

REVIEW

Open Access



Second-generation anti-amyloid monoclonal antibodies for Alzheimer's disease: current landscape and future perspectives

Byeong-Hyeon Kim^{1†}, Sujin Kim^{1,2†}, Yunkwon Nam^{1†}, Yong Ho Park^{1†}, Seong Min Shin^{1†} and Minho Moon^{1,2*} 

Abstract

Alzheimer's disease (AD) is the most common type of dementia. Monoclonal antibodies (MABs) serve as a promising therapeutic approach for AD by selectively targeting key pathogenic factors, such as amyloid- β (A β) peptide, tau protein, and neuroinflammation. Specifically, based on their efficacy in removing A β plaques from the brains of patients with AD, the U.S. Food and Drug Administration has approved three anti-amyloid MABs, aducanumab (Aduhelm[®]), lecanemab (Leqembi[®]), and donanemab (Kisunla[™]). Notably, lecanemab received traditional approval after demonstrating clinical benefit, supporting the A β cascade hypothesis. These MABs targeting A β are categorized based on their affinity to diverse conformational features of A β , including monomer, fibril, protofibril, and plaque forms of A β as well as pyroglutamate A β . First-generation MABs targeting the non-toxic monomeric A β , such as solanezumab, bapineuzumab, and crenezumab, failed to demonstrate clinical benefit for AD in clinical trials. In contrast, second-generation MABs, including aducanumab, lecanemab, donanemab, and gantenerumab directed against pathogenic A β species and aggregates have shown that reducing A β deposition can be an effective strategy to slow cognitive impairment in AD. In this review, we provide a comprehensive overview of the current status, mechanisms, outcomes, and limitations of second-generation MABs for the clinical treatment of AD. Moreover, we discuss the perspectives and future directions of anti-amyloid MABs in the treatment of AD.

Keywords Alzheimer's disease, Aducanumab, Lecanemab, Donanemab, Gantenerumab, Amyloid-related imaging abnormalities

Background

Monoclonal antibodies (MABs) were first developed in 1975 using the hybridoma technology invented by Köhler and Milstein [1], and have been essential in the diagnosis and treatment of debilitating diseases [2–4]. Numerous

MABs have been approved by the U.S. Food and Drug Administration (FDA) to treat a variety of diseases, starting with muromonab CD3 (Orthoclone OKT3[®]), a MAB that inhibits acute rejection in organ transplants [5]. Several MABs for Alzheimer's disease (AD) that target amyloid- β (A β) and tau, as well as inflammation, are currently in clinical trials [6]. Importantly, under the accelerated approval program, the FDA approved anti-amyloid MABs, such as aducanumab (Aduhelm[®]), lecanemab (Leqembi[®]), and donanemab (Kisunla[™]) based on surrogate markers of A β plaque removal in the brains of patients with AD [7, 8]. Lecanemab recently became the first disease-modifying drug to transition from accelerated approval to traditional approval, following the FDA's

[†]Byeong-Hyeon Kim, Sujin Kim, Yunkwon Nam, Yong Ho Park, and Seong Min Shin have contributed equally to this review.

*Correspondence:

Minho Moon

hominmoon@konyang.ac.kr

¹ Department of Biochemistry, College of Medicine, Konyang University, 158, Gwanjeodong-Ro Seo-Gu, Daejeon 35365, Republic of Korea

² Research Institute for Dementia Science, Konyang University, 158, Gwanjeodong-Ro Seo-Gu, Daejeon 35365, Republic of Korea



determination that it has demonstrated clinical benefits [9]. These findings provide valuable evidence upholding the A β cascade hypothesis that the aggregation and misfolding of A β leads to multiple pathological phenotypes and clinical dysfunctions in AD [10, 11].

Anti-amyloid MABs can be categorized based on affinity, with some showing a preference for binding to monomeric A β and others to fibrils. Unfortunately, the first-generation anti-amyloid MABs failed in clinical trials as they demonstrated no significant clinical benefit [12–15]. Notably, solanezumab failed to remove A β plaques or inhibit A β accumulation in all patients with preclinical AD to moderate AD in four clinical trials [16–18]. These results suggest that targeting monomeric A β may not be effective in the treatment of AD. The recently developed second-generation anti-amyloid MABs, such as aducanumab, lecanemab, donanemab, and gantenerumab, target the toxic form of A β aggregates (e.g., oligomers, protofibrils, fibrils, and plaques) [19]. These second-generation anti-amyloid MABs have been shown to considerably reduce A β deposition, as detected using amyloid positron emission tomography (PET), and slow cognitive decline in clinical trials [20–22].

In this review, we provide a comprehensive overview of recent advances in the clinical trials of four second-generation anti-amyloid MABs: aducanumab,

lecanemab, donanemab, and gantenerumab. In addition, we comparatively analyze their mechanisms and limitations. Finally, we explore future directions for innovation and novel technologies required to develop the next generation of anti-amyloid MABs.

Clinical trials of second-generation anti-amyloid monoclonal antibodies in AD

Currently, most treatments for AD are symptomatic and do not stop or reverse the progression of AD. Aducanumab, lecanemab, and donanemab are the second-generation anti-amyloid MABs currently approved for clinical use as disease-modifying treatments for AD [21, 23]. Disease-modifying treatments that target A β are becoming more popular due to the growing focus on targeting the early stages of disease. In the early stages of AD, disruption of continuous A β production and efficient clearance leads to the toxic aggregation of A β into misfolded aggregates. Particularly, A β aggregates contribute to neurodegeneration and neuroinflammation in the AD brain [24]. Recently, other disease-modifying treatments targeting A β , such as gantenerumab, have undergone clinical trials with the aim of treating AD (Fig. 1).

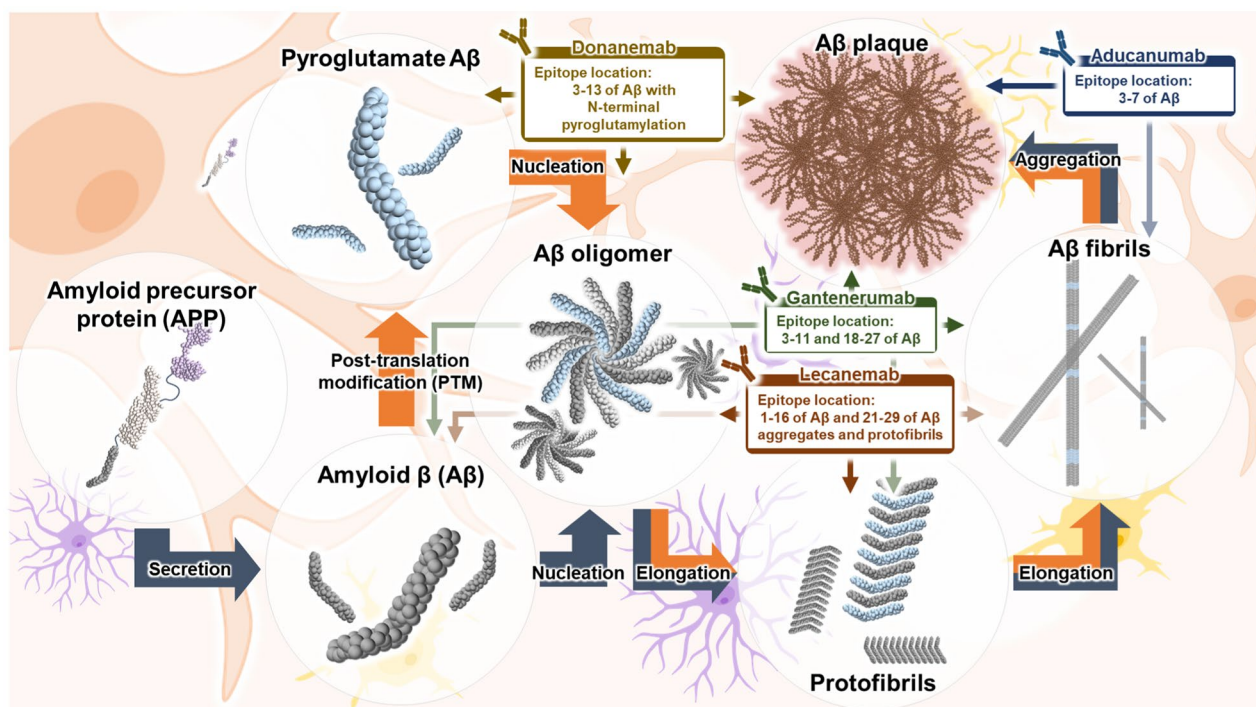


Fig. 1 Second-generation anti-amyloid monoclonal antibodies (MABs) target different forms of A β . Aducanumab targets A β fibrils and plaques. Lecanemab binds to soluble A β oligomers, protofibrils, fibrils, and plaques. Gantenerumab targets soluble A β oligomers, protofibrils, fibrils and plaques. Donanemab specifically targets the N-terminus of pyroglutamate present in A β plaques

Clinical trials of aducanumab

Aducanumab was developed from the memory B cells of peripheral blood lymphocytes obtained from healthy elderly individuals without cognitive decline and elderly individuals with cognitive impairment experiencing an unusually gradual decline in cognitive function [25]. Aducanumab mainly targets A β plaques by recognizing the amino acids 3–7 of A β (Fig. 1) [26]. A single ascending dose study (NCT01397539) of aducanumab, a phase 1 clinical trial, was conducted in 53 patients with mild-to-moderate AD. This study aimed to evaluate the pharmacokinetics, safety, and tolerability of different doses of aducanumab administered via a single intravenous injection in patients with AD [27]. The findings of this clinical trial demonstrated that at 30 mg/kg, patients exhibited excellent tolerance to aducanumab without experiencing severe or serious adverse effects (SAEs), whereas all patients experienced SAEs such as symptomatic amyloid-related imaging abnormalities (ARIA) at a dose of 60 mg/kg [27]. Subsequently, a multiple-dose PRIME study (NCT01677572) of aducanumab, a phase 1 clinical trial, was performed in 197 patients with prodromal-to-mild AD. The study demonstrated that the administration of aducanumab reduced A β deposition in the brains of patients with prodromal-to-mild AD in a dose- and time-dependent manner. Moreover, treatment with aducanumab delayed cognitive decline in a dose-dependent manner, as measured by the Clinical Dementia Rating-Sum of Boxes (CDR-SB) and Mini-Mental State Examination (MMSE) scores. However, ARIA-oedema/effusion (ARIA-E) abnormalities were found in groups administered with 1, 3, 6, or 10 mg/kg of aducanumab but not in the placebo group. ARIA-E particularly occurred more frequently in apolipoprotein E (*APOE*) ϵ 4 carriers than in non-carriers [25]. Based on the accumulated clinical results, the ENGAGE (NCT02477800) and EMERGE (NCT02484547) studies established the following clinical criteria: inclusion of patients with A β in the brain detected with amyloid PET, inclusion of patients with mild cognitive impairment (MCI), and inclusion of patients who consented to *APOE* genetic analysis (Table 1) [28]. The ENGAGE and EMERGE studies were phase 3 clinical trials in which low (3 or 6 mg/kg) and high (10 mg/kg) doses of aducanumab were administered to 1653 and 1643 patients with MCI due to AD and mild AD, respectively. In the ENGAGE study, aducanumab treatment did not meet the criteria for either primary or secondary clinical endpoints. In contrast, the results of the EMERGE study revealed significant changes in both primary and secondary endpoints in the high-dose aducanumab group; additionally, in the high-dose aducanumab group, the primary and secondary endpoints, such as CDR-SB, MMSE, Alzheimer's Disease

Assessment Scale-Cognitive Subscale (ADAS-Cog) 13, and Alzheimer's Disease Cooperative Study-Activities of Daily Living Scale for Mild Cognitive Impairment (ADCS-ADL-MCI) scores, decreased by 22%, 18%, 27%, and 40%, respectively. Furthermore, high-dose administration of aducanumab reduced brain A β accumulation by 59% and 71% from the baseline in ENGAGE and EMERGE studies, respectively [20]. The difference in results between ENGAGE and EMERGE may be due to the differences in the administration of high-dose aducanumab. In 2017, Biogen modified the protocol to increase the dose of aducanumab to 10 mg/kg for *APOE*4-positive patients. As a result of the protocol change, the number of participants in the high-dose ENGAGE study was smaller than that in the high-dose EMERGE study, and consequently, a smaller proportion of participants received all 14 doses of aducanumab at 10 mg/kg [29]. Specifically, 22% of ENGAGE participants received 14 doses at 10 mg/kg, while 29% of EMERGE participants received 14 doses at 10 mg/kg [30]. Moreover, ENGAGE participants had a shorter average duration of exposure to the 10 mg/kg dose because of the timing of the protocol change. These differences in cumulative exposure to the high-dose aducanumab may have contributed to the differences in efficacy outcomes observed between the two studies. Notably, aducanumab has been successful in slowing cognitive decline and reducing A β deposition in patients with AD and has received accelerated approval from the FDA based on the evaluation of surrogate markers for the reduction of amyloid plaques in the brain [8]. However, the most common SAE observed in the ENGAGE and EMERGE studies was ARIA-E, with an incidence of 35.2% in the high-dose aducanumab group [31]. Furthermore, the slowing effects of aducanumab on cognitive decline are still debated [32]. To verify the clinical benefits of aducanumab, the ENVISION study (NCT05310071) recently terminated a phase 3b/4 clinical trial in 1512 patients with MCI due to AD and mild AD.

