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Development of amyloid beta-directed antibodies against Alzheimer's disease: Twists and turns

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SUMMARY Alzheimer's disease (AD) is a severe and progressive neurodegenerative disease, and the treatment options that are currently available are limited. The amyloid cascade hypothesis has had a significant influence in explaining the pathology underlying AD. Inhibiting the production and aggregation of amyloid-beta (Aβ) and promoting its clearance have been important strategies in the development of anti-AD drugs over the past two decades. Specifically, Aβ directed antibodies have been highly anticipated, but drug development has been fraught with obstacles and challenges. Antibodies targeting the C-terminal or central region of Aβ, such as ponezumab, solanezumab, and crenezumab, primarily bind to Aβ monomers, yet no significant clearance of brain plaques or slowing of disease progression has been observed in clinical trials. Antibodies targeting the N-terminal region of Aβ, including aducanumab, lecanemab, and donanemab, primarily bind to aggregated forms of Aβ, and have shown efficacy in clearing brain plaques and slowing early-stage AD progression in clinical trials. However, clinical trials of gantenerumab, which targets conformational epitopes in the N-terminal and central sequences of Aβ and which selectively binds to aggregated forms, have failed, raising some new questions about the Aβ hypothesis. Advances in research on the pathological mechanisms of AD and advances in early diagnostic techniques may shift the time window for drug intervention and offer a potential pathway for developing effective drugs to delay the onset and progression of AD in the future.

Keywords Aβ hypothesis, AD, aducanumab, lecanemab, donanemab, gantenerumab

Alzheimer's disease (AD) is a severe neurodegenerative disease characterized by progressive memory loss, cognitive impairment, and neuropsychiatric symptoms (*1*). The incidence of AD is closely related to age (*1,2*). With the extension of the human lifespan and further aging of the population, the prevalence of AD is increasing (*3,4*). According to statistics from the World Health Organization, the number of dementia patients worldwide is expected to increase from 55 million in 2019 to 139 million in 2050, with AD patients accounting for approximately 60-70% of cases (*5*). This poses significant challenges to healthcare systems and global societal development.

The etiology and pathogenesis of AD remain elusive. The currently proposed hypotheses mainly include the Aβ cascade hypothesis, the tau hypothesis, the cholinergic hypothesis, and the excitotoxicity hypothesis (*1,6*). The drugs currently used to treat AD are mainly acetylcholinesterase inhibitors and N-methyl-*D*-aspartate (NMDA) receptor antagonists, which were developed based on the cholinergic hypothesis and the excitotoxicity hypothesis, respectively. These medications aim to ameliorate symptoms such as memory and cognitive impairments in AD patients, but they do not halt or reverse the progression of the disease, exhibiting limited clinical efficacy. The amyloid cascade hypothesis holds significant influence in explaining the pathogenesis of AD, suggesting that abnormal accumulation of Aβ in the brain leads to hyperphosphorylation of tau within neurons, thereby promoting the formation of neurofibrillary tangles, synaptic loss, and neuronal death, and ultimately resulting in cognitive impairment and other associated symptoms (*7*). Based on this hypothesis, inhibiting Aβ production and aggregation while promoting Aβ clearance has been a crucial strategy in the development of anti-AD drugs over the past two decades (*7,8*). Antibody-based therapeutics targeting Aβ in particular have held great promise, but drug development in this field has been fraught with challenges and setbacks (Table 1).

Aβ, derived from the amyloid precursor protein (APP) through cleavage by β-secretase 1 (BACE1)

