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Review

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Amyloid-β-targeted therapies for Alzheimer's disease: currently and in the future

Huimin Cai, Xiaofeng Fu, Shuiyue Quan, Ziye Ren, Changbiao Chu, Longfei Jia*

Innovation Center for Neurological Disorders and Department of Neurology, Xuanwu Hospital, Capital Medical University, National Clinical Research Center for Geriatric Diseases, Beijing 100053, China.

Correspondence to: Dr. Longfei Jia, Innovation Center for Neurological Disorders and Department of Neurology, Xuanwu Hospital, Capital Medical University, National Clinical Research Center for Geriatric Diseases, 45 Changchun St., Beijing 100053, China. E-mail: longfei@mail.ccmu.edu.cn

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Abstract

Alzheimer's disease (AD) is common and devastating. However, current symptomatic treatments are unable to alter the progression of the disease. Fortunately, many ongoing trials of disease-modifying therapies may provide new insights into the treatment and prevention of AD. Due to the long-held amyloid cascade hypothesis, the development of pharmacotherapies targeting amyloid-β (Aβ) has been a major focus in AD research. The recent positive results and approval of several anti- Aβ monoclonal antibodies seem to be a milestone for AD treatment. In this review, we highlight the rationale and status of different Aβ-targeted therapies for AD, including those now on the market and those in clinical trials. We also discuss the challenges and future perspectives of Aβ-targeted therapies for AD.

Keywords: Alzheimer's disease, disease-modifying therapy, amyloid

INTRODUCTION

An estimated 15 million older adults are living with dementia in China, among whom 9.8 million have Alzheimer's disease (AD)[1]. As the mean life expectancy increases and the aging population grows rapidly,

the global cost attributable to dementia will reach US $9.12 trillion in 2050[2]. As such, effective treatment and prevention for AD are eagerly awaited.

Currently, treatment options for AD include both pharmacological and non-pharmacological approaches. Non-pharmacological therapies such as cognition stimulation therapy, occupational therapy, and behavioral interventions aim to enhance cognitive performance and improve the overall quality of life for individuals with AD[3]. Other alternative non-pharmacological therapies, including deep brain stimulation, focused ultrasound, and acoustic/light stimulation at 40 Hz, are under investigation[4]. Pharmacological interventions for AD primarily focus on the underlying pathophysiology of the disease. In 1993, the US Food and Drug Administration (FDA) approved the first drug, tacrine, for AD. Tacrine is an acetylcholinesterase inhibitor (AChEI) but is no longer in use due to hepatotoxicity[5]. An improved second-generation AChEIs and an N-methyl-D-aspartate receptor antagonist entered the market during the decade after tacrine. Although widely used, these drugs only provide symptomatic relief[6]. Therefore, research efforts have focused on developing disease-modifying therapies for the treatment and prevention of AD. Most disease-modifying therapies tested for AD in the past 20 years have targeted amyloid-β (Aβ) peptide and tau protein since they are the two key pathological hallmarks of the disease[6,7]. Meanwhile, remarkable advances in understanding the pathophysiology of AD have led to multiple hypotheses, such as inflammatory hypothesis, metabolism hypothesis, and mitochondrial hypothesis. These emerging hypotheses have sparked clinical development for potential treatments targeting these pathways[7,8], which have been extensively reviewed in other literature[9,10]. Given the recent advancements in Aβ-targeted therapies, this review aims to summarize and provide an updated overview of these therapies, focusing on those that have been approved and those currently in clinical trials.

THE AMYLOID CASCADE HYPOTHESIS

The impetus for developing Aβ-targeted therapies originates from the prevailing amyloid cascade hypothesis[11]. The production of the Aβ peptide occurs through the cleavage of a type I transmembrane glycoprotein, amyloid precursor protein (APP). In the non-amyloidogenic pathway, cleavage of APP by α-secretase releases a soluble extracellular fragment called sAPPα and retains a membrane-bound C-terminal fragment known as CTFα. CTFα is subsequently processed by γ-secretase, resulting in the generation of a soluble N-terminal fragment called P3 that does not possess aggregating tendencies. Alternatively, when APP is cleaved by β-site APP cleaving enzyme (BACE), it results in the generation of a soluble extracellular fragment called sAPPβ and a 99-residue C-terminal fragment called CTFβ or C99. C99 is further cleaved by γ-secretase, leading to the production of the neurotoxic Aβ peptide (amyloidogenic pathway)[12]. Aβ exists in different lengths, with Aβ40 being the most abundant and Aβ42 being less soluble. Extracellular accumulation of Aβ triggers its aggregation, progressing from monomers, oligomers, and protofibrils and eventually forming insoluble plaques[13], which is a hallmark pathology of AD[14]. Aβ accumulation serves as the initiating event in AD pathogenesis and triggers a series of downstream events such as tau hyperphosphorylation, formation of the neurofibrillary tangle, oxidative stress, inflammatory responses, synaptic dysfunction, and cognitive impairment[15]. Hence, Aβ has been one of the most popular targets in AD drug research. Figure 1 illustrates the mechanism of action of the main Aβ-targeted therapies based on the amyloid cascade hypothesis. Aβ-targeted strategies attempt to antagonize Aβ aggregation (Aβ aggregation inhibitors), reduce Aβ production by inhibiting or modulating BACE and γ-secretase (BACE inhibitors, γ-secretase inhibitors, and γ-secretase modulators), promote clearance of Aβ via active or passive immunotherapies (anti-Aβ vaccines and anti-Aβ monoclonal antibodies) or decrease related protein expression (RNA-based therapies). Figure 2 illustrates the clinical development of Aβ-targeted therapies for AD.

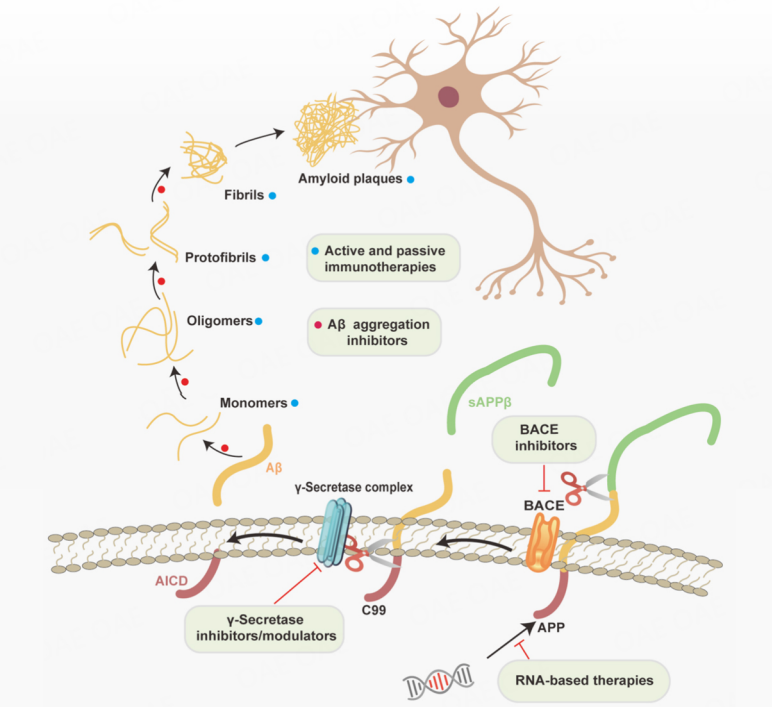

Figure 1. Illustration of amyloid cascade hypothesis and targets of anti-amyloid-β (Aβ) therapies for Alzheimer's disease. This figure illustrates the mechanism of action of the main Aβ-targeted therapies based on the amyloid cascade hypothesis. Blue circles indicate the targets of active and passive immunotherapies. Red circles indicate the targets of Aβ aggregation inhibitors. Aβ: amyloid-β; AICD: amyloid precursor protein intracellular domain; APP: amyloid precursor protein; BACE: β-site amyloid precursor protein cleaving enzyme; C99: a 99-residue C-terminal fragment; sAPPβ: soluble amyloid precursor protein-β.