Clinical trials of lecanemab

Lecanemab, an MAB that specifically detects the protofibril forms of A β , was developed from mouse mAb158 [33]. Lecanemab targets A β protofibrils by recognizing amino acids 1–16 of A β and 21–29 of A β aggregate/protofibrils. Since these epitopes are also exposed in monomers, oligomers, and fibrils, lecanemab binds weakly to them (Fig. 1) [26]. In a preclinical study, short- and long-term administration of mAb158, the murine version of lecanemab (BAN2401), reduced the levels of A β protofibrils in the brains and cerebrospinal fluid (CSF) of Tg-ArcSwe mice, a transgenic mouse line expressing the Swedish and Arctic mutant of human amyloid-beta precursor protein (*APP*) gene [34]. A single- and

Table 1 Comparison of aducanumab, lecanemab, donanemab, and gantenerumab in phase 3 clinical trials

| Anti-amyloid MABs | Aducanumab [20] | | | Lecanemab [21] | | Donanemab [22] | | Gantenerumab [53] | |
|---------------------|---------------------|---|---|--|--|---|---|-------------------|----------|
| | EMERGE | | ENGAGE | GRADUATE I | | GRADUATE II | | | |
| Criteria | AD stage | Early AD | Early AD | Early AD | Early AD | Early AD | Early AD | Early AD | Early AD |
| | Key characteristics | 50 to 85 years old, Presence of amyloid pathology, Stage 3 and 4 patients as described in the FDA 2018 Guidance, MMSE score: 24–30, CDR global score: 0.5 | | 50 to 90 years old, Presence of amyloid pathology, Objective impairment in episodic memory (WMS-IV LMII) | 60 to 85 years old, Presence of amyloid pathology (≥ 37 CL), Presence of tau pathology (> 1.10 SUVr), MMSE score: 20–28 | 50 to 90 years old, Presence of amyloid pathology, Ratio of phosphorylated tau181 to A β_{42} in CSF: 0.024 or higher, Free and Cued Selective Reminding Test (fCSRT): 0.67 or lower, MMSE score: 22–30, CDR global score: 0.5 or 1 | 50 to 90 years old, Presence of amyloid pathology, Ratio of phosphorylated tau181 to A β_{42} in CSF: 0.024 or higher, Free and Cued Selective Reminding Test (fCSRT): 0.67 or lower, MMSE score: 22–30, CDR global score: 0.5 or 1 | | |
| Endpoints | Primary endpoint | CDR-SB | | CDR-SB | iADRS | | CDR-SB | | |
| | | Low dose [Diff vs placebo]: –0.26 (15%), High dose [Diff vs placebo]: –0.39 (22%) | Low dose [Diff vs placebo]: –0.18 (12%), High dose [Diff vs placebo]: –0.03 (2%) | Diff vs placebo: –0.45 (27%) | Low/medium tau population [Diff vs placebo]: –0.68 (36%), Combined population [Diff vs placebo]: –0.67 (29%) | Diff vs placebo: –0.31 (8%) | Diff vs placebo: –0.19 (6%) | | |
| | Secondary endpoints | MMSE | | ADAS-Cog14 | ADCS-iADL | ADAS-Cog13 | | | |
| | | Low dose [Diff vs placebo]: –0.1 (3%), High dose [Diff vs placebo]: –0.6 (18%) | Low dose [Diff vs placebo]: –0.2 (6%), High dose [Diff vs placebo]: –0.1 (3%) | Diff vs placebo: –1.44 (26%) | Low/medium tau population [Diff vs placebo]: 1.83 (40%), Combined population [Diff vs placebo]: 1.70 (28%) | Diff vs placebo: –1.25 (13%) | Diff vs placebo: –1.28 (16%) | | |
| | | ADAS-Cog13 | | ADCOMS | ADCS-Cog13 | ADCS-ADL | | | |
| | | Low dose [Diff vs placebo]: –0.70 (14%), High dose [Diff vs placebo]: –1.40 (27%) | Low dose [Diff vs placebo]: –0.58 (11%), High dose [Diff vs placebo]: –0.59 (11%) | Diff vs placebo: –0.05 (24%) | Low/medium tau population [Diff vs placebo]: –1.52 (32%), Combined population [Diff vs placebo]: –1.33 (20%) | Diff vs placebo: 1.11 (9%) | Diff vs placebo: 0.82 (9%) | | |
| | | ADCS-ADL-MCI | | ADCS-ADL-MCI | MMSE | FAQ | | | |
| | | Low dose [Diff vs placebo]: 0.7 (16%), High dose [Diff vs placebo]: 1.7 (40%) | Low dose [Diff vs placebo]: 0.7 (18%), High dose [Diff vs placebo]: 0.7 (18%) | Diff vs placebo: –2.0 (37%) | Low/medium tau population [Diff vs placebo]: 0.48 (23%), Combined population [Diff vs placebo]: 0.47 (16%) | Diff vs placebo: –0.86 (11%) | Diff vs placebo: –0.86 (13%) | | |
| Biomarker endpoints | | Amyloid PET | | Amyloid PET | Amyloid PET | Amyloid PET | Amyloid PET | | |
| | | | | | | | | | |

Table 1 (continued)

| Anti-amyloid MABs | Aducanumab [20] | | Lecanemab [21] | | Donanemab [22] | | Gantenerumab [53] | |
|-------------------|--|--|---|--|--|--|---|---|
| | EMERGE | ENGAGE | | | | | GRADUATE I | GRADUATE II |
| Adverse effects | Low dose [Diff vs placebo]: –42 CL, High dose [Diff vs placebo]: –64 CL | Low dose [Diff vs placebo]: –39 CL, High dose [Diff vs placebo]: –54 CL | Diff vs placebo: –59.12 CL | | Low/medium tau population [Diff vs placebo]: –88.2 CL, Combined population [Diff vs placebo]: –86.3 CL | | Diff vs placebo: –47.5 CL, *Expected result at 84 weeks | Diff vs placebo: –41.5 CL, *Expected result at 84 weeks |
| | | | CSF A β_{42} | | Tau PET | | | |
| | | | Increased in lecanemab group | | | | | |
| | | | Plasma A β_{42} /A β_{40} ratio | | | | | |
| | | | Increased in lecanemab group | | No significant difference | | Diff vs placebo: –66.4 CL, * Result at 116 weeks | Diff vs placebo: –56.5 CL, * Result at 116 weeks |
| | | | CSF t-tau | | | | | |
| | | | Decreased in lecanemab group | | | | | |
| | | | CSF p-tau181 | | Plasma p-tau271 | | | |
| | | | Decreased in lecanemab group | | Decreased in donanemab group | | Tau PET | |
| | | | Plasma p-tau181 | | | | No significant difference | |
| Adverse effects | Low dose [Diff vs placebo]: NA, High dose [Diff vs placebo]: –0.667 | Low dose [Diff vs placebo]: NA, High dose [Diff vs placebo]: –0.777 | Decreased in lecanemab group | | | | | |
| | | | CSF NfL | | Plasma neurofilament light | | | |
| | | | Inconsistent results | | Inconsistent results | | | |
| | | | Plasma NfL | | | | | |
| | | | Decreased in lecanemab group | | | | | |
| | | | CSF neurogranin | | Plasma GFAP | | | |
| | | | Increased in lecanemab group | | Decreased in donanemab group | | | |
| | | | Plasma GFAP | | | | | |
| | | | Decreased in lecanemab group | | | | | |
| | | | 12.6%, (Symptomatic: 2.8%) | | 24% (Symptomatic: 6.1%) | | 23.9% (Symptomatic: 5.2%) | 25.8% (Symptomatic: 4.8%) |
| Adverse effects | Low dose: 26%, High dose: 35% Microhemorrhage | Low dose: 26%, High dose: 36% Microhemorrhage | Microhemorrhage | | Microhemorrhage | | 23.7% | 22.0% |
| | | | | | | | | |

Table 1 (continued)

| Anti-amyloid MABs | Aducanumab [20] | | Lecanemab [21] | | Donanemab [22] | | Gantenerumab [53] | |
|----------------------|-------------------------------|-------------------------------|-----------------------|--|-----------------------|--|-------------------|--|
| | EMERGE | ENGAGE | GRADUATE I | | GRADUATE II | | | |
| | Low dose: 10%, High dose: 13% | Low dose: 10%, High dose: 13% | 17.3% | | 26.8% | | | |
| | Superficial siderosis | | Superficial siderosis | | Superficial siderosis | | | |
| | Low dose: 10%, High dose: 13% | Low dose: 9%, High dose: 16% | 5.6% | | 15.7% | | | |

CL, centiloid scale; Diff, difference; Early AD means mild cognitive impairment due to AD Mild AD; NA, not available; MMSE, mini-mental state examination; CDR, clinical dementia rating; CDR-SB, clinical dementia rating-sum of boxes; ADAS-Cog 13, Alzheimer's disease assessment scale-cognitive subscale 13; ADCS-ADL-MCI, Alzheimer's disease cooperative study activities of daily living-mild cognitive impairment scale; PET, positron emission tomography; iADRS, integrated Alzheimer's disease rating scale; ADCS-iADL, Alzheimer's disease cooperative study-instrumental activities of daily living; ADAS-Cog 14, Alzheimer's disease assessment scale-cognitive subscale 14; ADCOMS, Alzheimer's disease composite score; CSF, cerebral spinal fluid; NfL, neurofilament light chain; GFAP, glial fibrillary acidic protein

multiple-ascending dose study (NCT01230853) of lecanemab, a phase 1 clinical trial, was performed in 80 patients with mild-to-moderate AD. The phase 1 clinical trial demonstrated the safety and tolerability of lecanemab at sequentially increasing doses, and that it was well-tolerated in all participating treatment groups. SAEs rarely occurred due to drug administration; in particular, the incidence of ARIAs was similar between the lecanemab and placebo groups [35]. Furthermore, Study 201 (NCT01767311), a phase 2 clinical trial, evaluated the safety, tolerability, and efficacy of lecanemab in 856 patients with MCI due to AD or mild AD. In this study, only patients with confirmed evidence of A β using PET or CSF testing were eligible for the clinical trial. The primary endpoint for the highest dose of lecanemab administration (twice-monthly, 10 mg/kg) was evaluated through Bayesian analysis of the Alzheimer's Disease Composite Score (ADCOMS) at 12 months. The calculated probability of showing more than 25% improvement compared with placebo was 64%, falling short of the 80% target. However, the secondary endpoints for the highest dose of lecanemab (twice-monthly 10 mg/kg) demonstrated even greater delay of cognitive deficits at 18 months, with a 30% improvement in ADCOMS, 47% improvement in ADAS-Cog14, and 26% improvement in CDR-SB compared with placebo. Moreover, regarding biomarkers, administration of lecanemab decreased the level of A β in the brain and the level of phosphorylated tau (p-tau) in the CSF. The incidence of ARIA-E with lecanemab was 9.9% in patients receiving the highest dose and 14.3% in patients who were *APOE* ϵ 4-carriers [36]. Based on the efficacy of lecanemab in clearing A β in the phase 2 clinical trial, the FDA approved lecanemab under the accelerated approval program [7]. A study predicting the long-term treatment with lecanemab using a disease simulation model based on the Study 201 reported that the longer the administration period of lecanemab, the better the clinical outcome [37]. This suggests that early initiation of lecanemab treatment has a potentially greater impact on the progression of AD. Finally, the Clarity AD study (NCT03887455) of lecanemab, a phase 3 clinical trial, was performed in 1906 patients with MCI due to AD and mild AD (Table 1). The primary endpoint, CDR-SB score, showed a 27% delay in clinical decline at 18 months in the lecanemab group compared with the placebo group. The secondary endpoints for lecanemab, including ADAS-Cog14, ADCOMS, and ADCS-ADL-MCI scores, exhibited 26%, 24%, and 37% slowing of cognitive impairment, respectively, in the lecanemab group compared with the placebo group. Furthermore, lecanemab reduced the levels of amyloid burden in the brain by 59.12 (77.92 \rightarrow 18.8) centiloids (CL) from baseline. In addition, the incidences of ARIA-E and

ARIA-haemosiderosis/microhaemorrhages (ARIA-H) in the lecanemab group were 12.6% (Symptomatic ARIA-E: 2.8%) and 17.3% (Symptomatic ARIA-H: 0.7%), respectively [21]. Based on the efficacy of lecanemab in slowing clinical deficits and clearing A β deposition in the phase 3 clinical trial, the FDA moved lecanemab to traditional approval [9].

