Review paper

Folia Neuropathologica

Current study on diagnosis and treatment of Alzheimer's disease by targeting amyloid β-protein

Kai-Ye Hua[1], Wei-Jiang Zhao[1,2]

[1]Wuxi School of Medicine, Jiangnan University, Wuxi, Jiangsu, P.R. China, [2]Cell Biology Department, Wuxi School of Medicine, Jiangnan University, Wuxi, Jiangsu, P.R. China

Folia Neuropathol 2023; 61 (1): 8-15 *DOI: https://doi.org/10.5114/fn.2022.119778*

Abstract

Alzheimer's disease (AD), also known as senile dementia, is a degenerative disease of the central nervous system and is characterized by insidious onset and a chronic progressive course. It is the most common type of senile dementia. Studies have proved that the deposition of amyloid β (Aβ) in the brain is one of the initiating factors correlated to the pathology of AD, and it acts as one of the critical factors leading to the onset of AD. A large number of long-term studies have shown that Aβ may be a therapeutic target for a breakthrough in the treatment of AD. This review elucidates the important role of Aβ in the development of AD, current research on the role of Aβ in AD pathogenesis, and treatment of AD by targeting Aβ.

Key words: *Alzheimer's disease, amyloid β, pathogenesis, treatment.*

Introduction

Alzheimer's disease (AD) is one of the most common degenerative diseases of the brain. It is caused by multiple genetic and environmental factors. AD occurs more frequently in the elderly over 65 years old. The main manifestation of AD is dementia. The course of AD varies, the average duration is seven years, and most people die of complications. In addition to the age of onset, the disease is also characterized by familial aggregation, resulting from the interaction between genetic and environmental factors [16]. In a 2018 survey, more than 10 million people in China suffered from AD, almost doubling every ten years [61]. Over the past 100 years, many pathophysiological aspects of AD have been discovered and identified. Many clinical studies have shown that the onset of AD may be induced by a variety of factors, including excessive aggregation of amyloid β (Aβ) and neurofibrillary tangles (NFTs) formed by hyperphosphorylation of tau protein, absence of the neurotransmitter acetylcholine (ACh), activation of microglia by inflammatory reactions, and brain cell damage caused by advanced glycation end products (AGEs) with their receptors (RAGE) [45,40], and so on. Also, researchers found that there were significant gender differences in the incidence of AD after the age of 85 years. At the age of 90, the incidence of AD is three times higher in women than in men [2]. There is a significant interaction between sex and $A\beta_{42}$ on longitudinal hippocampal atrophy, and longitudinal decline in memory and executive function [28]. A recent study shows that follicle-stimulating hormone (FSH), whose serum level is rising sharply in women before the last menstrual cycle, acts directly on hippocampal and cortical neurons to accelerate Aβ and Tau deposition and impairs cognition in mice displaying features of AD [66]. FSH can phosphorylate AKT, ERK1/2, and SRPK2, leading to the activation of C/EBPβ-AEP/δ-secretase and the cleavage of APP [66]. The study suggests that a highly targeted anti-FSH antibody could be a new way to treat AD [66]. Currently, AD can be alleviated by drugs but cannot be cured. With the aging issue

Communicating author:

Wei-Jiang Zhao, Cell Biology Department, Wuxi School of Medicine, Jiangnan University, 1800 Lihu Dadao, Wuxi 214122, Jiangsu, P.R. China, phone: +86 13794125386, e-mail: weijiangzhao@jiangnan.edu.cn

in China becoming increasingly severe and the number of AD patients increasing year by year, it is essential to explore the treatment of AD [65]. We here review the current research progress in the treatment of AD by targeting Aβ and hope to provide some references for developing new drugs for AD.