Aβ AGGREGATION INHIBITORS

Studies have demonstrated that soluble Aβ oligomers, instead of plaques, are a major source of neurotoxicity and cause neuronal damage at the nanomolar level[16]. Hence, Aβ aggregation inhibitors are presumed to be a rational therapeutic strategy for AD. They disrupt the interaction of Aβ peptides, prevent their aggregation into oligomers, and thus limit neurotoxicity. Agents with this mechanism of action include PBT1 and PBT2, scyllo-inositol (ELND005), and tramiprosate.

PBT1(clioquinol) is a small molecule agent functioning as a metal protein attenuating compound[17]. Metal ions, such as copper and zinc, drive Aβ towards fibrillization and amyloid plaque formation and result in a series of redox reactions[18-20]. By binding these metal chaperones, PBT1 disrupts the interaction between Aβ peptides and metals and therefore prevents toxic Aβ oligomerization[17]. PBT1 failed to exhibit significant improvement in cognition or clinical global function in participants with mild cognitive impairment (MCI) to AD but showed a positive effect in those with more severe cognitive impairment (as measured by the Alzheimer's Disease Assessment Scale-Cognitive [ADAS-cog] Subscale ≥ 25)[21]. The study investigator attributed this finding to the limited statistical power of the study and the nonlinear sensitivity of the ADAS-cog instrument, which hinders the detection of subtle cognitive differences in the less severely affected group. The second-generation clioquinol (PBT2), which has improved pharmacological prosperities and efficacy[22], also failed to show significant effects on cognition or function in MCI or mild to moderate AD[23,24].

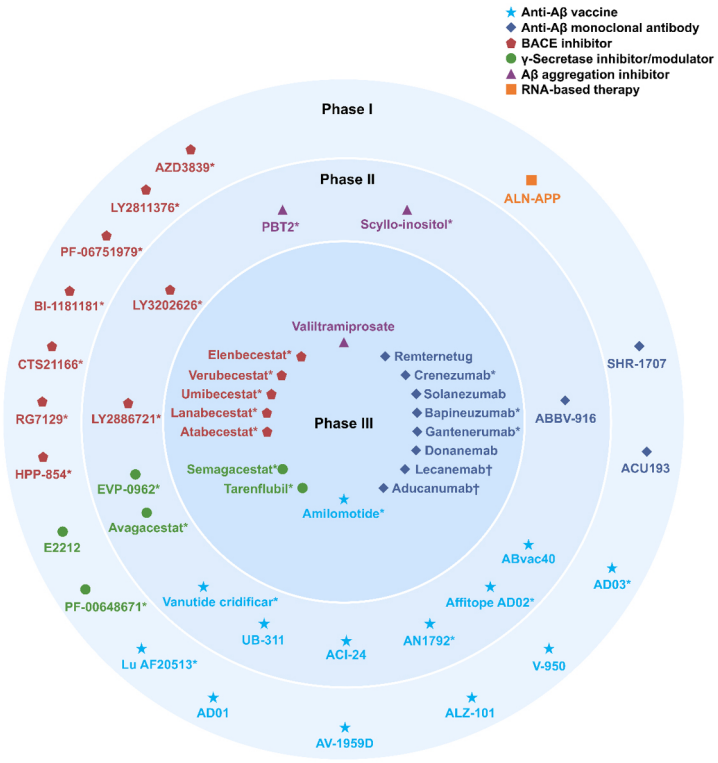

Figure 2. Development of amyloid-β (Aβ)-targeted therapy for Alzheimer's disease (AD). This figure summarizes the clinical development of Aβ-targeted pharmacotherapies for AD, reported according to the most advanced phase of the study and main therapeutic properties. Data were accessed on July 18, 2023. Agents with "*" indicate that the trials have been discontinued or are inactive. Agents with "†" indicate that the agents have been approved for the treatment of AD. Aβ: amyloid-β; AD: Alzheimer's disease; APP: amyloid precursor protein; BACE: β-site amyloid precursor protein cleaving enzyme.

Scyllo-inositol is an oral inositol stereoisomer that is thought to neutralize toxic Aβ oligomers and reduce aggregation[25]. This agent showed a dose-dependent reduction in Aβ pathology and ameliorated learning deficits in transgenic mice[25,26]. However, scyllo-inositol failed to show clinical benefits in participants with mild to moderate AD despite encouraging results in preclinical studies[27]. Severe adverse effects, including infections and death, were reported in the higher dosage (2,000 or 4,000 mg/day)[27], while the lower dosage (250 mg/day) appeared to have an acceptable safety profile[28]. Further development of the agent has not progressed in recent years.

Tramiprosate is a small aminosulfonate compound that functions by binding to and stabilizing Aβ42 monomers, thereby preventing oligomerization and plaque formation[29]. Tramiprosate did not show efficacy in mild to moderate AD in a phase III trial[30], but subgroup analyses demonstrated beneficial effects in the high-risk apolipoprotein E (*APOE*) ε4 carriers[31,32]. Then a prodrug version of tramiprosate, valiltramiprosate (ALZ-801), has developed with enhanced pharmacokinetic properties and tolerance[33]. A phase III trial is ongoing to validate the cognitive efficacy of valiltramiprosate on *APOE* ε4 homozygotes with early AD[34].

BACE INHIBITORS

BACE1 is the first and rate-limiting secretase responsible for the proteolysis of APP in the amyloidogenic pathway[12]. The level and activity of BACE1 are higher in AD compared to healthy subjects[35]. BACE1 suppression interferes with the upstream of the amyloid cascade and, therefore, reduces the production of

toxic Aβ, which may have important implications in the treatment of AD. Despite numerous studies conducted thus far, no BACE inhibitors have successfully completed phase III clinical trials [Table 1]. The concerning adverse effects observed in these trials include worsening cognitive function and weight loss. These effects may be attributed to the crucial role of BACE1 substrates beyond APP in neurodevelopment, as evidenced by the development of myelination deficits[36], cognitive impairment, and axon guidance defects in BACE1 knockout mice[36-38]. These findings suggest that developing a BACE inhibitor without these side effects could be challenging.

Verubecestat (MK-8931) is an orally administered BACE1 inhibitor characterized by its high cell permeability, excellent water solubility, and remarkable ability to cross the blood-brain barrier[39]. Verubecestat reduced cerebrospinal fluid (CSF) Aβ concentrations by over 90% in rodents and nonhuman primates[39]. Phase I trials[40-42] indicated that verubecestat was well-tolerated and capable of reducing Aβ protein levels in CSF. However, subsequent trials revealed that verubecestat failed to delay cognitive decline in participants with prodromal AD[43]. Moreover, it was associated with a range of treatment-related adverse events, including hippocampal atrophy, suicidal ideation, weight loss, and sleep disorders[44].

LY3202626 is a potent inhibitor of BACE1, known for its ability to cross the blood-brain barrier[45]. Preclinical studies demonstrated that LY3202626 dose-dependently reduced Aβ levels in primary cultured neurons of PDAPP mice[45]. Phase I trials[46-49] indicated that LY3202626 was generally well tolerated. Unfortunately, phase II trials[50] did not show any differences in tau PET, Aβ PET, or cognitive function between the treatment group and the placebo group. Additionally, a reduction in brain volume, particularly in the hippocampus region, was observed. Therefore, the study was discontinued.

