotrophic effects of insulin in the brain seems to make neurons vulnerable to stress and to reduce the brain's ability to repair damage that accumulates over time, resulting in impairments in synaptic, metabolic and immune response functions[140]. In the aged brain, insulin might lose its effectiveness as a growth factor, even in individuals without diabetes[144–146]. Therefore, anti-diabetes drugs might be an effective approach for treating AD[147]. Liraglutide, a glucagon-like peptide 1 receptor agonist, has produced impressive results in animal models of AD[148]. In a small placebo-controlled study in patients with MCI or AD, intranasal insulin had encouraging effects on cognition[149]. Several larger controlled clinical studies are underway.

Another promising target for intervention is neuroinflammation. Several studies have identified a link between the brain's immune system and AD[150], and microglia have a central role in the link between inflammation and neurodegeneration[151]. Epidemiological studies have shown that a high infectious burden during adult life increases the risk of cognitive impairment or dementia in later life[152,153]. Microglia might be activated by peripheral inflammation and/or gut microbiota, and chronic activation could prove detrimental under certain conditions. Conversely, an efficient adaptive immune system might prevent AD pathogenesis by modulating microglial function[154]. We need to fully understand the biology of microglia to explore whether immune-based therapies could be designed for AD.

Tau-targeting therapies are another active area of investigation. As tau pathology correlates better with cognitive impairments than do Aβ lesions, targeting of tau is expected to be more effective than Aβ clearance once the clinical symptoms are evident. Unfortunately, most of the initial anti-tau therapies based on inhibition of kinases or tau aggregation, or on stabilization of microtubules, have been discontinued because of toxicity and/or lack of efficacy[155]. Currently, the majority of tau-targeting therapies in clinical trials are immunotherapies, which have shown promise in numerous preclinical studies. We expect to see the outcomes of these immunization trials in the near future[155].

We cannot rule out the possibility that during the initial phase of the AD pathophysiological process, unknown neuronal noxious stimuli cause a compensatory change, leading to increased production or reduced clearance of Aβ as a secondary consequence of the initial insult. Interesting parallels can be drawn with sepsis, a complex disorder that develops as a dysregulated host response to an infection and is associated with high risk of death. Although leukocytosis is a typical initial laboratory finding, sepsis originates from an infectious agent, and therapy is aimed not at reducing the numbers of white blood cells (the host response to the microbial assault) but at removal of the underlying source of infection. Similarly, Aβ accumulation might be considered a reaction of the brain to neuronal damage (for example,

mitochondrial dysregulation), and therapy should be directed against the cause of the neuronal insult and not the host response. As in sepsis, where the initial leukocytosis can cause further biological dysfunction, prolonged and progressive Aβ accumulation in AD might have further toxic consequences.

Conclusions

The amyloid cascade hypothesis of AD was formulated 25 years ago on the basis of strong genetic, biochemical and histopathological evidence. The hypothesis was later strengthened by longitudinal biomarker, cognitive and clinical studies. However, >15 years of clinical failure with several classes of anti-Aβ drugs that affect the formation, aggregation and clearance of Aβ have followed. Some drugs that inhibit the production of 'nascent' Aβ, such as γ-secretase and BACE inhibitors, were found to accelerate cognitive decline, possibly owing to off-target effects. Aβ has a physiological role in facilitating neuronal function and memory consolidation, and increases in CNS Aβ production might occur in other clinical conditions, such as sleep deprivation, general anaesthesia and cardiac arrest, and in response to neuronal stress.

Whether Aβ accumulation causes AD or is a by-product of the AD process remains unknown. In their seminal article on the amyloid hypothesis[3], Beyreuther and Masters stated "the real test of this hypothesis will come when therapeutic strategies are developed around the concept of preventing the generation of βA4 amyloid or of removing the earliest para-amyloid aggregates from the brain." The results of ongoing studies in preclinical or asymptomatic stages of AD and cognitively healthy individuals at risk of AD should provide some answers. In the meantime, it is imperative that alternative hypotheses are considered and actively pursued.

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Author contributions

All authors researched the data for the article, provided substantial contributions to discussions of its content, wrote the article and undertook the review and/or editing of the manuscript before submission.

Competing interests

B.P.I. is an employee at Chiesi Farmaceutici and has developed anti-Alzheimer disease (AD) drugs. He is co-inventor of patents on anti-AD drugs. He does not hold stock options. The other authors declare no competing interests.

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