The formation mechanism and types of amyloid β

As early as last century, scientists had discovered the presence of Aβ fibrillary protein in the brain of AD patients by electron microscope observation and pointed out that Aβ was related to the occurrence of AD [26]. Aβ is a kind of polypeptide highly resistant to proteolysis and is produced when amyloid precursor protein (APP) is cleaved by corresponding proteases. Two pathways are responsible for APP cleavage, one of which is the usual pathway and the other Aβ cleavage pathway (Fig. 1). Aβ is composed of 37-43 amino acids, of which $Aβ_{40}$ and $Aβ_{42}$ are the most common subtypes [10]. Most Aβ peptides have a length of 40 residues ($Aβ_{40}$), while a small number have a length of 42 residues ($Aβ_{42}$). $Aβ_{42}$ is generally considered more neurotoxic due to the increased possibility of misfolding and protein aggregation caused by the binding of two residues [1]. In addition, the low solubility of $Aβ_{42}$ makes it more likely to accumulate and form fibrous substances, which then form Aβ plaques. Aβ protein can accumulate in the extracellular matrix after releasing from cells by exocytosis [4,60]. The lysosome can degrade the Aβ protein. However, as people get older, the permeability of the lysosome membrane changes, making Aβ protein outflow more easily to result in the accumulation of Aβ protein. Meanwhile, the lysozyme activity also decreases, leading to a decrease in dissolving efficiency and the accumulation of Aβ protein. The accumulation of Aβ protein leads to an increase in Golgi vesicles, causing dendrites to swell and shorten and impeding neurotransmitter transmission [38,41].

Neuronal effects of amyloid β on neurons at different levels

N-methyl-d-aspartate receptor (NMDAR) mediates transmission and plasticity changes between excitatory synapses. The subunits discovered so far are NR1, NR2 (A-D), and NR3 (A, B) [19]. Under normal circumstances, Aβ promotes the release of glutamate from vesicles into the synaptic cleft and acts on the NMDAR on the postsynaptic membrane. When excess glutamate is released and astrocytes are dysfunctional, glutamate can accumulate in the synaptic cleft and cause the activation of NMDAR outside the synapse, leading to calcium overload in postsynaptic neurons and inducing excitatory toxicity, neuronal apoptosis, and neurodegeneration [29].

In previous studies, researchers investigated the effects of $Aβ_{42}$ on neuronal excitability and voltage-gated sodium channels using a cellular patch-clamp, and they found that $Aβ_{42}$ can reduce the threshold of neuronal action potential and increase the firing frequency

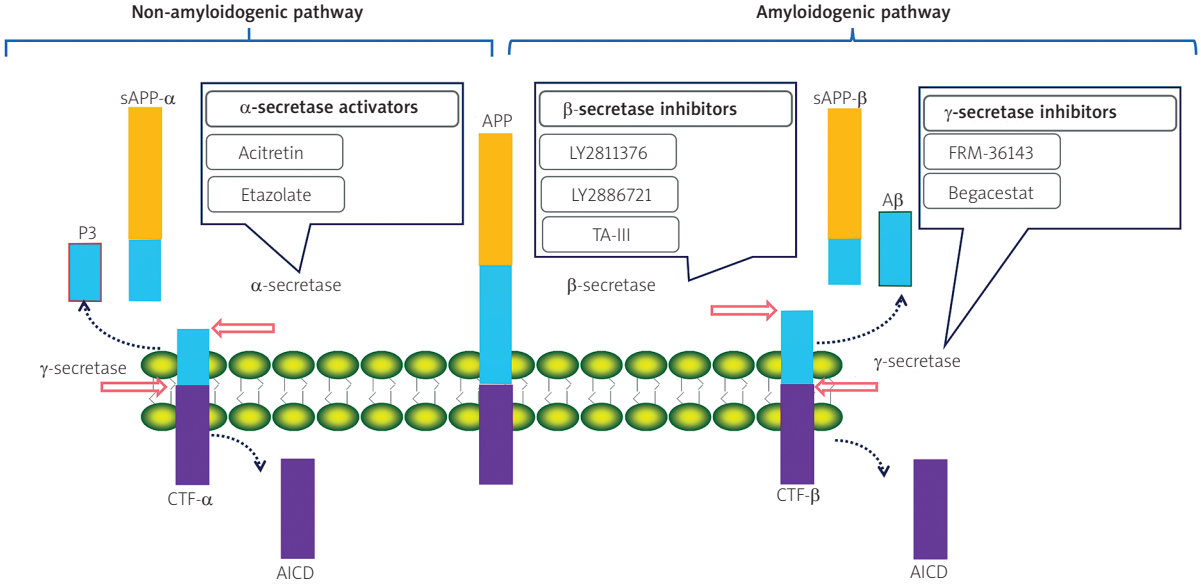

Fig. 1. Schematic diagram of the amyloid precursor protein (APP) cleavage pathway, based on which the drugs and corresponding targeting secretases are shown. sAPP – soluble amyloid precursor protein, AICD – APP intracellular domain, CTF – C-terminal fragment, Aβ – amyloid β-protein.

of neuronal action potential [17]. Inhibition of sodium channels and excitability may be a target for treating AD.