Umibecestat (CNP520) exhibits significant selectivity for BACE1 over BACE2 and other proteases[51]. Umibecestat reduced CSF Aβ and Aβ deposition in preclinical models[51]. Animal toxicology studies of umibecestat indicated its safety profile, showing no adverse effects such as hair depigmentation, retinal degeneration, hepatotoxicity, or cardiotoxicity[51]. However, subsequent findings revealed that participants in the treated group experienced cognitive decline, brain atrophy, and weight loss compared to the placebo group, leading to the discontinuation of the study[52,53].

Elenbecestat (E2609) is an orally bioavailable small molecule BACE1 inhibitor[54]. Earlier trials demonstrated that elenbecestat was generally well tolerated and reduced Aβ levels in CSF and plasma, which holds promise for improving cognitive function[54-58]. However, the follow-up study was discontinued due to the lack of effective cognitive improvement in the treatment group[59]. Additionally, some participants in the treatment group experienced adverse effects such as rash, neuropsychiatric symptoms, cognitive impairment, decreased brain volume, a transient decrease in white blood cells, and elevated liver enzymes compared to the placebo group[14,52,60-62].

Atabecestat (JNJ-54861911) exhibits effective penetration into the central nervous system (CNS)[63]. Phase I results reported up to a 95% reduction in Aβ levels in healthy subjects[64]. However, subsequent phase II and III studies were terminated due to abnormally elevated liver enzymes and a potential trend towards cognitive decline in participants[65-67]. Follow-up reports suggest that hepatotoxicity may be linked to the inflammatory response induced by atabecestat and its metabolites[68].

Lanabecestat (LY3314814, AZD3293) showed promising potential in preclinical studies with various animal models[69], leading to its progression into clinical trials. Phase I trial[70] demonstrated the safety of lanabecestat and its ability to significantly reduce Aβ levels in both plasma and CSF. However, the phase III

Table 1. Clinical trials of BACE inhibitors, γ-secretase inhibitors and modulators

Target/Mechanism	Sponsor	Drug	Study Population	Phase	Status	Clinical trial identifier	Start date	Estimated end date	Results	Remarks
BACE inhibitor	Merck	Verubecestat (MK-8931)	Mild to moderate AD	II/III	Terminated	NCT01739348	November 2012	April 2017	Lack of efficacy	No cognitive or functional benefits
			Prodromal AD	III	Terminated	NCT01953601	November 2013	April 2018	Toxicity and lack of efficacy	Worse cognition and functional ability; more adverse effects
	Eli Lilly	LY2811376	Healthy subjects	I	Completed	NCT00838084	December 2008	June 2009	Toxicity	nonclinical retinal toxicity
	Eli Lilly	LY2886721	Mild AD	I /II	Terminated	NCT01561430	March 2012	August 2013	Toxicity	Abnormally elevated liver enzymes
	Eli Lilly	LY3202626	Mild AD	II	Terminated	NCT02791191	June 2016	July 2018	Toxicity and lack of efficacy	-
	Amgen/Novartis	Umibecestat (CNP520)	Preclinical AD	II/III	Terminated	NCT03131453	August 2017	March 2020	Toxicity and lack of efficacy	Worse cognition; brain atrophy weight loss
			Preclinical AD	II/III	Terminated	NCT02565511	November 2015	April 2020		
	Eisai/Biogen	Elenbecestat (E2609)	Early AD	III	Terminated	NCT02956486	October 2016	January 2020	Toxicity and lack of efficacy	No cognitive benefits; more adverse effects
	Janssen	Atabecestat (JNJ-54861911)	Preclinical AD	II/III	Terminated	NCT02569398	October 2015	December 2018	Toxicity and lack of efficacy	Worse cognition; more adverse effects
	Astrazeneca/ Eil Lilly	Lanabecestat (LY3314814, AZD3293)	Early AD	III	Terminated	NCT02972658	March 2017	October 2018	Toxicity and lack of efficacy	Worse cognition; brain atrophy
			Mild AD	III	Terminated	NCT02783573	July 2016	September 2018		
	Boehringer Ingelheim	BI-1181181 (VTP-37948)	Healthy subjects	I	Terminated	NCT02254161	November 2014	February 2015	Toxicity	Skin reactions
			Healthy subjects	I	Completed	NCT02106247	April 2014	June 2014		
			Healthy subjects	I	Completed	NCT02044406	January 2014	September 2014		
	Pfizer	PF-06751979	Healthy subjects	I	Completed	NCT02793232	June 2016	January 2017	Discontinuing research and development in neurology	-
			Healthy subjects	I	Completed	NCT03126721	April 2017	July 2017		
			Healthy subjects	I	Completed	NCT02509117	July 2015	July 2016		
	Hoffmann-La Roche	RG7129 (RO5508887)	Healthy subjects	I	Completed	NCT01664143	July 2012	September 2012	No results have been published	-
			Healthy subjects	I	Completed	NCT01592331	May 2012	October 2012		
			Healthy subjects	I	Completed	NCT01461967	September 2011	December 2011		
	CoMentis	CTS21166	Healthy male	I	Completed	NCT00621010	June 2007	February 2008	No results have been published	-

	High Point Pharmaceuticals, LLC	HPP-854	Mild AD	I	Terminated	NCT01482013	October 2011	March 2012	No results have been published	-
	AstraZeneca	AZD3839	Healthy subjects	I	Completed	NCT01348737	June 2011	November 2011	Toxicity	-
γ-Secretase inhibitor	Eli Lilly	Semagacestat (LY450139)	Probable AD	III	Completed	NCT01035138	December 2009	April 2011	Toxicity and lack of efficacy	Worse cognition; risk of skin cancer and infections
			Mild to moderate AD	III	Completed	NCT00762411	September 2008	April 2011		
			Mild to moderate AD	III	Completed	NCT00594568	March 2008	May 2011		
	Bristol-Myers Squibb	Avagacestat (BMS708163)	Prodromal AD	II	Terminated	NCT00890890	May 2009	July 2013	Toxicity and lack of efficacy	Worse cognition; gastrointestinal and dermatological side effects
γ-Secretase modulator	Myrexis	Tarenflurbil (R-flurbiprofen, MPC-7869)	Probable AD	III	Terminated	NCT00380276	September 2006	December 2008	Toxicity and lack of efficacy inadequate ability to penetrate the brain	-
			Mild AD	III	Terminated	NCT00322036	May 2006	December 2008		
			Mild AD	III	Completed	NCT00105547	February 2005	May 2008		
	FORUM Pharmaceuticals	EVP-0962	MCI or early AD, healthy subjects	II	Completed	NCT01661673	November 2012	October 2013	Toxicity	-
	Eisai	E2212	Healthy subjects	I	Completed	NCT01221259	January 2010	November 2012	Acceptable safety and promising pharmacodynamic response	-
	Pfizer	PF-06648671	Healthy subjects	I	Completed	NCT02407353	October 2015	March 2016	Discontinuing research and development in neurology	-
			Healthy subjects	I	Completed	NCT02316756	December 2014	March 2015		
			Healthy subjects	I	Completed	NCT02440100	May 2015	October 2016		

Aβ: amyloid-β; AD: Alzheimer's disease; APP: amyloid precursor protein; BACE: β-site amyloid precursor protein cleaving enzyme; MCI: mild cognitive impairment.

study[71] revealed that lanabecestat treatment did not slow or prevent cognitive decline. Furthermore, some adverse events were reported, including brain atrophy, neuropsychiatric symptoms, weight loss, and hair depigmentation.

BI-1181181 (VTP-37948) is an inhibitor of both BACE1 and BACE2[72]. Results from the phase I trial in stages I and II indicated that a single dose of BI-1181181 was well-tolerated, and its long half-life supported a once-daily oral dosing regimen[73,74]. However, the phase I trial was terminated during stage III due to skin reactions observed in some participants[75].