Clinical trials of donanemab

Donanemab, developed from mouse mE8-IgG2a, recognizes amino acids 3–13 and specifically targets the N-terminal pyroglutamate of A β [24, 38]. While donanemab is often characterized as a plaque-specific antibody due to its binding to the N3pE epitope predominantly found in amyloid plaques, it has the potential to interact with various N3pE-containing A β species, including soluble, insoluble, and intracellular forms [39, 40]. The interaction between donanemab and various N3pE-containing A β species suggests that donanemab has the potential to contribute more comprehensively to A β reduction in AD (Fig. 1) [41–43]. Phase 1a (NCT01837641) and 1b (NCT02624778) clinical studies of donanemab have been completed in 100 and 61 patients with mild-to-moderate AD, respectively. Results from the phase 1 clinical trial of donanemab demonstrated that donanemab was well tolerated at both single and multiple doses [44]; however, ARIA was observed in one-quarter of patients receiving donanemab. Single administration of donanemab resulted in changes in amyloid PET only above the dose of 10 mg/kg, and repeated doses of 10 mg/kg and 20 mg/kg resulted in sustained reductions in the deposition of A β [44, 45]. Based on these results, the selected dose of donanemab was up to 1400 mg, approximately 20 mg/kg as used in the phase 2 clinical trial. The TRAILBLAZER-ALZ study (NCT03367403) on donanemab, a phase 2 clinical trial, was performed in 272 patients with early symptomatic AD and mild AD. The study only included patients with A β and tau depositions as detected using PET. Patients with low (standardized uptake value ratio [SUVR] < 1.10) or high (SUVR > 1.46) tau deposition were excluded from the TRAILBLAZER-ALZ study. Donanemab was intravenously administered to patients at 4-week intervals for 76 weeks (700 mg for the first three administrations and 1400 mg thereafter). The primary and secondary endpoints of donanemab treatment were assessed using the integrated Alzheimer's Disease Rating Scale (iADRS), CDR-SB, ADAS-Cog13, Alzheimer's Disease Cooperative Study-Instrumental Activities of Daily Living Inventory (ADCS-iADL), and MMSE scores. The primary outcome showed that there was a significant mean change difference of 3.25 in the iADRS score between the donanemab and the placebo groups. The secondary outcome showed mean changes

of -0.67 and -0.7 for CDR-SB, 1.83 and 1.70 for ADCS-iADL, and -1.52 and -1.33 for ADAS-Cog13 scores in the donanemab and the placebo groups, respectively. These results indicate a tendency toward delayed cognitive decline. Biomarker outcomes for donanemab showed a reduction in amyloid burden by 84.13 ($107.6 \rightarrow 23.47$) CL from baseline and a slight slowing of global tau load compared with placebo. Moreover, the group receiving donanemab had a higher incidence of ARIA-E and ARIA-H than the placebo group, whereas neither SEAs nor death was observed in the donanemab group [46, 47]. TRAILBLAZER-EXT (NCT04640077), a follow-up study to the TRAILBLAZER-ALZ study, is currently in progress. Unfortunately, the FDA denied the application for accelerated approval of donanemab based on phase 2 clinical trial data, as it did not meet the minimum requirement of 100 patients to have been treated with donanemab for at least 12 months. Finally, the TRAILBLAZER-ALZ 2 study (NCT04437511) of donanemab, a phase 3 clinical trial, was performed in 1800 patients with early symptomatic AD who had A β and tau pathologies (Table 1). The primary endpoint of the study, iADRS, exhibited a 35.1% delay in clinical decline at 72 weeks in the donanemab group compared with the placebo group. The secondary endpoints of donanemab administration, including CDR-SB, ADCS-iADL, ADAS-Cog13, and MMSE scores, also displayed a 37.0%, 39.9%, 32.4%, and 22.9% delayed cognitive decline, respectively, in the donanemab group compared with placebo. The level of amyloid plaques in the brain was reduced by 88.2 CL in the donanemab group compared to that in the placebo group. Notably, 80.1% of the patients treated with donanemab had reduced brain amyloid levels. In addition, the plasma levels of p-tau217 and glial fibrillary acidic protein (GFAP) in the donanemab group decreased by 39.3% and 21.3%, respectively, from baseline. The incidence of ARIA-E and ARIA-H in the donanemab group was 24.0% and 26.8%, respectively [22]. Additionally, the TRAILBLAZER-ALZ 3 study (NCT05026866) on the prophylactic effects of donanemab is currently being conducted in 3300 patients with plasma p-tau217 levels, consistent with early tau pathology, and without cognitive impairment. The TRAILBLAZER-ALZ 4 study (NCT05108922), a phase 3 clinical trial, is comparing the efficacy of donanemab and aducanumab. The TRAILBLAZER-ALZ 5 study (NCT05508789) is currently recruiting patients with early symptomatic AD to evaluate safety and efficacy of donanemab.

Clinical trials of gantenerumab

Gantenerumab is a novel human anti-A β MAB designed using HuCAL[®] phage display technology to specifically bind to A β fibrils [48]. Gantenerumab targets A β fibrils

by recognizing amino acids 3–11 and 18–27 of A β (Fig. 1) [48, 49]. In a preclinical study, intravenous injection of gantenerumab significantly reduced A β plaques in the hippocampus, cerebral cortex, and thalamus of PS2APP mice compared to the vehicle group [48]. A multiple-ascending-dose phase I study (NCT00531804) of gantenerumab was performed in 18 patients with mild AD. The patients received 2–7 infusions of intravenous gantenerumab at doses of 60 or 200 mg, or a placebo infusion every 4 weeks. Compared with the placebo group, the level of amyloid plaques in the cortical region was reduced by 15.6% in the group receiving 60 mg of gantenerumab and by 35.7% in the group receiving 200 mg of gantenerumab. Although amyloid plaque levels decreased with gantenerumab administration, two of the six patients in the group receiving 200 mg of gantenerumab developed neuroinflammation or ARIA in the left caudate nucleus and right temporal lobe with the highest level of amyloid reduction [49]. A subcutaneous formulation of gantenerumab (105 or 225 mg) was developed, and the SCarlet RoAD study, a phase 2 clinical trial, comprising 490 participants was initiated. Unfortunately, the SCarlet RoAD study was discontinued early as the target for changes in cognitive delay was not reached [50]. In between the termination of SCarlet RoAD and the initiation of phase 3 clinical trials, a collaborative study called DIAN-TU study (NCT04623242 and NCT01760005) was initiated. The study involved 52 participants with dominantly inherited AD in each treatment group who were assigned to receive either gantenerumab or solanezumab. ARIA-E was observed in 19.2% of patients in the gantenerumab group [17]. Following the termination of the SCarlet RoAD trial, the Marguerite RoAD trial (NCT02051608) evaluating gantenerumab in 389 participants diagnosed with mild AD entered phase 3 clinical testing. However, the Marguerite RoAD trial failed the futility analysis and was converted to an open-label extension (OLE) study. Gantenerumab administered at a high dose of 1200 mg in the SCarlet and Marguerite RoAD OLE studies reduced brain amyloid levels by an average of 59 CL as detected by florbetapir PET. Although one-third of the participants underwent ARIA-E, most were asymptomatic [51]. In addition, patients treated with gantenerumab continued to show reduced amyloid levels. At the end of the three-year SCarlet and Marguerite RoAD OLE study, 80% of the participants treated with gantenerumab showed a reduction in the amyloid burden, resulting in a transition to an amyloid-negative state [52]. Graduate I and II phase 3 clinical trials of gantenerumab efficacy and safety in 1053 and 975 participants with early AD (NCT03444870 and NCT03443973), respectively, failed to demonstrate a significant mean change in cognitive decline (-0.31 and -0.19 for CDR-SB from baseline) (Table 1) [53]. Interestingly, the administration of

gantenerumab resulted in the partial reduction of amyloid plaques compared to placebo at 116 weeks. Furthermore, gantenerumab treatment resulted in improvements in several soluble biomarkers of AD in the CSF, including reduced levels of total tau, p-tau181, and neurogranin [53]. The observation that gantenerumab reduced amyloid burden but did not improve cognitive function may be related to the characteristics of the patients enrolled in the study. The Graduate I and II clinical trials enrolled patients with MCI due to AD and mild AD who already had significant amyloid plaques in the brain. Moreover, gantenerumab was subcutaneously administered at gradually increasing concentrations, starting at 120 mg for the first 8 weeks, followed by 255 mg until week 20, 510 mg until week 32, and 1020 mg thereafter [53]. Notably, the clinical efficacy of anti-amyloid MABs is expected to be maximized by rapid reductions in brain amyloid levels [54]. Unfortunately, the initial low doses of gantenerumab, 120 and 255 mg through week 20, may have contributed to slower clearance of A β , potentially delaying the observation of clinical benefits. Future clinical trials of gantenerumab will need to address the challenges of optimizing dosing strategies and ensuring A β clearance to maximize therapeutic efficacy. The phase 3 Graduate I and II studies analyzed a phase 2 study (NCT04592341) evaluating the pharmacodynamic effects of short-term administration of gantenerumab in 192 patients with early AD and phase 3 studies (NCT04339413 and NCT04374253) evaluating the safety of long-term administration of gantenerumab. Furthermore, a new phase 3 clinical trial, the Skyline Study (NCT05256134), evaluating the efficacy and safety of gantenerumab in amyloid-positive, cognitively unimpaired patients at risk of early AD, is currently underway. In addition, a multiple-dose study (NCT01656525) of gantenerumab, a phase 1 trial with 28 participants, and the DIAN-TU study (NCT05552157), a phase 2/3 clinical trial with 220 participants, were discontinued. Future directions for the treatment of AD with MABs currently point to shuttling the transport of MABs across the blood–brain barrier (BBB) to increase target engagement. Trontinemab (NCT04639050), a gantenerumab conjugated to a human transferrin receptor 1 (TfR1)-directed Brainshuttle™ module, demonstrates significantly greater brain uptake than gantenerumab, with brain distribution coefficients reported to be sevenfold to 33-fold higher across various brain regions [55]; TfR1-mediated transcytosis is expected to alter the course of immunotherapy for AD.

A comprehensive analysis of second-generation anti-amyloid MABs in AD

Comparison of aducanumab, lecanemab, donanemab, and gantenerumab in phase 3 clinical trials

Phase 3 clinical trials of aducanumab, lecanemab, donanemab, and gantenerumab targeted the same cohort of participants, i.e., patients diagnosed with MCI due to AD or mild AD (Table 1). The criteria of AD diagnosis are based on the threshold hypothesis that removal of A β before amyloid pathology has reached a certain threshold will inhibit the spread of tau pathology [54]. The “CL” scale was developed to standardize amyloid PET imaging measurements. CL represents that the average value is zero in “high certainty” amyloid-negative patients and an average of 100 in “typical” patients with AD. The ideal range for CL in patients with AD participating in clinical trials of anti-amyloid MABs has been suggested to be typically between 20 and 67 CL [54]. Inclusion criteria for clinical trials of aducanumab were participants aged 50–85 years with (1) an MMSE score between 24 and 30, (2) a CDR global score of 0.5, and (3) disease progression at stages 3 and 4, as described in the 2018 FDA guidelines. In addition, participants between the ages of 50 and 90 with objective episodic memory impairment were included in lecanemab clinical trials. The inclusion criteria for the clinical trials of donanemab were participants aged 60 to 85 years with an MMSE score between 20 and 28, and the presence of tau pathology. Furthermore, participants in the gantenerumab clinical trials were required to be between 50 and 90 years of age, have an MMSE score between 22 and 30, a CDR global score of either 0.5 or 1, a p-tau181/A β ₄₂ ratio in CSF of 0.024 or higher, and a Free and Cued Selective Reminding Test score of 0.67 or lower. Taken together, the criteria for recruiting participants in anti-amyloid MAB clinical trials have become more rigorous, particularly with criteria excluding patients with tau pathology patterns different from those of typical patients with AD as well as those with either too little or too much accumulation.