In addition, exogenous $A\beta_{40}$ may reduce the survival rate of neural stem cells and reduce the proportion of neural stem cells that differentiate into neurons by inducing neural stem cell apoptosis. This can lead to a decrease in the number of neurons within the human brain and ultimately to the onset of AD [5].

Many studies in animal models and *ex vivo* cultures indicate that amyloid β-peptide oligomers induce synaptic damage early during the progression of AD [11]. And further research showed that the Aβ oligomers exhibit early dual effects by acutely enhancing dendritic complexity and spine density [50].

Amyloid β-based diagnostic method

In 2007, the International Working Group (IWG) launched a new study on AD diagnosis, presenting a conceptual framework with criteria introducing biomarkers into the diagnosis of AD [12]. With the development of non-invasive diagnostic techniques, a new method for improving diagnostic accuracy has emerged. Positron emission computed tomography was used to detect the deposition of Aβ in living brain plaques after injection of radiolabelled tracers [8]. For example, in recent years, several fluoro-18 (18F)-labelled amyloid tracers have been developed [68]. 18F-florbetaben Aβ imaging facilitates accurate detection of prodromal AD. In addition, Aβ detection in cerebrospinal fluid (CSF) can also assist in AD diagnosis, but the accuracy is slightly lower than that of 18F-florbetaben Aβ imaging [20]. Early this year, researchers used a functional probe responding to Aβ oligomer *via* second near-infrared window (NIR-II) fluorescence imaging, which is more sensitive to Aβ oligomer (Kd = 6.16 nM) than Aβ fibrils. In addition, this method showed no response to other small biological molecules, proving to be conducive to the early diagnosis of AD *in vivo* [33]. In addition, because Aβ can migrate from the blood-brain barrier to plasma, studies have assessed $A\beta_{40}$ and $A\beta_{42}$ levels in human red blood cells (RBC). With evidence of an age-dependent increase of Aβ concentration in RBCs, confirmation of RBC-associated Aβ may be a tool for early recognition of dementia and predicting cognitive impairment [32].

Treatment of Alzheimer's disease with amyloid β protein as a therapeutic target

Inhibition of Aβ generation

Multiple studies have shown that Aβ oligomers and plaques at all stages of AD can produce direct neurotoxic effects. It has been recently proposed that in the predementia stage of AD, removal of the negative impact of Aβ pathology may improve memory and hippocampal function [13]. Therefore, reducing the production of Aβ polypeptide to minimize the formation of Aβ oligomer and plaque expansion is of great significance for the treatment of AD [62].

Activation of α-secretase

A disintegrin and metalloproteinase (ADAM) is a family consisting of transmembrane proteins and secretive metalloproteinases composed of approximately 750 amino acids. It functions by promoting cell adhesion and proteolysis [23]. ADAM10 is a major α-secretase that inhibits Aβ peptide formation from APP in the non-amyloid pathway. Enhanced ADAM10 activation can promote the generation of soluble APP-α (sAPPα), a derivative fragment of APP, thus protecting the brain by inhibiting Aβ production [67]. This fragment has been shown to exert neuroprotective effects [36]. Since both Aβ and sAPPα are sequentially proteolysed products of APP protein, its production can be reduced by increasing the generation of sAPPα [1,10]. It was found that α-secretase can not only increase benign APP metabolism but also inhibit the activity of γ-secretase in the HEK293 cell line [56].

Since α-secretase has few side effects, enhancing its activity is a potentially effective treatment for AD. Previous studies have demonstrated that Acitretin can promote the increase of mature ADAM10, thus increasing the production of a benign metabolite of APP [14,52]. Since APP processing can regulate lipid metabolism, lipid homeostasis should be closely monitored during the application of Acitretin to avoid hyperlipidaemia [31]. Etazolate, a kind of neuroprotective drug linking GABA(A), was shown to activate α-secretase and increase sAPPα production [35] (Fig. 1).