LY2811376, the first small molecule BACE1 inhibitor entering clinical trials, demonstrated a significant reduction in Aβ levels in both plasma and CSF during the phase I trial[76]. However, subsequent toxicological data revealed retinal toxicity, leading to the discontinuation of further studies[45,77].

LY2886721, a second-generation BACE1 inhibitor developed after LY2811376, exhibits a 10-fold stronger inhibition of BACE1 without affecting other aspartic proteases like histone D, pepsin, and renin[78]. In a PDAPP transgenic mouse model, oral administration of LY2886721 significantly reduced Aβ levels in primary neurons[79]. Phase I trials[80-85] demonstrated the good tolerability of LY2886721 under different dosages and its ability to lower CSF Aβ levels. However, hepatotoxicity was observed in many participants during the phase II trial[86], leading to the discontinuation of the study.

The development of several BACE1 inhibitors has been terminated for various reasons. PF-06751979, developed by Pfizer, showed promising results in preclinical and phase I studies with a reduction in CSF Aβ 42 levels[87-92]. However, Pfizer decided to discontinue its development in neurology, leading to the drug not advancing to phase II or III trials[93]. RG7129 (RO5508887) was found to be hepatotoxic, resulting in the discontinuation of the study[94]. Only one registered phase I trial was found for CTS21166[95] and HPP-854[96], but no relevant trial results or records of further clinical studies are available.

γ-SECRETASE INHIBITORS AND MODULATORS

γ-Secretase, a transmembrane protein complex, cleaves more than 140 substrates, including APP and Notch[97]. APP is initially cleaved by BACE, producing C99, which is subsequently proteolyzed by γ-secretase at multiple sites to generate Aβ peptides[12,98]. Therefore, targeting γ-secretase is considered a promising strategy for AD. Agents under this rationale include γ-secretase inhibitors and modulators [Table 1].

Semagacestat (LY450139) is the most potent γ-secretase inhibitor tested in humans. A study using stable isotope-labeled amino acid (13C6-leucine) found that semagacestat reduced Aβ production in a dose-dependent manner in the human CNS[99]. However, clinical trials of semagacestat failed due to its non-selective inhibition of substrates, leading to suppressed Notch signaling and accumulation of CTFs. These side effects included an increased risk of skin cancer, infection, gastrointestinal symptoms, and a decline in cognitive performance, which ultimately resulted in the discontinuation of clinical development[100-103].

Avagacestat (BMS708163) was initially considered a potent and selective γ-secretase inhibitor with "Notch-sparing" activity[104]. Preclinical studies indicated that it was 193 times more selective for Aβ processing than Notch and effectively inhibited the formation of Aβ40 and Aβ42, suggesting it may have fewer adverse effects compared to non-selective agents[104]. However, subsequent research revealed that avagacestat exhibited only 3-7 fold selectivity for APP over Notch[105]. Consistent with the latter findings, avagacestat caused Notch-deficient toxicity similar to semagacestat, that is, higher incidence of skin cancer, worsened cognitive function, and higher brain atrophy rate in phase II trials[106,107]. Therefore, the development of avagacestat was terminated.

One reason for the extensive toxicities of γ-secretase inhibitors may be that these proteases regulate many physiological processes within and outside the nervous system[108]. Another explanation for the failure would be too much and too quick suppression of Aβ, as Aβ accumulates and causes neurotoxicity in a chronic manner. Novel strategies aimed at partial suppression of the proteases and gradual reduction of Aβ are under development[109]. γ-Secretase modulators selectively modulate the γ-secretase cleavage site of APP rather than the downstream ε-cleavage site[110]. Consequently, γ-secretase modulators that exert a lesser influence on Notch signaling or other substrates would result in fewer side effects[111].

Nonsteroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen, indomethacin, and sulindac sulfide, are the first identified γ-secretase modulators based on the epidemiological findings of lower prevalence of AD in NSAID users[112-114]. Short-term administration of NSAIDs reduced Aβ42 levels in the brain of APP transgenic mice and decreased Aβ42 secretion in cultured cells accompanied by an increase in Aβ38 isoforms, suggesting that NSAIDs subtly modulate γ-secretase activity without significantly affecting other APP processing pathways or Notch cleavage. Notably, the effect of NSAIDs on Aβ pathology in the brain is independent of their cyclooxygenase activity[115]. Tarenflurbil is the R-enantiomer of NSAIDs targeting the presenilin component of γ-secretase, which has less effect on cyclooxygenase activity and thus causes fewer gastrointestinal symptoms[115-118]. Tarenflurbil selectively reduced Aβ42 level and rescued the memory deficits of APP transgenic mice at chronic dosage in preclinical studies[116,119]. However, tarenflurbil failed to show significant efficacy in clinical studies, which is ascribed to its suboptimal pharmacodynamics, and the agent was no longer developed[120,121]. CHF507480, an NSAID-derived carboxylic acid analog, is initially considered a γ-secretase modulator but is now reported to act on multiple targets, including tau and neuroinflammation[122,123]. CHF507480 restored hippocampal neurogenesis, decreased brain Aβ burden, and improved memory in mouse models of AD[124,125]. CHF507480 showed clinical improvement in participants with MCI, especially in *APOE* ε4 carriers[126,127]. However, this differential clinical outcome could be attributed to the higher levels of neuroinflammation in *APOE* ε4 carriers compared to non-carriers[128], and this agent is being investigated as a microglial modulator[129].

E2012 is a non-NSAID-derived imidazole γ-secretase modulator. A preclinical study showed it dose-dependently reduced Aβ40 and Aβ42 levels in rat CSF, brain, and plasma[130]. It also increased shorter Aβ peptides such as Aβ37 and Aβ38, while maintaining the total amount of Aβ peptides[131]. It demonstrated a 50% efficacy in reducing plasma levels of Aβ42 during the phase I trial[132]. However, a high-dose group in a preclinical safety study exhibited lenticular opacity, leading to the suspension of the clinical trial[133]. E2212, an improved compound of E2012, demonstrated an acceptable safety profile and promising pharmacodynamic response in doses ranging from 10 mg to 250 mg in the phase I trial[134], but no further updates have been reported.

ACTIVE IMMUNOTHERAPIES AGAINST Aβ

Immunotherapies have emerged as a potential treatment approach to slow down or halt the progression of AD by eliminating Aβ aggregates. The focus is on generating antibodies (active immunization) or using existing antibodies (passive immunization) against Aβ antigens to facilitate the clearance of Aβ[135]. Active immunization allows the production of antibodies over a longer period by administering a few doses of vaccine. However, T-cell-mediated adverse effects may occur after active immunization and limit its use[136]. Figure 3 illustrates the Aβ epitope targeted by representative anti-Aβ vaccines.