The therapeutic efficacy of all four anti-amyloid MABs against AD was assessed using CDR-SB scores and amyloid PET scans (Table 1). In clinical trials of anti-amyloid MABs for AD, the changes of CDR-SB scores compared to placebo for aducanumab, lecanemab, donanemab, and gantenerumab were −0.39 (22%), −0.45 (27%), −0.68 (36%) and −0.31 (8%), respectively. Based on the CDR-SB scores, the order of efficacy of the four anti-amyloid MABs in delaying cognitive decline is as follows: donanemab, lecanemab, aducanumab, and gantenerumab. In clinical trials of anti-amyloid MABs for AD, the changes in amyloid PET values for aducanumab, lecanemab, donanemab, and gantenerumab were −64 (from 85 to 21) CL, −55.48 (from 77.92 to 22.44)

Table 2 Association between anti-amyloid MABs and incidence of ARIA

| Anti-amyloid MABs | Treatment dosage | Target | ARIA | Clinical benefits | References |
|-------------------|------------------|--|-------|--------------------|------------|
| Solanezumab | 6 mg/kg (Q4W) | Monomeric A β | 0.9% | No | [12] |
| Crenezumab | 60 mg/kg (Q4W) | Monomeric A β (Small extent oligomers) | 0.3% | No | [15] |
| ACU-193 | 25 mg/kg (Q2W) | Oligomeric A β ; ADDLs | 7.1% | Not available data | [60] |
| Lecanemab | 10 mg/kg (Q2W) | Oligomer / Protofibrils | 12.6% | Yes | [21] |
| Donanemab | 20 mg/kg (Q4W) | Pyroglutamate A β aggregates | 24% | Yes | [22] |
| Gantenerumab | 7 mg/kg (Q2W) | Fibrils/Plaques | 25.8% | No | [53] |
| Aducanumab | 10 mg/kg (Q4W) | Plaques | 35% | Conflicting | [20] |
| Bapineuzumab | 1 mg/kg (Q13W) | Plaques | 11.8% | No | [14] |

MABs, monoclonal antibodies; ARIA, amyloid-related imaging abnormalities; ADDLs, A β -derived diffusible ligands; Q2W; once every 2 weeks, Q4W; once every 4 weeks, Q13W; once every 13 weeks, ADDLs, A β -derived diffusible ligands

CL, -88.0 (from 102.4 to 14.4) CL, and -47.5 (from 94.44 to 46.9) CL, respectively. Notably, aducanumab, lecanemab and donanemab eliminated A β to levels below 20 CL, which is considered amyloid negative. Unfortunately, gantenerumab only reduced A β to 46.9 CL and did not reach the amyloid-negative threshold of less than 20 CL. These results from clinical trials suggest that removing A β to the negative threshold results in clinical benefit.

The four anti-amyloid MABs have shown different incidences of ARIA in clinical trials involving patients with AD. Aducanumab, lecanemab, donanemab, and gantenerumab showed ARIA-E incidence rates of 36%, 12.6%, 24%, and 25.8%, respectively (Table 1). In clinical trials of anti-amyloid MABs in AD, the A β epitope targeted by the antibody and the administered dose could be closely related to the incidence of ARIA-E. In particular, the correlation between the dose/frequency of antibody administration and the occurrence of ARIA is well documented in the clinical trial of lecanemab [36]. The incidence of ARIA increases proportionally with the dosage and frequency of antibody administration. In addition, ARIA occurrence may be related to the A β form targeted by anti-amyloid MABs (Table 2). First-generation anti-amyloid MABs mainly targeting A β monomers, such as solanezumab and crenezumab, are generally associated with a low incidence of ARIA in patients with AD. However, they showed no clinical benefits in clinical trials of AD [12, 15]. In particular, crenezumab, which significantly binds to multiple forms of A β , including monomers, oligomers, fibrils, and plaques [56], showed a lower incidence of ARIA than solanezumab even at higher doses. The primary reason is that crenezumab uses an IgG4 backbone, unlike other anti-amyloid MABs with a different IgG1 backbone [57]. Compared to the IgG1 isotype, the IgG4 isotype weakly binds to Fc γ R, minimally activates complement, and modulates immune responses. The

reduced binding affinity of the IgG4 isotype to Fc γ R results in decreased Fc γ R-mediated microglial phagocytic function and leads to anti-inflammatory properties, ultimately contributing to a decreased incidence of ARIA-E. Bapineuzumab, which targets A β plaques, showed a high prevalence of ARIA even at low doses and a low frequency of administration [14]. Although clinical studies of bapineuzumab did not detect A β deposition in the brain by imaging, the increased concentration of A β in plasma suggests that bapineuzumab actively degrades A β plaques in the brain. The occurrence of ARIA may be linked to the removal of A β plaques deposited within and around blood vessels [58]. In particular, second-generation anti-amyloid MABs that target the oligomeric form and fibrils, such as aducanumab, lecanemab, donanemab, and gantenerumab, have been shown to have a higher incidence of ARIA in the brains of AD patients compared to solanezumab and crenezumab, which target monomeric A β [20, 21, 46, 53]. This difference in ARIA incidence appears to be related to the specific A β forms targeted by anti-amyloid MABs. In this regard, the impact of anti-amyloid MABs targeting oligomeric A β on the incidence of ARIA has drawn substantial research attention. ACU193 is an antibody that specifically binds to A β -derived diffusible ligands. ACU193 binds to A β oligomers with more than 500-fold selectivity versus A β monomers and fibrils [59]. In a phase 1 clinical trial (NCT04931459), ACU193 was administered to 65 patients with MCI due to AD or mild AD. The ARIA-E incidence rate in patients treated with 25 mg/kg ACU193 was 7.1% [60], which was lower than the prevalence observed in patients administered with similar doses of lecanemab, donanemab, gantenerumab, or aducanumab. Therefore, the development of anti-amyloid MABs targeting the oligomeric forms of A β may receive increasing attention. However, the clinical efficacy of antibodies that selectively target

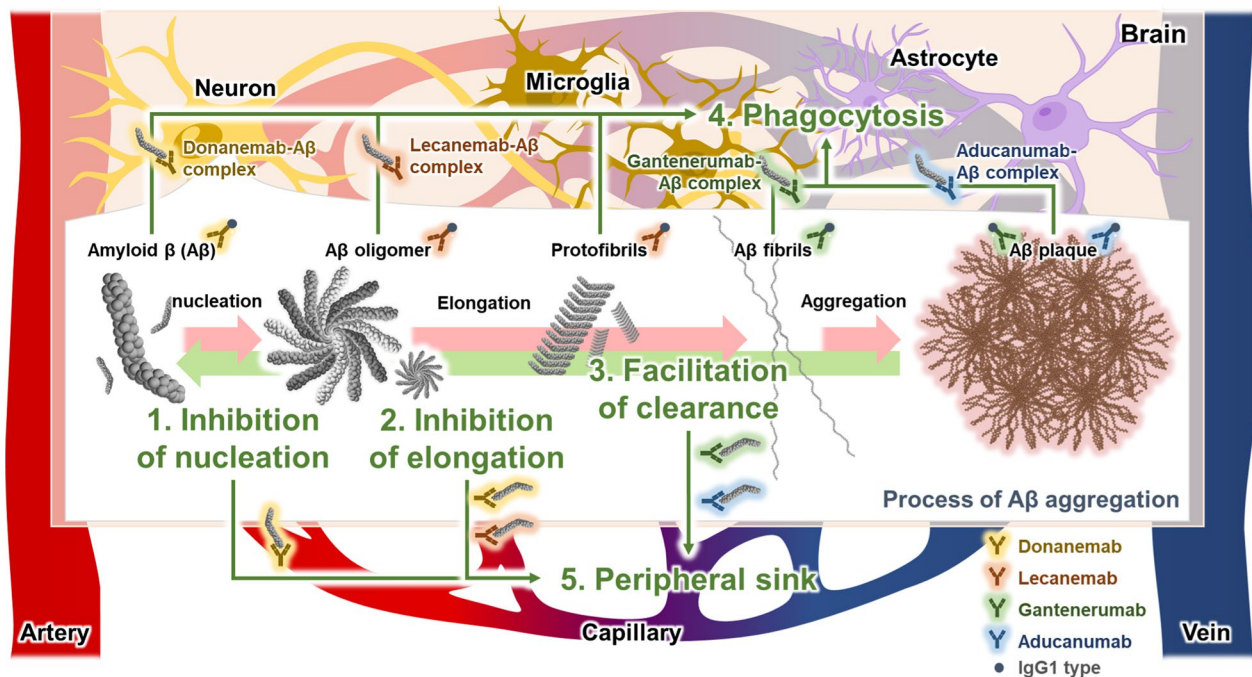


Fig. 2 The putative mechanisms by which second-generation anti-amyloid monoclonal antibodies (MABs) reduce the accumulation of Aβ in Alzheimer's disease (AD). Donanemab-Aβ and lecanemab-Aβ complexes inhibit Aβ accumulation by blocking nucleation and aggregation, and clear Aβ by glial cell-mediated phagocytosis and peripheral sink mechanisms. In addition, gantenerumab-Aβ and aducanumab-Aβ complexes degrade Aβ into sub-aggregates, inducing phagocytosis and peripheral sink mechanisms. Second-generation anti-amyloid MABs have been humanized to the IgG1 subclass

oligomers, such as ACU-193, has not been confirmed, and further research on antibodies selectively targeting oligomers is needed.

Mechanisms of action of anti-amyloid MABs

Five putative mechanisms by which anti-amyloid MABs reduce the accumulation of Aβ in individuals with AD have been proposed (Fig. 2) [61, 62]. The first putative mechanism involves binding to Aβ monomers, oligomers, and protofibrils, and interfering with the nucleation of Aβ [25, 48, 63]. Since Aβ oligomers and protofibrils produced by Aβ nucleation are the most toxic aggregates, reducing these forms with anti-amyloid MABs may attenuate the progression of AD [64, 65]. The second mechanism is the blocking of elongation of already-formed Aβ fibrils [66]. Elongation of Aβ fibrils occurs due to the binding of various Aβ forms, such as monomers and dimers, to the endpoints of the fibrils [67]. However, anti-amyloid MABs bind to Aβ fibrils and inhibit elongation by preventing further binding of other Aβ forms to fibril endpoints. The third mechanism is by specifically binding to Aβ aggregates, including fibrils and plaques, and facilitating Aβ degradation to lower-order aggregates. Anti-amyloid MABs break down Aβ into smaller aggregates, prevent reassembly of lower-aggregation-state Aβ,

and thus inhibit Aβ-induced neurodegeneration [68]. The fourth putative mechanism is the microglial cell-mediated phagocytosis [61, 62, 69]. Microglia-mediated phagocytosis is induced by microglial recognition of the fragment crystallizable region (FcR) of anti-amyloid MABs bound to Aβ. The four antibodies were humanized with the IgG1 isotype [70], the preferred candidate IgG subclass for therapeutic antibody engineering [71, 72]. The IgG1 isotype is known to act as an effector through the Fcγ receptor [73]. Since all four antibodies have been modified to IgG1 isotypes during the humanization process, differences in phagocytosis based on the IgG1 isotype are not considered to be significant. Interestingly, the efficiency of microglial phagocytosis may be influenced by the species of Aβ. A previous study reported that Aβ fibrils are cleared more efficiently than the oligomeric form of Aβ [74]. These findings suggest that aducanumab and gantenerumab, which target Aβ fibrils, might lead to higher rates of microglial phagocytosis. In other words, microglia target the FcR of anti-amyloid MABs bound to Aβ, removing Aβ from the parenchyma of brains with AD by FcR-mediated microglial phagocytosis. The final putative mechanism is the peripheral sink mechanism, whereby anti-amyloid MABs sequester or dissociate the Aβ in the blood [75, 76]. As a homeostatic