Inhibition of β-secretase

β-secretase (BACE) is a transmembrane aspartate protease that cleaves APP to generate sAPPβ and its C-terminal fragment CTF-β. BACE is the main target enzyme for treating AD [51].

β-secretase-1 (BACE-1) expression levels are increased in most patients with AD, and APP cleavage by β-secretase can produce pathological products [59]. Therefore, BACE may be a significant therapeutic target for AD. Over the years, β-secretase inhibitors, such as LY2811376 and LY2886721, have often been discontinued due to toxic side effects [39]. Researchers identified TA-III in Rhizoma Anemarrhenae (RA) as a novel BACE-1 inhibitor by combining cell extraction and chemical genomics target guidance, and verified its potential pharmacological target as BACE-1 using sur-

face plasmon resonance (SPR), enzyme activity measurement, and visual molecular docking method. TA-III was confirmed to prevent neuronal injury by improving memory impairment, reducing Aβ aggregation through the amygdala pathway, and impairing the NMDAR/ERK signalling pathway [63] (Fig. 1).

Inhibition of γ-secretase

γ-secretase is a kind of intramembrane aspartic acid protease, a member of the intramembrane-cleaving-proteases (I-Clips). γ-secretase is comprised of presenilin (PS), nicastrin (NCT), anterior pharynx-defective-1 (APH-1), and presenilin enhancer-2 (PEN-2) [27]. β-secretase cleaves APP to produce two fragments, while the final step of Aβ production requires γ-secretase to cleave the CTF-β fragment. Because of its important role in Aβ production, γ-secretase has also become an important drug target. Blocking γ-secretase cleavage of APP can reduce the amount of toxic Aβ and ultimately achieve the purpose of treating or alleviating the progression of AD [9]. Begacestat, a novel, 2,5-disubstituted thiophene sulfonamide from Wyeth (now Pfizer), has been identified as a potential γ-secretase inhibitor [37]. It has also shown promise in clinical trials [22]. In 2016, researchers reported the characterization of FRM-36143, a compound derived from optimizing a novel structural class of γ-secretase modulators (GSMs). It has been confirmed to reverse the effect of PS mutations, which may benefit the patients when treated early enough in the disease [6] (Fig. 1).

Drug that antagonizes amyloid β

Of the four targeted drugs with potential for approval [57], Alz-801 is an optimized precursor drug modified from taurine, a kind of Aβantagonist. The metabolic product of tramiprosate and the precursor drug ALZ-801 in humans is 3-sulfopropionic acid (3-SPA), whose level is 12.6 times higher in AD patients treated with tramiprosate than in untreated patients. Multiple 3-SPA molecules can interact with the Aβ42 molecule to inhibit its polymerization into oligomers, significantly affecting AD patients with the apolipoprotein E (ApoE) ε4 gene.

Promoting the clearance of amyloid β

Transportation through the blood-brain barrier

The deposition of Aβ may begin years or even more than ten years before the onset of AD, so early intervention to remove Aβ is crucial [48]. Extracellular deposits of Aβ can be removed from the brain by various clearance systems, most notably by transportation through the blood-brain barrier (BBB) [54]. Earlier studies in mice showed that about 75% of total extracellular Aβ can be cleared through the BBB, and only a few can be cleared by interstitial fluid (ISF) [43]. Subsequently, researchers found that CSF enters the parenchyma along paravascular spaces surrounding penetrating arteries and that brain interstitial fluid is cleared along paravenous drainage pathways. Loss of the Aquaporin 4 (Aqp4) gene inhibits the clearance of soluble Aβ during CSF mass flow, suggesting that this pathway may play a role in the removal of Aβ from the central nervous system [24].