The first Aβ-targeted active immunotherapy candidate, AN1792, was developed using full-length Aβ42 and formulated with QS21 adjuvant. The inclusion of QS21 adjuvant was aimed at shifting T cell response towards a TH1 phenotype and enhancing the antibody response[137]. In the phase I trial, AN1792 was able to

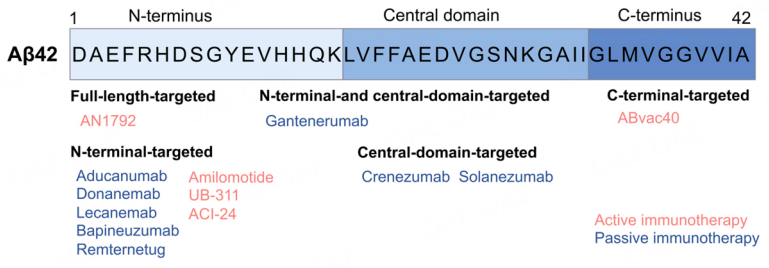

Figure 3. Amyloid-β (Aβ) epitopes of anti-Aβ vaccines and monoclonal antibodies tested in clinical trials for Alzheimer's disease. Aβ amino acid sequence is indicated in the one-letter code. Anti-Aβ vaccines and monoclonal antibodies are classified according to the targeting epitope of Aβ. Aβ: amyloid-β.

generate an antibody response against Aβ42, leading to a reduction in amyloid plaques in the brain and an improvement in cognitive function among participants[138]. However, the phase II trial was terminated after 6% of participants developed meningoencephalitis mediated by Aβ-specific T-cell inflammatory response[139]. The severe adverse effect is presumably due to the use of full-length Aβ42, which contains T-cell epitopes in the C-terminus. Additionally, AN1792 faced challenges with weak immunogenicity, resulting in low antibody titers and limited therapeutic efficacy[140].

To address the issue of self-reactive T cell response and achieve higher antibody titers, second-generation vaccines were developed to target the B cell epitope of Aβ while avoiding T cell-mediated response[141]. The B cell epitope is primarily found in the Aβ1-15 region, and the fragment Aβ3-10 retains sufficient immunogenicity to elicit strong immune responses while minimizing T cell response. Consequently, the Aβ3-10 fragment is employed as the key component in the peptide vaccine[142-144]. Several second-generation Aβ vaccines have been developed, including amilomotide (CAD106), Vanutide cridificar (ACC-001), Lu AF20513, UB-311, ACI-24, V-950, ABvac40, ALZ-101, AD01, Affitope AD02, and AD03[145,146]. These vaccines have not induced meningoencephalitis in clinical trials, but certain antibody-mediated adverse reactions such as vasogenic edema and microhemorrhages have been reported. Currently, only a few of these vaccines have advanced to late-phase clinical trials, namely amilomotide, ACI-24, UB-311, and ABvac40[146].

Amilomotide is composed of multiple copies of Aβ1-6 coupled to a virus-like particle derived from the bacteriophage Qβ[141]. Active immunization with amilomotide prevented brain amyloid plaque accumulation in a transgenic mouse model of AD, with an 80% reduction in plaque compared to controls[147]. Phase I/II clinical trials of amilomotide conducted thus far have shown a favorable safety profile, acceptable antibody response, and promising effects in slowing down Aβ deposition in humans[141,148]. Notably, no cases of meningoencephalitis, aseptic meningoencephalitis, or vasogenic edema were reported[141]. Given these positive outcomes, amilomotide holds great potential as an effective therapeutic agent for preventing Aβ deposition in high-risk groups. However, further phase II/III trial of amilomotide was terminated prematurely due to unpredicted cognitive changes, brain atrophy, and weight loss[149].

ACI-24 is an Aβ vaccine consisting of a tetra-palmitoylated Aβ1-15 peptide in β conformation, coupled with liposomes containing monophosphorylated lipid A as an adjuvant[150]. Preclinical studies using mouse models of amyloidosis[150] and Down syndrome[151] demonstrated the efficacy of ACI-24 in rescuing memory deficits. Having achieved satisfactory results in the preclinical trial[152], a phase I/II trial[153] was conducted in mild to moderate AD to compare different doses of ACI-24 with placebo and assess its safety, immunogenicity, and efficacy. However, the trial ultimately enrolled only 48 participants, and the planned

phase II efficacy stage was canceled due to limited antibody response. A phase II trial[154] with a new formulation was then initiated in participants with mild AD. Results presented in 2021 showed a clear IgM antibody response, and increased CSF Aβ levels, but no observable change in amyloid-PET scans. Another phase I study[155] investigated the safety, tolerability, and immune response of ACI-24 in Down syndrome. The study showed that the ACI-24 vaccine was safe and well tolerated, with no serious adverse events, CNS inflammation, or T-cell activation observed. A dose-dependent IgG response was seen at the higher doses. However, the study had a small sample size of only 16 subjects, and long-term follow-up is necessary to assess the cognitive function of the participants.

UB-311 contains two Aβ1-14-targeting peptides (B-cell epitopes), each linked to a different helper T-cell peptide epitope. These chimeric peptides are formulated to enhance immunogenicity within a Th2-biased delivery system, thereby minimizing T-cell inflammatory reactivity[156]. In a phase I study, UB-311 elicited a strong antibody response against Aβ without stimulating a cytotoxic T-cell response[156]. The safety and good tolerability of UB-311 were further confirmed in a phase II study in participants with mild to moderate AD. Exploratory endpoints of the study demonstrated a slower rate of cognitive decline in AD participants and a reduction in brain Aβ. The extension of the phase II trial was terminated due to treatment assignment error[157,158] and a phase III trial is planned[159].

While the majority of Aβ vaccine research has focused on N-terminal epitopes, alternative approaches are being explored. One such candidate is ABvac40, which consists of multiple repetitions of Aβ33-40, a C-terminal fragment of Aβ40, conjugated to keyhole limpet hemocyanin[160]. Aβ40 is the most common Aβ isoform and the predominant component of cerebral amyloid angiopathy[161]. Furthermore, it can also generate toxic aggregate[162,163] and is associated with the severity of AD[164,165]. The phase I trial demonstrated that ABvac40 was well tolerated and elicited the production of specific antibodies against Aβ40[160]. According to the topline results of the phase II trial presented recently, ABvac40 met its primary safety and efficacy outcome, displayed an excellent safety and tolerability profile, and elicited a robust immune response in participants with MCI or mild AD[166]. More details of the study are pending.

DNA vaccines, also known as genetic vaccines, represent the third generation of vaccines and offer distinct advantages over traditional peptide vaccines. Unlike peptide vaccines, DNA vaccines do not require the addition of adjuvant-like peptides. They are relatively safe, cost-effective, and capable of maintaining a sustained level of antigen expression within host cells[167]. AV-1959D is a DNA vaccine incorporating three copies of Aβ1-11, along with 12 T-cell-activating epitopes derived from various sources such as tetanus toxin, hepatitis B, and influenza viruses. These foreign antigens help enhance antibody responses by activating naïve and memory lymphocytes, which is particularly beneficial for older individuals who typically have weaker responses to vaccines[168,169]. AV-1959D demonstrated a robust cellular immune response and generated potent anti-Aβ antibodies in animal models, without triggering T-cell infiltration[170-173]. Furthermore, the early version of the vaccine effectively inhibited amyloid plaque accumulation, reduced glial activation, and prevented behavior deficits in aged mice[167]. No toxicities or amyloid-related imaging abnormalities (ARIA) were observed in mice susceptible to cerebral amyloid angiopathy[169]. These findings highlight the vaccine's potential as a safe and effective treatment for AD, leading to its advancement to a clinical trial in December 2022. The phase I clinical trial[174] aims to enroll 48 participants with early AD, who will receive different dosages of AV-1959D or placebo to assess its safety and tolerability.

PASSIVE IMMUNOTHERAPIES AGAINST Aβ

Many active immunotherapies against Aβ were discontinued either because of severe T-cell-mediated meningoencephalitis or for futility[139,148,175]. Consequently, efforts have shifted towards passive immunotherapies to avoid T-cell-mediated responses. Passive anti-Aβ immunotherapies involve humanized monoclonal or polyclonal antibodies targeting Aβ. The anti-Aβ antibodies used in clinical trials have varied in their specificity, targeting different domains of the Aβ peptide [Figure 3]. They have different affinities for various forms of Aβ, including monomers, oligomers, protofibrils, and plaques [Figure 4]. Several monoclonal antibodies have entered phase III trials: aducanumab, lecanemab, donanemab, remternetug, gantenerumab, bapineuzumab, solanezumab, and crenezumab. In addition, three monoclonal antibodies including SHR-1707[176], ACU193[177], and ABBV-916[178] are under phase I and II trials.