Table 1. Research on and development of Aβ-directed antibodies against AD

Antibody	Epitope	Isotype	Targeted Aβ form	Study cohort in clinical trial	Outcome	*Ref.*
Ponezumab (Pfizer)	C-terminal sequence of Aβ (residues 33-40)	IgG_2	Primarily monomers	Mild-to-moderate AD (a phase 2 study), treatment for 18 months.	No effects on cognitive/functional outcomes; No dose response regarding cerebrospinal fluid biomarkers.	(*16,17*)
Solanezumab (Eli Lilly)	Central region of Aβ (residues 16-26)	IgG_1	Primarily monomers	(i) Mild to moderate AD (EXPEDITION 1 and EXPEDITION 2 studies), treatment for 80 weeks; (ii) Mild AD (EXPEDITION 3 study), treatment for 76 weeks; (iii) Asymptomatic or mildly symptomatic elderly individuals with biomarker evidence of brain amyloid deposition (A4 study), treatment for 3 years.	Increased levels of total $Aβ_{40}$ and $Aβ_{42}$ in plasma and CSF but no clearance of brain amyloid plaque; No significant cognitive and functional improvement compared to placebo.	(*19-21*)
Crenezumab (Roche)	Central regions of Aβ (residues 13-24)	IgG_4	Monomers and aggregates	Prodromal to mild AD (CREAD and CREAD2 study), treatment for 100 weeks.	No meaningful changes in AD biomarkers and no improvement of clinical decline compared to placebo (CREAD and CREAD2).	(*22,23*)
Bapineuzumab (Janssen and Pfizer)	N-terminal sequence of Aβ (residues 1-5)	IgG_1	Monomers and aggregates	Mild-to-moderate AD with or without apolipoprotein E (*APOE*) ε4 allele, treatment for 78 weeks.	Reducing AD biomarkers in patients with *APOE* ε4 allele but no functional improvement in patients with or without *APOE* ε4 allele.	(*26*)
Aducanumab (Biogen and Eisai)	N-terminal sequence of Aβ (residues 3-7)	IgG_1	Mainly aggregates (soluble and insoluble)	Mild cognitive impairment or the mild dementia stage of AD (EMERGE and ENGAGE studies)	Reducing amyloid β plaques but inconsistency in reducing clinical decline compared to placebo.	(*15,27*)
Lecanemab (Biogen and Eisai)	N-terminal sequence (residues 1-16)	IgG_1	Mainly aggregates (soluble and insoluble)	Mild cognitive impairment or the mild dementia stage of AD (Clarity AD study), treatment for 18 months	Reducing amyloid burden and clinical decline compared to placebo.	(*30*)
Donanemab (Eli Lilly)	N-terminal modified Aβ peptide (Aβ3-42)	IgG_1	Plaque	Mild cognitive impairment or the mild dementia stage of AD (TRAILBLAZER-ALZ 2 study), treatment for 72 weeks	Reducing amyloid burden and slowing clinical progression compared to placebo.	(*31*)
Gantenerumab (Roche)	Both N-terminal and central regions of Aβ (conformational epitope; residues 3-11, 18-27)	IgG_1	Mainly aggregates (soluble and insoluble)	Mild cognitive impairment or mild dementia due to AD (GRADUATE I and II), treatment for 116 weeks.	Reducing amyloid plaque burden but no clinical improvement compared to placebo.	(*34*)

and γ-secretase, is a product consisting of 30-51 amino acid residues in length (*7,9*). The main forms of Aβ are $A\beta_{1-40}$ and $A\beta_{1-42}$, with additional N-terminally truncated products such as $A\beta_{3-42}$ (*10*). Aβ monomers are proposed to possess certain physiological functions, such as regulating learning and memory, angiogenesis, neurogenesis, repairing leaks in the blood-brain barrier, promoting recovery from injury, and acting as an antimicrobial peptide and tumor suppressor (*11,12*). However, due to excessive production or other unknown reasons, Aβ monomers aggregate abnormally, forming soluble oligomers and protofibrils that are highly neurotoxic (*13,14*). The protofibrils can further aggregate into insoluble fibrils and ultimately be deposited as plaques, which are considered pathological hallmarks of AD. The transition of Aβ monomers to plaques is thought to be a dynamic and reversible process, as reducing the levels of soluble Aβ can shift the equilibrium and lead to plaque dissolution (*7*). Aβ-directed antibodies aim to bind to Aβ and promote immune-mediated clearance. Depending on the antigen epitope targeted, the form of Aβ bound by the antibody also differs. Since the N-terminal sequence of Aβ is exposed after aggregation, antibodies targeting the epitope in this region can theoretically bind to all forms of Aβ (including monomers, oligomers, protofibrils, and amyloid fibrils) (*8*). That said, the central and C-terminal regions are buried within the aggregates, making antibodies targeting these regions primarily bind to Aβ monomers (*8*). Over the past 20 years, different antibodies designed to target various antigenic epitopes have been tested for their ability to clear Aβ and potentially slow or reverse the progression of AD. These successful or unsuccessful cases may provide valuable lessons and insights for the development of future Aβ-targeting drugs.

Typical antibodies targeting the C-terminal or central region of Aβ include ponezumab, solanezumab, and crenezumab (*15*). These antibodies bind to Aβ monomers, aiming to shift the equilibrium by reducing the quantity of Aβ monomers and consequently decreasing the quantity of highly cytotoxic forms of Aβ. Ponezumab, which targets the C-terminal sequence of Aβ (*16*), did not demonstrate a dose-response in terms of changes in AD biomarkers in cerebrospinal fluid and did not result in cognitive improvement in a phase 2 clinical trial involving patients with mild to moderate AD (*17*). As a result, further clinical research was discontinued. Solanezumab and crenezumab, both of which target the central region of Aβ, share some similarities in the amino acid composition of their Fab fragments (*18*). Solanezumab only binds to Aβ monomers, whereas crenezumab can also bind to the oligomer's lateral and edge residues (*18*). Four phase 3 clinical trials investigated the efficacy of solanezumab in patients in different stages of AD progression. EXPEDITION 1 and 2 included patients with mild to moderate AD, EXPEDITION 3 included patients with mild AD, and the A4 trial included asymptomatic or mildly symptomatic elderly individuals with biomarker evidence of brain amyloid deposition (*19-21*). Results indicated that solanezumab increased the concentration of $A\beta_{40}$ and $A\beta_{42}$ in plasma and cerebrospinal fluid, suggesting movement of Aβ within the central compartment and some transfer of Aβ to the periphery (*19-21*). However, solanezumab had no effect on removing deposited amyloid plaques and slowing disease progression in any of the studies (*19-21*). Two phase 3 clinical studies (CREAD and CREAD2) on the efficacy of crenezumab included participants with prodromal to mild AD (*22,23*). Results indicated that total $A\beta_{42}$ and $A\beta_{40}$ concentrations in plasma and cerebrospinal fluid significantly increased following the administration of crenezumab and remained elevated throughout the study (*22,23*). However, there were no significant changes in brain Aβ imaging, and crenezumab did not reduce clinical decline in participants (*22,23*). These research findings suggest that antibodies primarily targeting Aβ monomers may have limited efficacy in clearing amyloid plaques and slowing disease progression.