Amyloid β clearance by microglia and astrocytes

Studies have shown that Aβ aggregation induces the activation of microglia. Although activated microglia have the role of phagocytosis and clearance of Aβ to reduce the damage of Aβ to brain tissues, they can secrete a large number of cytotoxic substances, thus aggravating the inflammatory response [21]. Aβ induced reduction of lysosomes possibly through inhibition of mTOR/TFEB signalling pathway in both neurons and microglia in AD transgenic mice. The promotion of lysosome degradation can effectively reduce immature autophagosome accumulation, accelerating autophagosome clearance of Aβ and other pathological proteins [53]. This study also offers new ideas for treating AD.

Recent reports demonstrated that low-density lipoprotein receptor-related protein-1 (LRP1) plays a dominant role in clearing excessive Aβ production in an astrocyte-dependent clearance pathway [58]. LRP1 regulates the uptake of Aβ and degradation in astrocytes, and loss of low-density LRP in astrocytes can reduce the clearance of Aβ [34].

Immunotherapy targeting amyloid β

The results using animal AD models suggested that active and passive immunotherapy targeting Aβ is promising [64]. However, many side effects have been reported about the active immunotherapy for AD, such as encephalitis and cerebral haemorrhage [42]. AN-1792 was the first active immunotherapy strategy for AD. However, it was interrupted by meningoencephalitis in 6% of immunized patients in a Phase IIa immunotherapy trial [18]. In 2006, researchers constructed an AD mouse model in which Aβ derivative was co-administered with alum adjuvant to promote humoral immunity. This immunotherapy can reduce Aβ load without increasing vascular Aβ deposits or microhaemorrhages at 11-24 months post-treatment [3]. This study also suggests that the Aβ derivative, when used with an adjuvant suitable for humans, can reduce Aβ load and promote cognitive improvement without

increasing vascular microbleeds compared to other Aβ antibody studies [3]. In 2021, Fang *et al*. [15] reported a novel recombinant chimeric 12×(Aβ1-15-Th) antigen targeting the pathological confirmation of Aβ oligomer for AD treatment. They tested the immunogenicity of the chimeric vaccine in C57/BL6 mice and demonstrated its efficacy in AD mice. The results showed that the chimeric vaccine showed a noticeable neuroprotective effect by reducing the levels of soluble Aβ oligomers and soluble Aβ in the brain, suggesting that the chimeric vaccine is a good candidate for AD prevention [15]. ABvac40 is an active vaccine against the C-terminal end of Aβ40, and an ongoing Phase II clinical trial is needed to explore the clinical efficacy of ABvac40 [30]. In an active immunotherapy study, Aβ-targeted antibodies prevent the fibrosis of Aβ peptides, destroy preformed fibres, and interfere with the aggregation of Aβ [47].

Passive immunization is defined as a prefabricated antibody injection to provide immunity to the host. Examples include Aducanumab, a molecule screened from the human memory B cell family that binds specifically to Aβ aggregates, and Gantenerumab, an experimental immunoglobulin G1 (IgG1) antibody that binds specifically to Aβ [7]. Both drugs have the role of activating immune response and removing antigen-antibody complexes [46]. In addition, BAN2401 is a humanized IgG1 monoclonal antibody that selectively binds to soluble Aβ aggregated species. Previous studies have shown that BAN2401 can reduce amyloid loading on PET examination, slowing cognitive decline in a Phase II study in early symptomatic AD. It is currently tested in Phase III clinical trials to treat AD [44].

In addition to monotherapy, Aβ deposition can also be eliminated through passive Aβ immunotherapy and β secretase inhibitors. By combining an antibody treatment with chronic BACE1 inhibitor treatment, researchers demonstrate significant clearance of pre-existing amyloid deposits in Balb/c transgenic mice brains without inducing microhaemorrhages and other histopathological findings [25]. Overcoming the BBB is vital for most therapeutic drugs entering brain tissues. Nanoparticle (NP) mediated drug delivery systems prove to be a potential means of enhancing brain tissue-targeted drug transport through BBB [55]. Since nanoparticles can be used as drug delivery carriers across the BBB, the combination of nanoparticles and antibody fragments has been used to diagnose AD [49].

Conclusions

The pathogenesis of AD may be caused by multiple pathways and aetiology. Although there are side effects or insignificant effects of drugs in Phase I and Phase II clinical trials, new targets of drug action have been constantly discovered with the development of scientific research and technology and the deepening of related studies on the pathological mechanism of AD, and relevant drugs have been approved. There are still many candidate proteins targeting Aβ under investigation, which brings hope to cure AD. This review is expected to provide some references for developing treatments against AD by targeting Aβ.