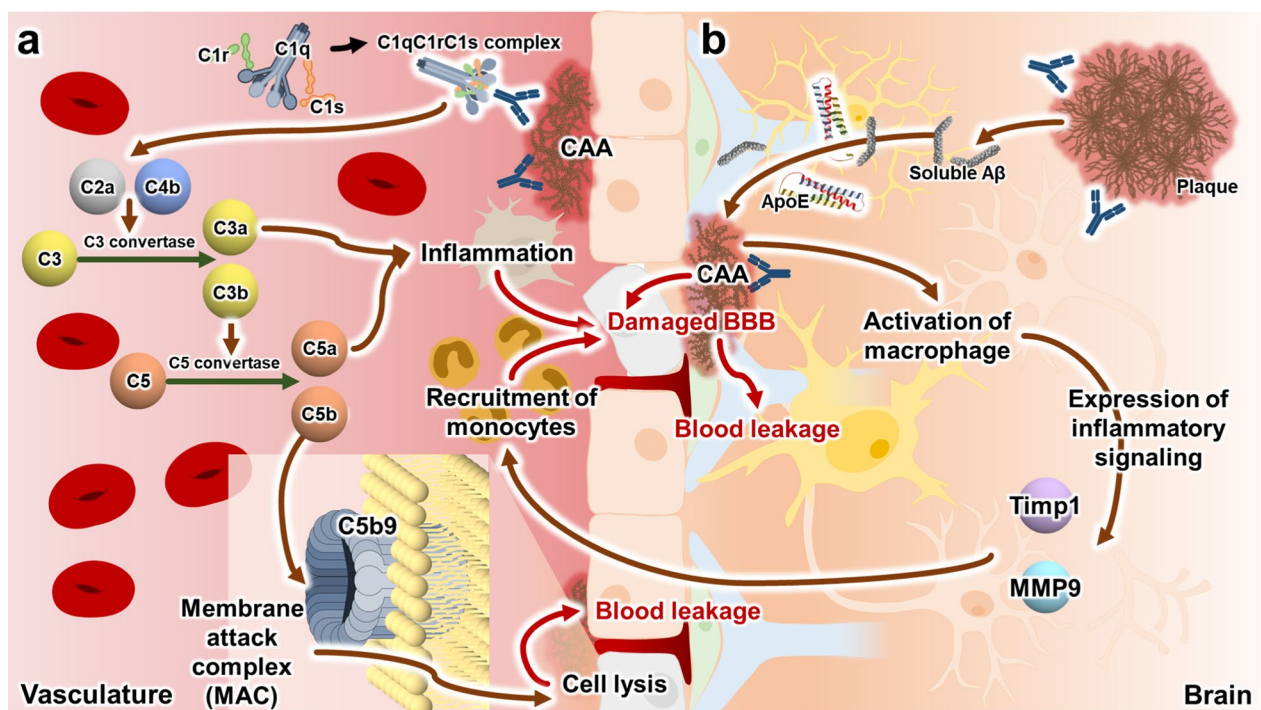


Fig. 3 The putative pathophysiological mechanisms leading to the incidence of amyloid-related imaging abnormalities (ARIA) in the brains of patients with Alzheimer's disease (AD). **a** The schematic diagram shows the occurrence of ARIA by the classical complement cascade in the vasculature. Anti-amyloid monoclonal antibodies (MABs) induce the formation of C1 complex. The C1 complex disrupts the blood brain barrier (BBB) by forming the membrane attack complex (MAC) C5b-9 through multiple complement signaling pathways. **b** The schematic diagram shows the incidence of ARIA by the FcR-mediated signaling pathways in the brain. During the removal process of Aβ aggregates by antibodies, detritus, such as soluble Aβ, is transported towards the BBB by ApoE. This transported Aβ contributes to the formation of cerebral amyloid angiopathy (CAA). The antibody-mediated clearance of formed CAA not only damages the BBB but also activates macrophages through the FcR-mediated signaling pathway. The activated macrophages induce inflammatory signaling, such as tissue inhibitor of metalloproteinases-1 (TIMP1) and matrix metalloproteinase 9 (MMP9), leading to an increased recruitment of monocytes around the BBB. The BBB is damaged by this inflammatory response

mechanism to maintain Aβ levels, the brain with AD effluxes Aβ into the periphery, reducing the formation of Aβ plaques in the parenchyma after anti-amyloid MAB administration.

In summary, anti-amyloid MABs can reduce parenchymal Aβ accumulation and Aβ-induced neuronal death in the brain through five different mechanisms to alleviate Aβ-mediated pathogenesis in AD (Fig. 2): (1) inhibition of Aβ plaque formation; (2) inhibition of Aβ fibril extension; (3) facilitation of Aβ aggregate clearance; (4) microglial-mediated phagocytosis of Aβ; and (5) peripheral clearance of Aβ.

Limitations of second-generation anti-amyloid MABs

A significant limitation of current antibodies targeting Aβ is the incidence of ARIA. The occurrence of ARIA was first observed in a phase 1 trial of bapineuzumab, one of the first-generation anti-amyloid MABs targeting Aβ plaques [77], and has been reported in the clinical trials of most second-generation anti-amyloid MABs [19].

ARIAs are divided into ARIA-E and ARIA-H based on types of magnetic resonance imaging signal abnormalities. ARIA-E refers to the leakage and accumulation of fluid in the brain, resulting in vasogenic edema or sulcal effusion, which causes hyperintensity abnormalities on imaging scans, such as changes in the cortical folds. ARIA-H refers to an intracerebral hemorrhage resulting in superficial siderosis, observed as hypointense hemosiderin deposition. Almost half of ARIA-E cases are accompanied by ARIA-H, indicating a significant overlap in the pathophysiological mechanisms of these conditions (Fig. 3) [78]. One putative pathophysiological mechanism leading to ARIA involves the formation of the membrane attack complex C5b-9 via the classical complement cascade [79]. Second-generation anti-amyloid MABs circulating in the blood of AD patients first react with accumulated Aβ aggregates in the cerebrovascular wall. This antigen–antibody interaction triggers formation of the C1qC1rC1s complex. The C1qC1rC1s complex activates the complement system via the classical

complement pathway, ultimately leading to the formation of the membrane attack complex C5b-9. The membrane attack complex C5b-9 perforates cell membranes, leading to leakage and microhemorrhages. Additionally, anti-amyloid MABs entering the brain cause the dissolution of A β plaques, forming soluble A β that are transported by ApoE to the vasculature, contributing to the formation of cerebral amyloid angiopathy (CAA) [78]. Anti-amyloid MABs bound to the formed CAA activate perivascular macrophages through FcR-mediated signaling, and these activated macrophages induce the expression of inflammatory signaling molecules, such as Timp1 and matrix metalloproteinase-9 [80]. The recruitment of peripheral monocytes by induced inflammatory signals damages the cerebrovascular wall, resulting in ARIA (Fig. 3b) [81]. This process supports clinical findings that patients carrying the *APOE* ϵ 4 allele have an increased incidence of ARIA, suggesting a genetic predisposition that influences the pathophysiological mechanisms leading to ARIA. Moreover, the presence of the *APOE* ϵ 4 allele exacerbates the process, potentially leading to increased ARIA incidence in patients with AD, as the level of A β oligomers in the brains of healthy individuals is approximately 2.7-fold higher than that in the brains of patients with AD and *APOE* ϵ 3/ ϵ 3 [82].

It is challenging to safely and effectively deliver peripherally administered anti-amyloid MABs to the brain. When administered intravenously or subcutaneously, only a small fraction of the injected dose crosses the BBB, with only 0.01% to 0.11% of the anti-amyloid MABs present in the plasma known to effectively reach the brain [83–85]. These limitations are important when determining the dose of anti-amyloid MABs in clinical trials for AD. There is a close relationship between amyloid clearance/clinical benefits and the administered doses. Aducanumab, lecanemab, donanemab, and gantenerumab have demonstrated dose-dependent effects on amyloid clearance and clinical benefits. However, high-dose administration of these anti-amyloid MABs also causes side effects, such as an increased prevalence of ARIA [54]. Consequently, these limitations have played a pivotal role in the rejection of both aducanumab and lecanemab by the European Medicines Agency (EMA). Although aducanumab and lecanemab have demonstrated efficacy in reducing A β plaques, the relatively modest cognitive benefits were not sufficient to overcome the significant risks associated with ARIA. The EMA's decision highlights the ongoing challenge of balancing the therapeutic benefits of amyloid clearance with patient safety, especially given the potential for serious adverse events like ARIA-E and ARIA-H.

Several attempts have been made to overcome the limitations of BBB penetration by anti-amyloid MABs. The

combination of an antibody recognizing BBB receptors with another antibody targeting A β in a bispecific antibody construct, which has two distinct binding domains that can simultaneously bind to different antigens, can enhance BBB penetration [86]. Strategies to facilitate BBB penetration include the construction of bispecific antibodies that enhance TfR1-mediated transcytosis, such as trontinemab, which consists of gantenerumab conjugated to a human TfR1-targeting BBB shuttle fragment. Furthermore, single-chain variable-fragment (scFv) antibodies [87] and nanobodies [88, 89] have superior properties, such as small size, high stability, strong antigen-binding affinity, and high BBB permeability, compared with conventional antibodies. In addition, certain manipulations, such as magnetic or ultrasonic stimulation can temporarily open the BBB in a non-invasive manner to improve the BBB permeability for antibodies [90, 91].

Loss of brain tissue is a critical factor contributing to cognitive dysfunction in AD [92]. Brain atrophy is an objective indicator of disease progression and severity of neurodegeneration. In particular, anti-amyloid MABs, especially second-generation MABs, have been reported to accelerate brain atrophy [20, 36, 46]. It has been suggested that brain atrophy induced by anti-amyloid MABs may be related to the clearance of amyloid plaques from the brains of individuals with AD. However, given that the amount of A β peptide present in the AD brain is not abundant [93], it is difficult to induce brain atrophy by clearing amyloid plaques using anti-amyloid MABs. Results from clinical studies on aducanumab, lecanemab, and donanemab in patients with AD indicated that a reduction in plaque volume had minimal impact on changes in global brain volume [94–96]. In addition to clearing amyloid plaques, neuronal loss induced by anti-amyloid MABs results in brain atrophy, which may exacerbate cognitive deterioration in patients with AD. Unfortunately, the molecular mechanisms underlying brain atrophy caused by anti-amyloid MABs, as well as their long-term consequences on AD brain health, remain unknown. Consequently, there are growing concerns on the potential adverse effects of anti-amyloid MABs on cognitive dysfunction and AD progression in patients with AD.

Perspective and future directions of anti-amyloid MABs: what's next?

Second-generation anti-amyloid MABs have provided valuable insights that can help resolve the debate surrounding the amyloid hypothesis and improve our understanding of AD pathogenesis and drug development. The following are some of the insights that will be useful for future development and improvement of anti-amyloid MABs for the treatment of AD.

The future direction of next-generation anti-amyloid MABs is to progress toward modifying the structure of antibodies to minimize adverse effects and enhance therapeutic efficacy for the treatment of AD. The prevalent form of antibodies used in recent cancer treatments is a conjugated structure of both antibodies and drugs, and these types of drugs are called antibody–drug conjugates (ADC). An ADC is an immunoconjugate consisting of an antibody conjugated to a payload via a chemical linker [97]. Whether this concept of an ADC can help AD is still on debate. In particular, since small molecules are easier to pass through the BBB than antibodies, the question arises as to why these small molecules should be attached to antibodies. However, these small molecules have limitations, such as low binding to target, short half-lives, off-target effects, and widespread delivery. Antibodies can deliver small molecules more locally and effectively due to their ability to specifically bind to their targets, and they are delivered precisely to the target cell/region, unlike when small molecules are randomly spread and act in the body. This spatial targeting of antibodies can help improve therapeutic effectiveness by delivering small molecules exactly where they are needed, potentially overcoming some of the limitations associated with the broader distribution of such agents within the brain. Moreover, when small molecules are administered alone, they can diffuse or metabolize rapidly in the body. However, such small molecules bound to antibodies can be delivered stably through the antibody and remain in the body for a longer time. Thus, the half-lives of small molecules in the blood are longer, increasing the possibility of reaching target cells. Furthermore, small molecules, when administered alone, can affect normal tissues, potentially leading to increased side effects. However, ADCs can reduce off-target effects by allowing drugs to reach their target. Although antibodies can overcome the limitations of small molecules, in practice, coupling small molecules to antibodies can significantly decrease drug exposure, potentially compromising the therapeutic activity when small molecules already have low target affinity. Hence, several criteria should be considered when selecting a payload in an ADC. These criteria of drugs include solubility, possessing reactive sites for linker conjugation, potency, safety in combination with the linker, and pharmacokinetics after release. Therefore, the concept of ADC, which attaches payloads that reduce the side effects caused by the antibody or maximize the efficacy of the antibody, can help improve the pharmacology of existing antibodies. For example, ADC that combines anti-amyloid MABs with anti-inflammatory corticosteroids (e.g., methylprednisolone and dexamethasone) could effectively reduce the occurrence of ARIA and delay AD progression more than current antibody

therapies [19]. ADC allows corticosteroids to target over-activated microglia, which can reduce the development of ARIA by alleviating the inflammatory response caused by activated microglia, without the broader immunosuppressive effects of corticosteroids. Additionally, ADC combining anti-amyloid MABs and Mannitol, which is used to lower increased intracranial pressure, can effectively suppress the occurrence of ARIA based on local efficacy [98, 99]. Moreover, Morphomer[®] ADC (morADC), a drug candidate unveiled by AC Immune SA at the recent Alzheimer's Association International Conference, can maximize the efficacy of existing antibodies. The morADC combines monoclonal antibodies and brain-penetrant small molecules developed through the SupraAntigen[®] and Morphomer[®] platforms, respectively, to target toxic proteins in neurodegenerative diseases. morADC synergistically increases the aggregation inhibition and clearance of these proteins compared to small molecules or antibodies alone. Recently, a review has proposed the structure of ideal ADC for AD treatment and their possible mechanisms [100]. A possible mechanism for such ADC in AD is as follows: (1) ADC crosses the BBB and accumulates in the brain, (2) the linker of the ADC in the brain is cleaved by extracellular proteases to separate the antibody and payload, and (3) the antibody binds to the antigen and the payload binds to another target, resulting in dual action. Unfortunately, since the efficacy of ADCs based on this mechanism is limited without improved BBB penetration, efforts should focus on the development of antibodies that enhance BBB penetration. In addition, identification of extracellular proteases capable of efficiently cleaving the linker to separate antibodies and payloads in the brain is critical to optimizing this approach.