Aducanumab is a recombinant human IgG1 monoclonal antibody targeting N-terminal Aβ3-7[179]. It preferentially directs against soluble Aβ aggregates and insoluble fibrils Aβ. Aducanumab received its first approval for AD in the US in June 2021 based on significant biomarker outcomes in EMERGE and ENGAGE studies[179,180]. Evidence of clinical efficacy is discordant and conflicting in the two identically designed studies[181]. EMERGE study (1,638 MCI or early AD) showed a significant 22% slowing of decline on the Clinical Dementia Rating-Sum of Boxes (CDR-SB) in the high-dose group, while no significant cognitive benefit was observed in either the low- or the high-dose group in ENGAGE study (1,647 MCI or early AD)[180,181]. The approval of aducanumab is controversial, and concerns have been raised about insufficient clinical efficacy and the misuse of statistics[182]. Notably, aducanumab was granted via an accelerated approval mechanism, which means that clinical benefits must be verified in postmarketing trials to obtain continued approval. Aducanumab exerts effects by decreasing amyloid plaques in the brain and, to some extent, phosphorylated tau (p-tau), representing the downstream tau pathology[180]. According to the prescribing information, aducanumab is recommended to be initiated in participants with MCI or mild AD[179]. The most worrisome adverse reactions are ARIA, including ARIA edema (ARIA-E; 35% *vs.* 3%), ARIA hemosiderin deposition (ARIA-H) microhemorrhage (19% *vs.* 7%), and ARIA-H superficial siderosis (15% *vs.* 2%). These adverse reactions are dose-dependent, leading to a greater withdrawal rate in the high-dose group[179,183]. Recipients must be pre-evaluated for susceptibility to these adverse reactions and closely monitored with scheduled magnetic resonance imaging (MRI) scans[184]. The marketing application for aducanumab was rejected by the European Medicines Agency and Japan's Health Ministry in 2021.

To obtain more data on the long-term safety and tolerability of aducanumab, a phase IIIb EMBARK study[185,186] is ongoing. Furthermore, a phase IV confirmatory trial ENVISION[187] began in June 2022. The trial will enroll 1,500 individuals with early AD and last 18 months. The primary outcome is CDR-SB and secondary outcomes include cognitive and functional measures, as well as Aβ and tau PET. Results are expected by 2026.

Lecanemab is a humanized IgG1 version of the mouse monoclonal antibody mAb158, which possesses a specific affinity for soluble Aβ protofibrils[188]. mAb158 exhibited the ability to reduce Aβ protofibrils in the brain and CSF in preclinical studies. Furthermore, mAb158 has the potential to protect neurons by mitigating the toxic effects of Aβ protofibrils by reducing their pathological accumulation in astrocytes[189]. Lecanemab was granted accelerated approval in January 2023 and full approval in July 2023 by the US FDA based on the results from phase II[190,191] and phase III trials (CLARITY AD)[192]. The CLARITY AD trial, a large global clinical study involving 1,795 participants with early AD, demonstrated that lecanemab successfully achieved its primary endpoints. The lecanemab treatment group exhibited a 27% slower clinical deterioration compared to the placebo group after 18 months, which corresponded to a treatment difference of -0.45 in the CDR-SB change in the intent-to-treat population. Additionally, all secondary endpoints,

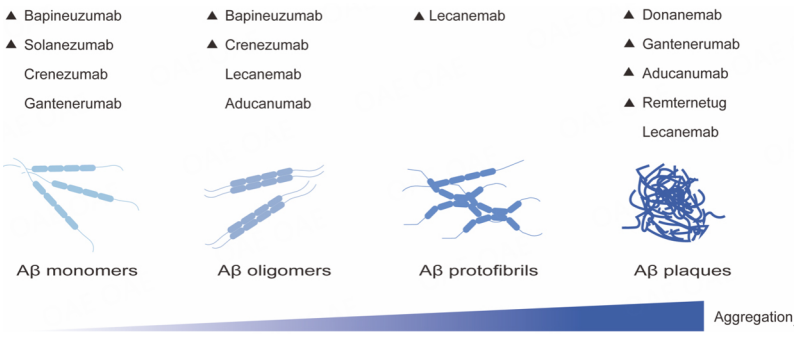

Figure 4. Different forms of amyloid-β (Aβ) targeted by different monoclonal antibodies. This figure illustrates the different Aβ forms targeted by current anti-Aβ monoclonal antibodies for Alzheimer's disease. Agents with a "triangle" indicate that the agent preferentially targets the form of Aβ below. Aβ: amyloid-β.

including global cognition and activities of daily living, showed statistically significant improvement compared to placebo. Furthermore, lecanemab effectively reduced Aβ burdens measured by PET scans in a subset of 698 participants. Biomarkers related to tau pathology and neurodegeneration in CSF and plasma also favored lecanemab over placebo. These findings support the positive correlation between the extent of Aβ reduction and the degree of clinical benefit. Lecanemab treatment, like other Aβ-targeted monoclonal antibodies, had side effects including ARIA-E and ARIA-H. ARIA-E incidence was 12.6% (2.8% symptomatic) in the lecanemab group and 1.7% (no symptomatic cases) in the placebo group. ARIA-H incidence was 17.3% (0.7% symptomatic) in the lecanemab group and 8.7% (0.2% symptomatic) in the placebo group. Overall, ARIA events were less frequent with lecanemab compared with other monoclonal antibodies[193]. Fatal brain hemorrhage cases were reported with lecanemab and concomitant anticoagulant treatment[194,195], suggesting caution in treating participants on anticoagulants with anti-Aβ monoclonal antibodies until more safety data are available[196].

The recent approval of lecanemab is encouraging, particularly to clinicians, patients, and caregivers. However, the statistically positive results were considered well below the minimal clinically important difference, which could result from biases due to loss of follow-up or functional unblinding[197]. Therefore, it is too early to conclude since we expect more evidence from postmarketing trials. A phase III study of lecanemab called AHEAD 3-45 began in July 2020[198]. It is a four-year study consisting of two sub-studies, involving a total of 1,400 cognitively normal individuals with elevated brain Aβ. The A3 sub-study (400 participants) focuses on those with Aβ burden below the positivity threshold; they will receive lecanemab at a dose of 5 mg/kg titrated to 10 mg/kg or a placebo every four weeks for 216 weeks with a change in brain Aβ PET as the primary outcome. The A45 sub-study (1,000 participants) enrolls those with positive Aβ PET; they will receive lecanemab titrated to 10 mg/kg every two weeks for 96 weeks, followed by the same dose every four weeks until week 216 with a change in Preclinical Alzheimer Cognitive Composite 5 score as the primary outcome. Notably, blood Aβ42/40 will be used to prescreen participants for elevated brain Aβ before PET imaging.