Antibodies targeting the N-terminal sequence of Aβ include bapineuzumab, aducanumab, lecanemab, and donanemab. Bapineuzumab has the ability to bind to all forms of Aβ, including monomers, oligomers, protofibrils, fibrils, and plaques (*24,25*). Two phase 3 clinical trial investigated the efficacy of bapineuzumab in mild to moderate AD patients with or without the apolipoprotein E (*APOE*) ε4 allele (*26*). Results indicated that bapineuzumab reduced AD biomarkers such as brain plaques and cerebrospinal fluid phosphorylated tau in *APOE* ε4 allele carriers but not in noncarriers (*26*). However, bapineuzumab did not improve clinical outcomes in patients either with or without the *APOE* ε4 allele (*26*). The failure of these trials prompts us to think that the deposition of Aβ in the brain and its pathological damage to neurons may occur much earlier than the onset of AD symptoms, and selecting patients in the middle to late stages of the disease for clinical trials may miss the optimal time window for anti-Aβ drug intervention. Two phase 3 clinical trials evaluating aducanumab (EMERGE and ENGAGE) included patients with mild cognitive impairment or the mild dementia stage of AD (*27*). Aducanumab demonstrated the ability to clear brain Aβ plaques and delayed disease progression in the EMERGE but not in the ENGAGE study (*27*). Aducanumab is the first drug based on the Aβ hypothesis to receive Accelerated Approval from the US Food and Drug Administration (FDA) in nearly two decades (*28*), but its efficacy still requires further confirmation in additional clinical trials. Lecanemab is another antibody that primarily targets aggregates of Aβ (mainly oligomers and protofibrils) (*29*). In a phase 3 clinical trial, lecanemab showed the ability to clear Aβ plaques and delay disease progression in patients with mild cognitive impairment or the mild dementia stage of AD (*30*). Based on robust

clinical data, lecanemab has received full FDA approval for the treatment of mild AD patients. Coincidentally, donanemab, an antibody targeting N-terminal modified Aβ peptides, has also shown efficacy in delaying disease progression in patients with mild cognitive impairment or the mild dementia stage of AD in a phase 3 clinical trial (TRAILBLAZER-ALZ 2) (*31*). It specifically targets $A\beta_{3\text{-}42}$ (*32*), which accumulates early in the deposition cascade, and it demonstrates the ability to effectively clear brain plaques (*10,31*). Results of clinical trials on aducanumab, lecanemab, and donanemab support the Aβ hypothesis and suggest that drugs targeting the N-terminal sequence and aggregated forms of Aβ, capable of plaque clearance, may be efficacious in early-stage AD patients.

Surprisingly, two recent phase III clinical trials, GRADUATE I and II, have demonstrated that gantenerumab, an antibody targeting the conformational epitopes of both the N-terminal and central sequences of Aβ and selectively binding to aggregated forms of Aβ (*33*), did not slow disease progression in patients with mild cognitive impairment or mild dementia due to AD, despite its reduction in cerebral Aβ plaque deposition (*34*). The reasons for the clinical trial failures are still unknown. Was gantenerumab's efficacy in clearing Aβ plaques insufficient, or did the recruited patients have more severe disease compared to studies of lecanemab and donanemab? Whatever the answer, these failures have raised new questions about the Aβ hypothesis.

In conclusion, recent trials have demonstrated that Aβ antibodies are efficacious in slowing disease progression, which to some extent dispels doubts surrounding the Aβ hypothesis. However, further experimental validation is still needed to confirm this hypothesis, particularly in terms of whether the strategies targeting Aβ will exhibit the same efficacy in additional clinical trials. Advances in research on the pathological mechanisms of AD and advances in early diagnostic techniques may shift the time window for drug intervention and offer a potential pathway for developing effective drugs to delay the onset and progression of AD in the future.

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