Targeted protein degradation (TPD) has recently emerged as a promising therapeutic approach for AD. TPD removes protein molecules from inside and outside the cells by engaging in protein degradation pathways [101]. Lysosome-targeting chimera (LYTAC) is a conjugate that binds to lysosome-shuttling receptors on cell surfaces and to the extracellular domains of target proteins, enabling the targeted degradation of extracellular and membrane proteins. It has been known that LYTAC, produced with an anti-ApoE4 antibody, increases lysosomal uptake of ApoE4, with the potential to treat AD [100]. Moreover, a previous study showed that the attachment of a lysosomal targeting ligand to an antibody induces the antibody to degrade extracellular proteins [102]. Hence, the functionalization of anti-amyloid MABs as LYTACs would allow for the efficient removal of extracellular A β aggregates through lysosomes. Proteolysis-targeting chimera (PROTAC), a subtype of TPD, induces the ubiquitination and degradation of target proteins via

the ubiquitin–proteasome system [103]. Another specific variation of PROTAC is antibody-based PROTAC (AbTAC). Unlike the traditional PROTACs, AbTACs target membrane proteins. AbTACs utilize bispecific antibodies that bind one of their arms to the target protein and the other to the transmembrane E3 ligase [104]. By modifying anti-amyloid MABs to replace the arm of the antibody that binds to the target protein, AbTAC can also effectively remove intracellular A β aggregates through the endosome-lysosome pathway or ubiquitin–proteasome system.

It has been shown that a modification of aducanumab, a second-generation anti-amyloid MAB, provides an effective therapeutic strategy against AD [105]. α A β -Gas6 is a fusion protein in which the aducanumab scFv that recognizes and binds to A β is substituted to the Gla domain of the Gas6 protein. Gas6 is a protein that bridges the interaction between phosphatidylserine and the Tyro3, Axl, and MerTK (TAM) receptors and plays a critical role in mediating efferocytosis by phagocytes [106]. α A β -Gas6 facilitates the clearance of A β through phagocytosis via both microglia and astrocytes, whereas aducanumab exclusively removes A β via microglial cells. Unlike aducanumab, α A β -Gas6 significantly reduces the secretion of pro-inflammatory cytokines in microglia and astrocytes [107]. Furthermore, administration of α A β -Gas6, compared to aducanumab, reduces the occurrence of CAA and microhemorrhage in AD mouse models [105]. Taken together, the modifications of anti-amyloid MABs are expected to overcome the limitations of existing anti-amyloid MABs and advance their therapeutic efficacy in AD, thereby establishing the next generation of MABs.

With the advancement of anti-amyloid MABs, the identification of biomarkers capable of detecting individuals in the early stages of AD has become crucial for prognostic evaluation. In clinical trials, aducanumab, lecanemab, and donanemab have removed more than 60% of A β deposition; however, although the cognitive decline in patients with AD has been slowed, cognitive impairment has not been halted. Furthermore, the levels of neurofilament light chain (NfL), a marker of neurodegeneration, were increased in the CSF and plasma of APP/PS1 mice, although at a slower rate [108, 109]. These findings suggest that while A β immunotherapy may be effective against A β pathology, it may have a negative effect on other pathological factors. Therefore, it is important to develop biomarkers capable of identifying these other pathological features and to develop therapeutic agents targeting them to optimize the efficacy of A β immunotherapy.

Currently, biomarkers commonly used as entry criteria or outcome measures in clinical trials are A β and tau

[6, 110]. The most common approach for measuring the deposition of A β and tau within the brain is using PET scans. Moreover, biomarkers in CSF or plasma, such as the A $\beta_{42/40}$ ratio and the levels of total tau, p-tau181, p-tau217, p-tau231, and microtubule-binding region (MTBR)-tau243, can reflect the pathology of A β and tau in the brain [111–113]. In addition to A β and tau, there are other biomarkers of AD that are associated with neurodegeneration and inflammation. Biomarkers associated with neurodegeneration in CSF and plasma include NfL, neurogranin, visinin-like protein 1, and synaptosomal-associated protein-25 [114]. Biomarkers related to inflammation, especially gliosis, include GFAP, chitinase-3-like protein 1, and soluble triggering receptor expressed on myeloid cell 2 [114]. The use of both existing and novel biomarkers could enable more precise screening and medication prescription for patients with AD, as well as individuals with MCI due to AD and preclinical AD, who may benefit from early intervention. Given that the annual rate of progression from MCI to dementia is estimated to be 10%–15%, with more than 80% of MCI patients progressing to dementia within six years [115], early diagnosis and intervention are critical to slowing or preventing the progression to AD. Interestingly, circulating RNAs in the blood, especially microRNAs (miRNAs), have shown promise as biomarkers for the diagnosis of preclinical or early stages of AD [116–119]. Several miRNAs have been identified as potential biomarkers for predicting the progression from MCI to AD [120]. Moreover, in preclinical AD, the combination of biomarkers, such as microRNA, p-tau181, NfL, GFAP, and A $\beta_{42/40}$ with amyloid PET or cognitive function tests, including ADAS-Cog13 and MMSE, allows for earlier and more accurate detection of AD [120–122].

Although anti-amyloid MABs have been shown to remove already formed A β plaques and ameliorate the toxic events already initiated by these A β aggregates, they do not slow plaque formation by inhibiting A β production, which is the first step in the amyloid cascade in the pathogenesis of AD. Therefore, combination therapies should be considered, depending on the clinical stage of the disease. First, β -site amyloid precursor protein cleaving enzyme inhibitors and γ -secretase inhibitors/modulators could be considered for patients in the preclinical stage where there is elevated A β yet low levels of other tau or neurodegeneration-related biomarkers. This administration can help delay other pathology and the onset of AD by inhibiting excessive A β production. Then, when patients enter the prodromal stage due to A β accumulation, they are shifted to anti-amyloid MABs to remove the A β that has already accumulated. The stage-targeted approach would maximize the benefits of both drugs to create a synergistic effect [123]. In addition,

given that A β accumulation contributes to tau phosphorylation and neurofibrillary tangle formation [124], combined therapies with drugs that target tau pathologies, such as tau aggregation inhibitors, phosphorylation inhibitors, and antisense oligonucleotides that bind to tau mRNA and block protein expression, may be useful as part of a multi-target combination therapy. Collectively, since AD is a multifactorial disorder involving multiple pathological processes, combination therapies targeting multiple pathological targets are likely to be more effective than single-target therapies once the disease has progressed and various pathologies have been initiated.

Conclusions

Second-generation anti-amyloid MABs, including aducanumab, lecanemab, donanemab, and gantenerumab, have attracted significant interest in the development of treatments for AD. In clinical trials, anti-amyloid MABs have demonstrated efficacy in reducing A β deposition and slowing cognitive decline in patients with AD. However, challenges such as the risk of ARIA, low BBB permeability, low efficacy, and brain atrophy remain unsolved. Our perspectives and future research directions should focus on addressing these challenges. The next generation of anti-amyloid MABs is expected to be developed and introduced through modifications that enhance BBB penetration and therapeutic efficacy while reducing adverse effects. Aligned with these advancements, the identification of biomarkers for the early detection of AD will contribute to increasing the efficacy of anti-amyloid MABs in the treatment of AD. Furthermore, given the complex pathology of AD, drugs targeting other pathologies along with anti-amyloid MABs should be prescribed to patients with AD as a combination therapy.

Abbreviations

| | |
|--------------|--|
| AbTAC | Antibody-based PROTAC |
| AD | Alzheimer's disease |
| ADAS-Cog | Alzheimer's Disease Assessment Scale-Cognitive Subscale |
| ADCOMS | Alzheimer's Disease Composite Score |
| ADCS-ADL-MCI | Alzheimer's Disease Cooperative Study-Activities of Daily Living Scale for Mild Cognitive Impairment |
| ADCS-iADL | Alzheimer's Disease Cooperative Study-Instrumental Activities of Daily Living Inventory |
| A β | Amyloid- β |
| ARIA | Amyloid-related imaging abnormalities |
| ARIA-E | ARIA-oedema/effusion |
| ARIA-H | Haemosiderosis/microhaemorrhages |
| MABs | Monoclonal antibodies |
| ApoE | Apolipoprotein E |
| BBB | Blood-brain barrier |
| CDR-SB | Clinical Dementia Rating-Sum of Boxes |
| CSF | Cerebrospinal fluid |
| FDA | Food and Drug Administration |
| FcR | Fragment crystallizable region |
| iADRS | Integrated Alzheimer's Disease Rating Scale |
| MMSE | Mini-Mental State Examination |
| MCI | Mild cognitive impairment |
| OLE | Open-label extension |

| | |
|--------|-------------------------------|
| p-tau | Phosphorylated tau |
| PET | Positron emission tomography |
| PROTAC | Proteolysis-targeting chimera |
| SAEs | Serious adverse effects |
| TfR1 | Transferrin receptor 1 |

Author contributions

B.-H.K., S.K., Y.N., Y.H.P., and S.M.S. contributed to writing the original draft. M.M. contributed to the conceptualization, supervision, and the writing and editing of the manuscript. All authors read and approved the final version of the manuscript.

Funding

This review was funded by Basic Science Research Program of the National Research Foundation of Korea (NRF), which is funded by the Ministry of Science, ICT & Future Planning (RS-2023-00240010 to M.M., NRF-2022R1A6A3A13053190 and RS-2024-00450135 to Y.N., RS-2023-00212388 to S.K., and RS-2023-00273557 to Y.H.P.), and a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (RS-2023-KH138733 to M.M.).

Availability of data and materials

Not Applicable.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not Applicable.

Competing interests

Authors declare no competing interests.

Received: 6 June 2024 Accepted: 20 December 2024

Published online: 27 January 2025

References

- Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature*. 1975;256(5517):495–7.
- Chi XK, Xu XL, Chen BY, Su J, Du YZ. Combining nanotechnology with monoclonal antibody drugs for rheumatoid arthritis treatments. *J Nanobiotech*. 2023;21(1):105.
- Zahavi D, Weiner L. Monoclonal antibodies in cancer therapy. *Antibodies (Basel)*. 2020;9(3):34.
- Breedveld FC. Therapeutic monoclonal antibodies. *Lancet*. 2000;355(9205):735–40.
- Todd PA, Brogden RN. Muromonab CD3 A review of its pharmacology and therapeutic potential. *Drugs*. 1989;37(6):871–99.
- Cummings J, Zhou Y, Lee G, Zhong K, Fonseca J, Cheng F. Alzheimer's disease drug development pipeline: 2023. *Alzheimers Dement (N Y)*. 2023;9(2): e12385.
- Food and Drug Administration. FDA grants accelerated approval for Alzheimer's disease treatment. 2023. <https://www.fda.gov/news-events/press-announcements/fda-grants-accelerated-approval-alzheimers-disease-treatment>.
- Cavazzoni P. FDA's decision to approve new treatment for Alzheimer's disease. 2021. <https://www.fda.gov/drugs/our-perspective/fdas-decision-approve-new-treatment-alzheimers-disease>.
- Food and Drug Administration. FDA converts novel Alzheimer's disease treatment to traditional approval. 2023. <https://www.fda.gov/news-events/press-announcements/fda-converts-novel-alzheimers-disease-treatment-traditional-approval>.

10. Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science*. 2002;297(5580):353–6.
11. Karran E, Mercken M, De Strooper B. The amyloid cascade hypothesis for Alzheimer's disease: an appraisal for the development of therapeutics. *Nat Rev Drug Discov*. 2011;10(9):698–712.
12. Doody RS, Thomas RG, Farlow M, Iwatsubo T, Vellas B, Joffe S, et al. Phase 3 trials of solanezumab for mild-to-moderate Alzheimer's disease. *N Engl J Med*. 2014;370(4):311–21.
13. Salloway S, Sperling R, Fox NC, Blennow K, Klunk W, Raskind M, et al. Two phase 3 trials of bapineuzumab in mild-to-moderate Alzheimer's disease. *N Engl J Med*. 2014;370(4):322–33.
14. Vandenberghe R, Rinne JO, Boada M, Katayama S, Scheltens P, Vellas B, et al. Bapineuzumab for mild to moderate Alzheimer's disease in two global, randomized, phase 3 trials. *Alzheimers Res Ther*. 2016;8(1):18.
15. Ostrowitzki S, Bittner T, Sink KM, Mackey H, Rabe C, Honig LS, et al. Evaluating the safety and efficacy of crenezumab versus placebo in adults with early Alzheimer's disease: two phase 3 randomized placebo-controlled trials. *JAMA Neurol*. 2022;79(11):1113–21.
16. Kwan ATH, Arfaie S, Theriault J, Rosa-Neto P, Gauthier S. Lessons learnt from the second generation of anti-amyloid monoclonal antibodies clinical trials. *Dement Geriatr Cogn Disord*. 2020;49(4):334–48.
17. Salloway S, Farlow M, McDade E, Clifford DB, Wang G, Llibre-Guerra JJ, et al. A trial of gantenerumab or solanezumab in dominantly inherited Alzheimer's disease. *Nat Med*. 2021;27(7):1187–96.
18. Siemers ER, Sundell KL, Carlson C, Case M, Sethuraman G, Liu-Seifert H, et al. Phase 3 solanezumab trials: Secondary outcomes in mild Alzheimer's disease patients. *Alzheimers Dement*. 2016;12(2):110–20.
19. Withington CG, Turner RS. Amyloid-related imaging abnormalities with anti-amyloid antibodies for the treatment of dementia due to Alzheimer's disease. *Front Neurol*. 2022;13: 862369.
20. Budd Haeberlein S, Aisen PS, Barkhof F, Chalkias S, Chen T, Cohen S, et al. Two randomized phase 3 studies of aducanumab in early Alzheimer's disease. *J Prev Alzheimers Dis*. 2022;9(2):197–210.
21. van Dyck CH, Swanson CJ, Aisen P, Bateman RJ, Chen C, Gee M, et al. Lecanemab in early Alzheimer's disease. *N Engl J Med*. 2023;388(1):9–21.
22. Sims JR, Zimmer JA, Evans CD, Lu M, Ardayfio P, Sparks J, et al. Donanemab in early symptomatic Alzheimer's disease: the TRAIL-BLAZER-ALZ 2 randomized clinical trial. *JAMA*. 2023;330(6):512–27.
23. Yang P, Sun F. Aducanumab: the first targeted Alzheimer's therapy. *Drug Discov Ther*. 2021;15(3):166–8.
24. Zhang Y, Chen H, Li R, Sterling K, Song W. Amyloid β -based therapy for Alzheimer's disease: challenges, successes and future. *Signal Transduct Target Ther*. 2023;8(1):248.
25. Sevigny J, Chiao P, Bussiere T, Weinreb PH, Williams L, Maier M, et al. The antibody aducanumab reduces Abeta plaques in Alzheimer's disease. *Nature*. 2016;537(7618):50–6.
26. Arndt JW, Qian F, Smith BA, Quan C, Kilambi KP, Bush MW, et al. Structural and kinetic basis for the selectivity of aducanumab for aggregated forms of amyloid-beta. *Sci Rep*. 2018;8(1):6412.
27. Ferrero J, Williams L, Stella H, Leitermann K, Mikulskis A, O'Gorman J, et al. First-in-human, double-blind, placebo-controlled, single-dose escalation study of aducanumab (BIB037) in mild-to-moderate Alzheimer's disease. *Alzheimers Dement (N Y)*. 2016;2(3):169–76.
28. Budd Haeberlein S, O'Gorman J, Chiao P, Bussiere T, von Rosenstiel P, Tian Y, et al. Clinical development of aducanumab, an anti-Abeta human monoclonal antibody being investigated for the treatment of early Alzheimer's disease. *J Prev Alzheimers Dis*. 2017;4(4):255–63.
29. Knopman DS, Jones DT, Greicius MD. Failure to demonstrate efficacy of aducanumab: an analysis of the EMERGE and ENGAGE trials as reported by Biogen, December 2019. *Alzheimers Dement*. 2021;17(4):696–701.
30. Haeberlein SB, Von Hehn C, Tian Y, Chalkias S, Muralidharan K, Chen T, et al. EMERGE and ENGAGE topline results: two phase 3 studies to evaluate aducanumab in patients with early Alzheimer's disease. *Clinical Trials Alzheimer's Disease*. 2019. <https://doi.org/10.1002/alz.047259>.
31. Salloway S, Chalkias S, Barkhof F, Burkett P, Barakos J, Purcell D, et al. Amyloid-related imaging abnormalities in 2 phase 3 studies evaluating aducanumab in patients with early Alzheimer's disease. *JAMA Neurol*. 2022;79(1):13–21.
32. Dickson SP, Hennessey S, Nicodemus Johnson J, Knowlton N, Hendrix SB. Avoiding future controversies in the Alzheimer's disease space through understanding the aducanumab data and FDA review. *Alzheimers Res Ther*. 2023;15(1):98.
33. Englund H, Sehlin D, Johansson AS, Nilsson LN, Gellerfors P, Paulie S, et al. Sensitive ELISA detection of amyloid-beta protofibrils in biological samples. *J Neurochem*. 2007;103(1):334–45.
34. Tucker S, Moller C, Tegerstedt K, Lord A, Laudon H, Sjodahl J, et al. The murine version of BAN2401 (mAb158) selectively reduces amyloid-beta protofibrils in brain and cerebrospinal fluid of tg-ArcSwe mice. *J Alzheimers Dis*. 2015;43(2):575–88.
35. Logovinsky V, Satlin A, Lai R, Swanson C, Kaplow J, Osswald G, et al. Safety and tolerability of BAN2401—a clinical study in Alzheimer's disease with a protofibril selective Abeta antibody. *Alzheimers Res Ther*. 2016;8(1):14.
36. Swanson CJ, Zhang Y, Dhadda S, Wang J, Kaplow J, Lai RYK, et al. A randomized, double-blind, phase 2b proof-of-concept clinical trial in early Alzheimer's disease with lecanemab, an anti-Abeta protofibril antibody. *Alzheimers Res Ther*. 2021;13(1):80.
37. Tahami Monfared AA, Tafazzoli A, Ye W, Chavan A, Zhang Q. Long-term health outcomes of lecanemab in patients with early Alzheimer's disease using simulation modeling. *Neurol Ther*. 2022;11(2):863–80.
38. Plotkin SS, Cashman NR. Passive immunotherapies targeting Abeta and tau in Alzheimer's disease. *Neurobiol Dis*. 2020;144: 105010.
39. Amano A, Sanjo N, Araki W, Anraku Y, Nakakido M, Matsubara E, et al. Peripheral administration of nanomicelle-encapsulated anti-Abeta oligomer fragment antibody reduces various toxic Abeta species in the brain. *J Nanobiotech*. 2023;21(1):36.
40. Rijal Upadhaya A, Kosterin I, Kumar S, von Arnim CA, Yamaguchi H, Fandrich M, et al. Biochemical stages of amyloid-beta peptide aggregation and accumulation in the human brain and their association with symptomatic and pathologically preclinical Alzheimer's disease. *Brain*. 2014;137:887–903.
41. Demattos RB, Lu J, Tang Y, Racke MM, Delong CA, Tzaferis JA, et al. A plaque-specific antibody clears existing beta-amyloid plaques in Alzheimer's disease mice. *Neuron*. 2012;76(5):908–20.
42. Saido TC, Iwatsubo T, Mann DM, Shimada H, Ihara Y, Kawashima S. Dominant and differential deposition of distinct beta-amyloid peptide species, a beta N3(pE), in senile plaques. *Neuron*. 1995;14(2):457–66.
43. Karran E, De Strooper B. The amyloid hypothesis in Alzheimer's disease: new insights from new therapeutics. *Nat Rev Drug Discovery*. 2022;21(4):306–18.
44. Lowe SL, Willis BA, Hawdon A, Natanegara F, Chua L, Foster J, et al. Donanemab (LY3002813) dose-escalation study in Alzheimer's disease. *Alzheimers Dement (N Y)*. 2021;7(1): e12112.
45. Lowe SL, Duggan Evans C, Shcherbinin S, Cheng YJ, Willis BA, Gueorguieva I, et al. Donanemab (LY3002813) phase 1b study in Alzheimer's disease: rapid and sustained reduction of brain amyloid measured by florbetapir F18 imaging. *J Prev Alzheimers Dis*. 2021;8(4):414–24.
46. Mintun MA, Lo AC, Duggan Evans C, Wessels AM, Ardayfio PA, Andersen SW, et al. Donanemab in early Alzheimer's disease. *N Engl J Med*. 2021;384(18):1691–704.
47. Shcherbinin S, Evans CD, Lu M, Andersen SW, Pontecorvo MJ, Willis BA, et al. Association of amyloid reduction after donanemab treatment with tau pathology and clinical outcomes: the TRAILBLAZER-ALZ randomized clinical trial. *JAMA Neurol*. 2022;79(10):1015–24.
48. Bohrmann B, Baumann K, Benz J, Gerber F, Huber W, Knoflach F, et al. Gantenerumab: a novel human anti-Abeta antibody demonstrates sustained cerebral amyloid-beta binding and elicits cell-mediated removal of human amyloid-beta. *J Alzheimers Dis*. 2012;28(1):49–69.
49. Ostrowitzki S, Deptula D, Thurfjell L, Barkhof F, Bohrmann B, Brooks DJ, et al. Mechanism of amyloid removal in patients with Alzheimer's disease treated with gantenerumab. *Arch Neurol*. 2012;69(2):198–207.
50. Ostrowitzki S, Lasser RA, Dorflinger E, Scheltens P, Barkhof F, Nikolcheva T, et al. A phase III randomized trial of gantenerumab in prodromal Alzheimer's disease. *Alzheimers Res Ther*. 2017;9(1):95.
51. Klein G, Delmar P, Voyle N, Rehal S, Hofmann C, Abi-Saab D, et al. Gantenerumab reduces amyloid-beta plaques in patients with prodromal