Donanemab is a humanized IgG1 monoclonal antibody derived from the mouse mE8-IgG2a. It specifically targets N-truncated pyroglutamate Aβ peptide (AβpE), a form of Aβ that aggregates in amyloid plaques[199]. Therefore, donanemab aims to eliminate existing plaques rather than prevent their formation. In a phase II trial (TRAILBLAZER-ALZ) involving 257 participants with early symptomatic AD who had brain tau and Aβ deposition, donanemab showed positive effects on delaying the clinical progression, as evidenced by a better composite score for cognition and functional ability (change from baseline in the Integrated Alzheimer's Disease Rating Scale [iADRS] score at 76 weeks: -6.86 in the treatment group *vs.* -10.06 in the

placebo group)[200]. The slowing in clinical decline was accompanied by a considerable reduction in amyloid plaques, with a mean change difference of 85 centiloids between donanemab and placebo at 76 weeks. This led to approximately two-thirds of the participants receiving donanemab becoming Aβ-PET negative by week 76[200,201]. A subsequent post hoc analysis[201] revealed that individuals with higher baseline Aβ experienced greater plaque reduction in the first six months but were less likely to achieve complete clearance compared to those with lower Aβ levels. The degree of Aβ reduction was correlated with changes on the iADRS clinical endpoint only in *APOE* ε4 carriers. The slowing of tau accumulation was more prominent in individuals who achieved complete Aβ clearance and in brain regions affected later in the disease progression. The remarkable clearance of amyloid plaques is attributed to the exclusive presence of AβpE within cerebral Aβ plaques[202]. The specific targeting of plaques by the antibody is expected to minimize the risk of ARIA by not affecting normal neurons. However, the occurrence of ARIA was significantly higher in the treatment group compared to the placebo group (ARIA-E:26.7% *vs.* 0.8%; ARIA-H: 8.4% *vs.* 3.2%). Furthermore, 7.6% of participants in the treatment group experienced infusion-related reactions, whereas no such reactions were observed in the placebo group. Approximately 90% of the participants treated with donanemab tested positive for antidrug antibodies[200].

Recently, the results for the phase III trial (TRAILBLAZER-ALZ2)[203] of donanemab were released, in which the treatment group demonstrated a significant slowing in cognitive decline (35.1% difference on iADRS measures in the low/medium tau population and 22.3% in the overall population) and showed improvements in all secondary clinical endpoints. The incidence of ARIA in the study was higher in the treatment group (ARIA-E:24.0% donanemab *vs.* 2.1% placebo and ARIA-H: 31.4% donanemab *vs.* 13.6% placebo). A series of trials are underway to explore its effect and safety in diverse populations[204-207].

Following donanemab, remternetug (LY3372993) is another monoclonal antibody targeting AβpE[208]. This agent is designed to share similar properties with donanemab while addressing some of the safety concerns related to antidrug antibodies and infusion reactions reported in donanemab[209]. Investigation of the agent started with a phase I trial in 2018 but was prematurely terminated for undisclosed reasons[210]. Another phase I trial[211] was initiated in 2020 involving multiple escalating doses(250-2,800 mg) in healthy subjects and participants with MCI or AD. Interim analysis[209] (41 participants) of the trial showed a dose-dependent reduction in amyloid plaque, with all participants in the 2,800 mg group dropping below the amyloid positivity threshold within three months. Although safety data remained blind, the study reported 10 cases of ARIA-E and 7 cases of ARIA-H, with no apparent connection to the dosage. No antidrug antibodies or systemic infusion reactions were detected. A phase III trial of remternetug (TRAILRUNNER-ALZ 1)[212] is ongoing to evaluate its safety, tolerability, and efficacy on participants with early symptomatic AD and is expected to be completed in 2025. Similarly, another monoclonal antibody, ABBV-916, also targets AβpE and is recently under investigation in a phase II trial[178].

Gantenerumab is a fully human IgG1 monoclonal antibody that targets both the amino-terminal and central regions of Aβ[213]. Gantenerumab preferentially binds to aggregated Aβ, with limited affinity to soluble Aβ. The antibody works by activating microglial phagocytosis and degrading the plaque[213-215]. Despite undergoing development for over a decade and being tested in 9 clinical trials involving 4,135 participants, gantenerumab has not demonstrated significant clinical benefits [214]. In November 2022, the results of the GRADUATE 1 and 2 trials[216-218] were released, revealing that gantenerumab missed its primary endpoint of a 20% reduction in clinical disease progression. The pooled analysis demonstrated an approximately 8% slowing of clinical decline by gantenerumab. Biomarkers of tau pathology and neurodegeneration favored gantenerumab over placebo, suggesting its potential for modifying the disease. However, further results demonstrated that gantenerumab cleared less plaque (46.8-57.6 centiloids at week

116) and had fewer participants achieving Aβ negativity on PET scans compared to previous trials. The incidence of ARIA was relatively high (ARIA-E: 23.9%-25.8% *vs.* 1.7%-3.8%; ARIA-H: 22.0%-23.7% *vs.* 12.2%-12.4%). Notably, gantenerumab was assessed in a phase II/III trial conducted by the Dominantly Inherited Alzheimer Network Trials Unit (DIAN-TU)[219]. The trial aims to prevent dementia in individuals at risk for autosomal-dominant AD. During the trial, gantenerumab dosage was increased by fivefold. However, the completed trial in November 2019 did not achieve its primary endpoint. Given the dismal failure of the agent, studies of gantenerumab have been discontinued.

Several other monoclonal antibodies targeting Aβ did not show promising results. Bapineuzumab was the first passive immunotherapy to be tested in late-phase clinical trials for mild to moderate AD. It binds to Aβ oligomers and monomers with similar affinity[220,221]. While it showed a reduction in Aβ accumulation in AD participants based on PET scans, the clinical trials did not demonstrate significant changes in CSF Aβ levels or meaningful clinical benefits for participants with mild to moderate AD[222-225]. Further, bapineuzumab raised safety concerns due to an increased incidence of ARIA at higher doses[222,226,227]. Consequently, further research on bapineuzumab was discontinued.

Solanezumab is a humanized IgG1 monoclonal antibody that targets the central region of Aβ (Aβ16-24). It works by stabilizing Aβ monomers and preventing the formation of Aβ oligomers, but it does not target fibrils[228,229]. Solanezumab demonstrated a safer profile with a lower frequency of ARIA occurrence[230-235]. However, it did not show significant improvements in cognitive and functional outcomes in phase III trials in mild or mild to moderate AD, including the aforementioned phase II/III trial conducted by DIAN-TU[219,236-238]. Recently updated results from the A4 study showed solanezumab failed to slow cognitive decline in preclinical AD or reduce the risk of progression to symptomatic AD[239,240].

Crenezumab, an IgG4 antibody, targets the mid-region of Aβ and can bind to various forms of Aβ, including monomers, oligomers, fibrils, and plaques, particularly oligomers[241-244]. It reduces the activation of microglia and minimizes adverse effects such as vasogenic edema and brain microhemorrhage[243,245]. Clinical trials showed that crenezumab reduced CSF levels of Aβ oligomers[246] and increased CSF Aβ42[247]. However, larger trials were discontinued as interim analysis indicated that cognitive decline was unlikely to be slowed, and the drug was unlikely to meet its primary endpoint[248]. API Colombian prevention trial[249-251] results, released in June 2022, were negative on the primary and secondary endpoints but showed positive trends for crenezumab. Crenezumab showed a 20% decline slowing in primary outcomes with variability, and some measures including CDR-SB, FDG-PET, CSF and plasma biomarkers had a trend towards improvement but did not reach statistical significance. Therefore, the development of crenezumab has been stopped.