Acknowledgements

This research was funded by Jiangsu Province Shuangchuang Talent Plan (Grant No. JSSCRC 2021533), College Students' Innovation and Entrepreneurship Training Program of Jiangsu Province (Grant No. 202110295144Y), the Research Start-up Fund of Jiangnan University (Grant No. 1285081903200020), and Research Start-up Fund of Wuxi School of Medicine, Jiangnan University (Grant No. 1286010242190060).

Disclosure

The authors report no conflict of interest.

References

1. Ahmed M, Davis J, Aucoin D, Sato T, Ahuja S, Aimoto S, Elliott JI, Van Nostrand WE, Smith SO. Structural conversion of neurotoxic amyloid-beta(1-42) oligomers to fibrils. Nat Struct Mol Biol 2010; 17: 561-567.
2. Andersen K, Launer LJ, Dewey ME, Letenneur L, Ott A, Copeland JR, Dartigues JF, Kragh-Sorensen P, Baldereschi M, Brayne C, Lobo A, Martinez-Lage JM, Stijnen T, Hofman A. Gender differences in the incidence of AD and vascular dementia: The EURODEM Studies. EURODEM Incidence Research Group. Neurology 1999; 53: 1992-1997.
3. Asuni AA, Boutajangout A, Scholtzova H, Knudsen E, Li YS, Quartermain D, Frangione B, Wisniewski T, Sigurdsson EM. Vaccination of Alzheimer's model mice with Abeta derivative in alum adjuvant reduces Abeta burden without microhemorrhages. Eur J Neurosci 2006; 24: 2530-2542.
4. Bateman RJ, Xiong C, Benzinger TL, Fagan AM, Goate A, Fox NC, Marcus DS, Cairns NJ, Xie X, Blazey TM, Holtzman DM, Santacruz A, Buckles V, Oliver A, Moulder K, Aisen PS, Ghetti B, Klunk WE, McDade E, Martins RN, Masters CL, Mayeux R, Ringman JM, Rossor MN, Schofield PR, Sperling RA, Salloway S, Morris JC. Clinical and biomarker changes in dominantly inherited Alzheimer's disease. N Engl J Med 2012; 367: 795-804.
5. Bernabeu-Zornoza A, Coronel R, Palmer C, López-Alonso V, Liste I. Oligomeric and fibrillar species of Aβ42 diversely affect human neural stem cells. Int J Mol Sci 2021; 22: 9537.
6. Blain JF, Bursavich MG, Freeman EA, Hrdlicka LA, Hodgdon HE, Chen T, Costa DE, Harrison BA, Kapadnis S, Murphy DA, Nolan S, Tu Z, Tang C, Burnett DA, Patzke H, Koenig G. Characterization of FRM-36143 as a new γ-secretase modulator for the potential treatment of familial Alzheimer's disease. Alzheimers Res Ther 2016; 8: 34.
7. Bohrmann B, Baumann K, Benz J, Gerber F, Huber W, Knoflach F, Messer J, Oroszlan K, Rauchenberger R, Richter WF, Rothe C, Urban M, Bardroff M, Winter M, Nordstedt C, Loetscher H. Gantenerumab: a novel human anti-Aβantibody demonstrates