- to moderate Alzheimer's disease: a PET substudy interim analysis. *Alzheimers Res Ther.* 2019;11(1):101.
52. Klein G, Delmar P, Kerchner GA, Hofmann C, Abi-Saab D, Davis A, et al. Thirty-six-month amyloid positron emission tomography results show continued reduction in amyloid burden with subcutaneous gantenerumab. *J Prev Alzheimers Dis.* 2021;8(1):3–6.
53. Bateman RJ, Smith J, Donohue MC, Delmar P, Abbas R, Salloway S, et al. Two phase 3 trials of gantenerumab in early Alzheimer's disease. *N Engl J Med.* 2023;389(20):1862–76.
54. Karran E, De Strooper B. The amyloid hypothesis in Alzheimer disease: new insights from new therapeutics. *Nat Rev Drug Discov.* 2022;21(4):306–18.
55. Grimm HP, Schumacher V, Schäfer M, Imhof-Jung S, Freskgård PO, Brady K, et al. Delivery of the Brainshuttle™ amyloid-beta antibody fusion trontinemab to non-human primate brain and projected efficacious dose regimens in humans. *MAbs.* 2023;15(1):2261509.
56. Ultsch M, Li B, Maurer T, Mathieu M, Adolfsson O, Muhs A, et al. Structure of crenezumab complex with A β shows loss of β -hairpin. *Sci Rep.* 2016;6(1):39374.
57. Adolfsson O, Pihlgren M, Toni N, Varisco Y, Buccarello AL, Antonello K, et al. An effector-reduced anti-beta-amyloid (Abeta) antibody with unique abeta binding properties promotes neuroprotection and glial engulfment of abeta. *J Neurosci.* 2012;32(28):9677–89.
58. Sperling RA, Jack CR Jr, Black SE, Frosch MP, Greenberg SM, Hyman BT, et al. Amyloid-related imaging abnormalities in amyloid-modifying therapeutic trials: recommendations from the Alzheimer's association research roundtable workgroup. *Alzheimers Dement.* 2011;7(4):367–85.
59. Krafft G, Hefti F, Goure W, Jerecic J, Iverson K, Walicke P. O2–06–03: ACU-193: a candidate therapeutic antibody that selectively targets soluble beta-amyloid oligomers. *Alzheimers Dement.* 2013;9:326.
60. Acumen Pharmaceuticals Presents Positive Topline Results from First-in-Human Phase 1 Study of ACU193 for Early Alzheimer's Disease at the Alzheimer's Association International Conference (AAIC). 2023.
61. Mantile F, Prisco A. Vaccination against beta-Amyloid as a Strategy for the prevention of Alzheimer's disease. *Biology (Basel).* 2020;9(12):425.
62. Panza F, Lozupone M, Seripa D, Imbimbo BP. Amyloid-beta immunotherapy for Alzheimer disease: Is it now a long shot? *Ann Neurol.* 2019;85(3):303–15.
63. Hasecke F, Niyangoda C, Borjas G, Pan J, Matthews G, Muschol M, et al. Protofibril-fibril interactions inhibit amyloid fibril assembly by obstructing secondary nucleation. *Angew Chem Int Ed.* 2021;60(6):3016–21.
64. Jamasbi E, Wade JD, Separovic F, Hossain MA. Amyloid beta (abeta) peptide and factors that play important roles in Alzheimer's disease. *Curr Med Chem.* 2016;23(9):884–92.
65. Sengupta U, Nilson AN, Kaye R. The role of amyloid-beta oligomers in toxicity, propagation, and immunotherapy. *EBioMedicine.* 2016;6:42–9.
66. Linse S, Scheidt T, Bernfur K, Vendruscolo M, Dobson CM, Cohen SLA, et al. Kinetic fingerprints differentiate the mechanisms of action of anti-beta antibodies. *Nat Struct Mol Biol.* 2020;27(12):1125–33.
67. Kumar S, Walter J. Phosphorylation of amyloid beta (Abeta) peptides—a trigger for formation of toxic aggregates in Alzheimer's disease. *Aging (Albany NY).* 2011;3(8):803–12.
68. Zampar S, Wirths O. Immunotherapy targeting amyloid- β peptides in Alzheimer's disease, Chapter 2. In: Huang X, editor. *Alzheimer's Disease: Drug Discovery* [Internet]. Brisbane (AU): Exon Publications; 2020.
69. Das P, Howard V, Loosbrock N, Dickson D, Murphy MP, Golde TE. Amyloid-beta immunization effectively reduces amyloid deposition in FcRgamma/knock-out mice. *J Neurosci.* 2003;23(24):8532–8.
70. Loeffler DA. Antibody-mediated clearance of brain amyloid-beta: mechanisms of action, effects of natural and monoclonal anti-beta antibodies, and downstream effects. *J Alzheimers Dis Rep.* 2023;7(1):873–93.
71. Alyanekian MA, Bernatowska E, Scherrmann JM, Aucouturier P, Poplavsky JL. Pharmacokinetics of total immunoglobulin G and immunoglobulin G subclasses in patients undergoing replacement therapy for primary immunodeficiency syndromes. *Vox Sang.* 2003;84(3):188–92.
72. Salfeld JG. Isotype selection in antibody engineering. *Nat Biotechnol.* 2007;25(12):1369–72.
73. Kapur R, Einarsdottir HK, Vidarsson G. IgG-effector functions: "the good, the bad and the ugly." *Immunol Lett.* 2014;160(2):139–44.
74. Pan XD, Zhu YG, Lin N, Zhang J, Ye QY, Huang HP, et al. Microglial phagocytosis induced by fibrillar beta-amyloid is attenuated by oligomeric beta-amyloid: implications for Alzheimer's disease. *Mol Neurodegener.* 2011;6:45.
75. Bittar A, Sengupta U, Kaye R. Prospects for strain-specific immunotherapy in Alzheimer's disease and tauopathies. *NPJ Vaccines.* 2018;3:9.
76. Wisniewski T, Konietzko U. Amyloid-beta immunisation for Alzheimer's disease. *Lancet Neurol.* 2008;7(9):805–11.
77. Black RS, Sperling RA, Safirstein B, Motter RN, Pallay A, Nichols A, et al. A single ascending dose study of bapineuzumab in patients with Alzheimer disease. *Alzheimer Dis Assoc Disord.* 2010;24(2):198–203.
78. Hampel H, Elhage A, Cho M, Apostolova LG, Nicoll JAR, Atri A. Amyloid-related imaging abnormalities (ARIA): radiological, biological and clinical characteristics. *Brain.* 2023;146(11):4414–24.
79. Holste X, Xia F, Garton HJL, Wan S, Hua Y, Keep RF, et al. The role of complement in brain injury following intracerebral hemorrhage: a review. *Exp Neurol.* 2021;340: 113654.
80. Taylor X, Clark IM, Fitzgerald GJ, Oluoch H, Hole JT, DeMattos RB, et al. Amyloid-beta (Abeta) immunotherapy induced microhemorrhages are associated with activated perivascular macrophages and peripheral monocyte recruitment in Alzheimer's disease mice. *Mol Neurodegener.* 2023;18(1):59.
81. Aldea R, Grimm HP, Gieschke R, Hofmann C, Lott D, Bullain S, et al. In silico exploration of amyloid-related imaging abnormalities in the gantenerumab open-label extension trials using a semi-mechanistic model. *Alzheimers Dement (N Y).* 2022;8(1): e12306.
82. Hashimoto T, Serrano-Pozo A, Hori Y, Adams KW, Takeda S, Banerji AO, et al. Apolipoprotein E, especially apolipoprotein E4, increases the oligomerization of amyloid beta peptide. *J Neurosci.* 2012;32(43):15181–92.
83. Lemere CA. Immunotherapy for Alzheimer's disease: hoops and hurdles. *Mol Neurodegener.* 2013;8:36.
84. St-Amour I, Pare I, Alata W, Coulombe K, Ringuette-Goulet C, Drouin-Ouellet J, et al. Brain bioavailability of human intravenous immunoglobulin and its transport through the murine blood-brain barrier. *J Cereb Blood Flow Metab.* 2013;33(12):1983–92.
85. Thom G, Hatcher J, Hearn A, Paterson J, Rodrigo N, Beljeau A, et al. Isolation of blood-brain barrier-crossing antibodies from a phage display library by competitive elution and their ability to penetrate the central nervous system. *MAbs.* 2018;10(2):304–14.
86. Pardridge WM. Re-engineering therapeutic antibodies for Alzheimer's disease as blood-brain barrier penetrating bi-specific antibodies. *Expert Opin Biol Ther.* 2016;16(12):1455–68.
87. Martin-Pena A, Rincon-Limas DE, Fernandez-Funez P. Anti-Abeta single-chain variable fragment antibodies restore memory acquisition in a *Drosophila* model of Alzheimer's disease. *Sci Rep.* 2017;7(1):11268.
88. Abskharon R, Pan H, Sawaya MR, Seidler PM, Olivares EJ, Chen Y, et al. Structure-based design of nanobodies that inhibit seeding of Alzheimer's patient-extracted tau fibrils. *Proc Natl Acad Sci U S A.* 2023;120(41): e2300258120.
89. Jovcevska I, Muyldermans S. The therapeutic potential of nanobodies. *BioDrugs.* 2020;34(1):11–26.
90. Abrahao A, Meng Y, Llinas M, Huang Y, Hamani C, Mainprize T, et al. First-in-human trial of blood-brain barrier opening in amyotrophic lateral sclerosis using MR-guided focused ultrasound. *Nat Commun.* 2019;10(1):4373.
91. Burgess A, Shah K, Hough O, Hynynen K. Focused ultrasound-mediated drug delivery through the blood-brain barrier. *Expert Rev Neurother.* 2015;15(5):477–91.
92. DeTure MA, Dickson DW. The neuropathological diagnosis of Alzheimer's disease. *Mol Neurodegener.* 2019;14(1):32.
93. Roberts BR, Lind M, Wagen AZ, Rembach A, Frugier T, Li QX, et al. Biochemically-defined pools of amyloid-beta in sporadic Alzheimer's disease: correlation with amyloid PET. *Brain.* 2017;140(5):1486–98.
94. Ayton S. Brain volume loss due to donanemab. *Eur J Neurol.* 2021;28(9):e67–8.
95. Alves F, Kalinowski P, Ayton S. Accelerated brain volume loss caused by anti-beta-amyloid drugs: a systematic review and meta-analysis. *Neurology.* 2023;100(20):e2114–24.
96. Barkhof F, Knopman DS. Brain shrinkage in anti-beta-amyloid Alzheimer trials: Neurodegeneration or pseudoatrophy? *Neurology.* 2023;100(20):941–2.

97. Zhao P, Zhang Y, Li W, Jeanty C, Xiang G, Dong Y. Recent advances of antibody drug conjugates for clinical applications. *Acta Pharm Sin B*. 2020;10(9):1589–600.
98. Diringier MN, Scalfani MT, Zazulia AR, Videen TO, Dhar R, Powers WJ. Effect of mannitol on cerebral blood volume in patients with head injury. *Neurosurgery*. 2012;70(5):1215–8.
99. Haluska M, Anthony ML. Osmotic blood-brain barrier modification for the treatment of malignant brain tumors. *Clin J Oncol Nurs*. 2004;8(3):263–7.
100. Punyakoti P, Behl T, Sehgal A, Yadav S, Sachdeva M, Anwer MK, et al. Postulating the possible cellular signalling mechanisms of antibody drug conjugates in Alzheimer's disease. *Cell Signal*. 2023;102: 110539.
101. Alabi SB, Crews CM. Major advances in targeted protein degradation: PROTACs, LYTACs, and MADTACs. *J Biol Chem*. 2021;296: 100647.
102. Banik SM, Pedram K, Wisnovsky S, Ahn G, Riley NM, Bertozzi CR. Lysosome-targeting chimaeras for degradation of extracellular proteins. *Nature*. 2020;584(7820):291–7.
103. He M, Cao C, Ni Z, Liu Y, Song P, Hao S, et al. PROTACs: great opportunities for academia and industry (an update from 2020 to 2021). *Signal Transduct Target Ther*. 2022;7(1):181.
104. Zhao L, Zhao J, Zhong K, Tong A, Jia D. Targeted protein degradation: mechanisms, strategies and application. *Signal Transduct Target Ther*. 2022;7(1):113.
105. Jung H, Lee SY, Lim S, Choi HR, Choi Y, Kim M, et al. Anti-inflammatory clearance of amyloid-beta by a chimeric Gas6 fusion protein. *Nat Med*. 2022;28(9):1802–12.
106. Myers KV, Amend SR, Pienta KJ. Targeting Tyro3, Axl and MerTK (TAM receptors): implications for macrophages in the tumor microenvironment. *Mol Cancer*. 2019;18(1):94.
107. Jung H, Lee SY, Kang MS, Jung H-J, Shin A-Y, Song J-Y, et al. Anti-inflammatory clearance of amyloid beta by a chimeric Gas6 fusion protein. *Alzheimers Dement*. 2023;19(S21): e072368.
108. Jucker M, Walker LC. Alzheimer's disease: from immunotherapy to immunoprevention. *Cell*. 2023;186(20):4260–70.
109. Bacioglu M, Maia LF, Preische O, Schelle J, Apel A, Kaeser SA, et al. Neurofilament light chain in blood and CSF as marker of disease progression in mouse models and in neurodegenerative diseases. *Neuron*. 2016;91(1):56–66.
110. Cummings J, Lee G, Nahed P, Kambar M, Zhong K, Fonseca J, et al. Alzheimer's disease drug development pipeline. *Alzheimers Dement (N Y)*. 2022;8(1): e12295.
111. Blennow K. Phenotyping Alzheimer's disease with blood tests. *Science*. 2021;373(6555):626–8.
112. Horie K, Salvado G, Barthelemy NR, Janelidze S, Li Y, He Y, et al. CSF MTBR-tau243 is a specific biomarker of tau tangle pathology in Alzheimer's disease. *Nat Med*. 2023;29(8):1954–63.
113. Mila-Aloma M, Ashton NJ, Shekari M, Salvado G, Ortiz-Romero P, Montoliu-Gaya L, et al. Plasma p-tau231 and p-tau217 as state markers of amyloid-beta pathology in preclinical Alzheimer's disease. *Nat Med*. 2022;28(9):1797–801.
114. Li Y, Yen D, Hendrix RD, Gordon BA, Dlamini S, Barthélemy NR, et al. Timing of biomarker changes in sporadic Alzheimer's disease in estimated years from symptom onset. *Ann Neurol*. 2024;95(5):951–65.
115. Rosenberg PB, Lyketsos C. Mild cognitive impairment: searching for the prodrome of Alzheimer's disease. *World Psychiatry*. 2008;7(2):72–8.
116. Kumar P, Dezso Z, MacKenzie C, Oestreicher J, Agoulnik S, Byrne M, et al. Circulating miRNA biomarkers for Alzheimer's disease. *PLoS ONE*. 2013;8(7): e69807.
117. Swarbrick S, Wragg N, Ghosh S, Stolzing A. Systematic review of miRNA as biomarkers in Alzheimer's disease. *Mol Neurobiol*. 2019;56(9):6156–67.
118. Lim J, Kim S, Oh SJ, Han SM, Moon SY, Kang B, et al. miRNA sensing hydrogels capable of self-signal amplification for early diagnosis of Alzheimer's disease. *Biosens Bioelectron*. 2022;209: 114279.
119. Liu S, Fan M, Zheng Q, Hao S, Yang L, Xia Q, et al. MicroRNAs in Alzheimer's disease: potential diagnostic markers and therapeutic targets. *Biomed Pharmacother*. 2022;148: 112681.
120. Krüger DM, Pena-Centeno T, Liu S, Park T, Kaurani L, Pradhan R, et al. The plasma miRNAome in ADNI: Signatures to aid the detection of at-risk individuals. *Alzheimer's & Dementia*.
121. Palmqvist S, Tideman P, Mattsson-Carligen N, Schindler SE, Smith R, Ossenkoppele R, et al. Blood biomarkers to detect Alzheimer disease in primary care and secondary care. *JAMA*. 2024;332(15):1245–1257.
122. Vergallo A, Lista S, Zhao Y, Lemercier P, Teipel SJ, Potier MC, et al. MiRNA-15b and miRNA-125b are associated with regional Aβ-PET and FDG-PET uptake in cognitively normal individuals with subjective memory complaints. *Transl Psychiatry*. 2021;11(1):78.
123. Madras K, Das R, Mohmmadabdul H, Lin L, Hyman BT, Lauffenburger DA, et al. Systematic in silico analysis of clinically tested drugs for reducing amyloid-beta plaque accumulation in Alzheimer's disease. *Alzheimers Dement*. 2021;17(9):1487–98.
124. Busciglio J, Lorenzo A, Yeh J, Yankner BA. Beta-amyloid fibrils induce tau phosphorylation and loss of microtubule binding. *Neuron*. 1995;14(4):879–88.