RNA-BASED THERAPIES

The rationale behind inhibiting APP expression for AD stems from its ability to decrease the amount of available APP for processing, thereby reducing the production of Aβ peptides. This can be achieved through a gene-silencing technique that employs intrathecal injection of small interfering RNA (siRNA) designed to target *APP* mRNA[252]. ALN-APP, the first siRNA therapy tested for CNS diseases, combines synthetic siRNA with a lipophilic conjugate called 2'-O-hexadecyl (C16), which enhances its ability to penetrate the CNS[253,254]. In nonhuman primate studies, a single intrathecal injection of ALN-APP reduced *APP* mRNA in the spinal cord and brain, resulting in a 75% decrease in sAPPα and sAPPβ levels. These reductions persisted for 2-3 months, returning to normal after 9 months. In an APP transgenic mouse model for AD, ALN-APP treatment resulted in a 50% decrease in *APP* mRNA and sAPPα, along with a reduction in Aβ40 deposition and inflammation. Furthermore, it normalized glutamate levels and improved behavioral outcomes[254]. A

phase 1 trial of ALN-APP[255] is ongoing on participants with MCI or early-onset AD to evaluate its safety, tolerability, pharmacokinetics, and pharmacodynamics. In April 2023, preliminary results[256] from single-dose data on 20 participants were released, indicating that ALN-APP dose-dependently reduced CSF sAPPα and sAPPβ, with the highest dose achieving a median reduction of over 70% in both biomarkers for at least three months. Adverse events were mild to moderate. Multiple-dose evaluation is on hold in the US but approved in Canada.

CURRENT CHALLENGES AND FUTURE DIRECTIONS OF Aβ-TARGETED THERAPIES

In recent years, substantial progress has been made in developing Aβ-targeted therapies for AD. Although previous studies have largely not led to success, valuable experiences have been learned and used to optimize the following research.

Numbers of negative clinical trials with Aβ-targeted therapy for AD have led to the questioning of this long-held hypothesis. Many studies propose that the accumulation of Aβ may be a downstream phenomenon or a compensatory protective mechanism, rather than the central trigger for the onset of the disease[257-259]. AD is a complicated disease with many unanswered questions. Further studies are warranted to uncover the pathophysiology of AD and to find novel effective therapeutic targets for the disease. The development of effective therapies is hampered by a lack of appropriate models. AD is a disease specific to humans, and no animal model can fully recapitulate the manifestations and mechanisms of the disease[260], which may explain why most of the encouraging results in animal models fail to be replicated in clinical trials. One key to bridging the gap is developing more reliable translational animal models to better explicate the disease.

For Aβ-targeted strategies, the correlation between Aβ clearance and clinical benefit is undetermined. Pooled evidence from clinical trials suggests that Aβ reduction strategies do not substantially enhance cognition[261], while some researchers insisted that complete clearance of plaques is necessary for the brain to respond gradually[262,263]. In this context, early initiating treatment might be important for Aβ reduction strategies to be effective[264,265]. Most trials recruit participants with mild to moderate AD, in which the disease could already be too advanced since neuropathological changes of AD have occurred decades before clinical manifestations[266]. Alternatively, recruiting asymptomatic participants with causal mutations of familial AD may be a future trend to meet the full potential of therapies as a preventative treatment, as shown in the DIAN-TU[267].

Many Aβ-targeted therapies with distinct rationales have exhibited enhanced efficacy among *APOE* ε4 carriers, suggesting that *APOE* ε4 may play a pivotal role in Aβ-targeted therapies, as evidenced by a pooled analysis[268]. Several hypotheses have been postulated to explain the phenomenon. First, the greater treatment response in carriers could be ascribed to the imbalance in the occurrence of ARIA-E and potential functional unblinding[269]. Additionally, the more favorable therapeutic outcomes observed among *APOE* ε4 carriers could be partially attributed to accelerated decline within this subgroup. However, the pooled analysis revealed that *APOE* ε4 non-carriers displayed a non-significantly faster rate of clinical progression[268]. Furthermore, differential reduction of APOE-mediated tau pathology between carriers and non-carriers may account for the disparate effects observed in Aβ-targeted therapies, given the association of *APOE* ε4 with tau pathology[268]. Moreover, *APOE* ε4 non-carriers may have additional coexisting pathologies[270], or their cognitive impairment may be influenced to a lesser extent by the underlying amyloidosis[268]. To sum up, the mechanisms underlying the phenomenon are undetermined and these hypotheses warrant further focused investigations.

In addition to Aβ, other factors also contribute to the pathophysiology of AD and are potential targets for disease-modifying therapies, including tau, neuroinflammation, and metabolism[7]. Furthermore, tau pathology is demonstrated to be more correlated with cognitive decline and clinical progression than Aβ[271]. As AD is a multifactorial disorder with various types of interrelated neuropathology[6], single-targeted agents might be insufficient to delay progression. Combined treatment and multitargeted agents have gained more attention in recent years and might be a rational direction. Multitargeted pharmacotherapies under clinical investigation for AD include Ginkgo biloba[272] and AD-35[273]. The DIAN-TU has initiated a concurrent trial[274] combining anti-Aβ and anti-tau therapies. In this study, 168 individuals with familial AD mutations will receive lecanemab, with half of them also receiving the anti-tau antibody E2814, while the other half will receive a placebo.

Passive anti-Aβ immunotherapy is currently regarded as the most promising direction for anti-Aβ treatment. However, there remain many pressing issues that must be addressed to fully harness its potential. Firstly, it is still debatable which form, isoform, or epitope is appropriate to target for clearance. Growing evidence indicates that soluble Aβ oligomers, rather than fibrillary aggregates, are more neurotoxic and better associated with AD clinical symptoms[13,275-277]. Research has demonstrated that selective antibodies targeting soluble Aβ oligomers can block synaptotoxicity and restore memory deficits in animal models[13]. However, some researchers have raised concerns regarding the therapeutic role of Aβ oligomer-specific antibodies, suggesting that they might increase the toxicity of Aβ oligomers[278]. Apart from Aβ40 and Aβ42, additional Aβ isoforms, including pyroglutamate Aβ3-42 and Aβ4-42, have been recognized as significant factors in the development of AD[199]. Treatment targeting these isoforms include donanemab, remternetug, ABBV-916, and ACI-24, and some of them have shown promising outcomes in clinical trials. Novel findings from the pseudo β-hairpin conformation of the N-terminal region of pyroglutamate Aβ monomers also have implications for active and passive immunization strategies in AD[279]. Additionally, ARIA is a common adverse effect of anti-Aβ monoclonal antibody treatment. The exact biological mechanisms underlying ARIA are still not fully understood, but they may result from increased permeability of cerebrovascular structures due to enhanced clearance of Aβ plaques, a saturation of perivascular drainage, direct interaction of monoclonal antibodies with vascular Aβ deposits, and weakening of blood vessel walls[263]. Although most cases of ARIA are transient, it is essential to closely monitor participants for ARIA, particularly after treatment initiation, and consider additional MRI scans if they develop new symptoms suggestive of ARIA[227,280]. Brain atrophy following anti-Aβ immunotherapies has also been overlooked[281]. Considering their association with cognitive decline and AD pathology, it is important to ensure that these changes do not indicate worsening neurodegeneration after treatment. Finally, as these recent trials use biomarkers for enrollment, monitoring, and outcome assessment, healthcare systems must be prepared for this disease-modifying treatment. Blood-based biomarkers appear to be a low-invasive and cost-effective approach for large-scale screening and primary care centers[282]. However, further validation by larger and more diverse populations and the establishment of relevant cut-offs are necessary before they can be implemented into regular usage.

With evolving experience, the development strategies and recommendations for the appropriate use of these Aβ-targeted therapies are continuously updated[184,196]. Recent approvals of the two monoclonal antibodies as the first disease-modifying therapy for AD are encouraging. However, it is too early to draw a conclusion and there is still a long way to go. In conclusion, we expect many results from trials in the next few years. With further research, effective treatment and prevention for AD are possible and anticipated.

DECLARATION

Authors' contributions

Study conception and design: Jia L, Cai H

Literature search and review: Cai H, Fu X, Quan S, Ren Z, Chu C

Draft manuscript and preparation: Cai H, Fu X, Quan S, Ren Z

Review and revision of paper: Jia L, Cai H, Chu C

Approval of final version: Jia L, Cai H, Fu X, Quan S, Ren Z, Chu C

Availability of data and materials

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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