sustained cerebral amyloid-β binding and elicits cell-mediated removal of human amyloid-β. J Alzheimers Dis 2012; 28: 49-69.
8. Clark CM, Pontecorvo MJ, Beach TG, Bedell BJ, Coleman RE, Doraiswamy PM, Fleisher AS, Reiman EM, Sabbagh MN, Sadowsky CH, Schneider JA, Arora A, Carpenter AP, Flitter ML, Joshi AD, Krautkramer MJ, Lu M, Mintun MA, Skovronsky DM. Cerebral PET with florbetapir compared with neuropathology at autopsy for detection of neuritic amyloid-β plaques: a prospective cohort study. Lancet Neurol 2012; 11: 669-678.
9. Crump CJ, Johnson DS, Li YM. Development and mechanism of γ-secretase modulators for Alzheimer's disease. Biochemistry 2013; 52: 3197-3216.
10. Deane R, Bell RD, Sagare A, Zlokovic BV. Clearance of amyloid-beta peptide across the blood-brain barrier: implication for therapies in Alzheimer's disease. CNS Neurol Disord Drug Targets 2009; 8: 16-30.
11. DeKosky ST, Scheff SW. Synapse loss in frontal cortex biopsies in Alzheimer's disease: correlation with cognitive severity. Ann Neurol 1990; 27: 457-464.
12. Dubois B, Feldman HH, Jacova C, Dekosky ST, Barberger-Gateau P, Cummings J, Delacourte A, Galasko D, Gauthier S, Jicha G, Meguro K, O'Brien J, Pasquier F, Robert P, Rossor M, Salloway S, Stern Y, Visser PJ, Scheltens P. Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS-ADRDA criteria. Lancet Neurol 2007; 6: 734-746.
13. Düzel E, Ziegler G, Berron D, Maass A, Schütze H, Cardenas-Blanco A, Glanz W, Metzger C, Dobisch L, Reuter M, Spottke A, Brosseron F, Fliessbach K, Heneka MT, Laske C, Peters O, Priller J, Spruth EJ, Ramirez A, Speck O, Schneider A, Teipel S, Kilimann I, Jens W, Schott B-H, Preis L, Gref D, Maier F, Munk MH, Roy N, Ballarini T, Yakupov R, Haynes JD, Dechent P, Scheffler K, Wagner M, Jessen F. Amyloid pathology but not APOE ε4 status is permissive for tau-related hippocampal dysfunction. Brain 2022; 145: 1473-1485.
14. Endres K, Fahrenholz F, Lotz J, Hiemke C, Teipel S, Lieb K, Tüscher O, Fellgiebel A. Increased CSF APPs-α levels in patients with Alzheimer disease treated with acitretin. Neurology 2014; 83: 1930-1935.
15. Fang H, Zhou G, Li Q, Wang H, Pang X, Xu Q, Yu Y. Active Immunotherapy with 12-mer Aβ1-42-like assembly vaccine shows efficacy in aged 3×Tg-AD mice. Curr Mol Med 2021; 21: 45-55.
16. Fratiglioni L, Ahlbom A, Viitanen M, Winblad B. Risk factors for late-onset Alzheimer's disease: a population-based, case-control study. Ann Neurol 1993; 33: 258-266.
17. George AA, Vieira JM, Xavier-Jackson C, Gee MT, Cirrito JR, Bimonte-Nelson HA, Picciotto MR, Lukas RJ, Whiteaker P. Implications of oligomeric amyloid-beta (oAβ(42)) signaling through α7β2-nicotinic acetylcholine receptors (nAChRs) on basal forebrain cholinergic neuronal intrinsic excitability and cognitive decline. J Neurosci 2021; 41: 555-575.
18. Gilman S, Koller M, Black RS, Jenkins L, Griffith SG, Fox NC, Eisner L, Kirby L, Rovira MB, Forette F, Orgogozo JM. Clinical effects of Abeta immunization (AN1792) in patients with AD in an interrupted trial. Neurology 2005; 64: 1553-1562.
19. Goebel DJ, Poosch MS. NMDA receptor subunit gene expression in the rat brain: a quantitative analysis of endogenous mRNA levels of NR1Com, NR2A, NR2B, NR2C, NR2D and NR3A. Mol Brain Res 1999; 69: 164-170.
20. Hampel H, Bürger K, Teipel SJ, Bokde AL, Zetterberg H, Blennow K. Core candidate neurochemical and imaging biomarkers of Alzheimer's disease. Alzheimers Dement 2008; 4: 38-48.
21. Hong S, Beja-Glasser VF, Nfonoyim BM, Frouin A, Li S, Ramakrishnan S, Merry KM, Shi Q, Rosenthal A, Barres BA, Lemere CA, Selkoe DJ, Stevens B. Complement and microglia mediate early synapse loss in Alzheimer mouse models. Science 2016; 352: 712-716.
22. Hopkins CR. ACS chemical neuroscience molecule spotlight on Begacestat (GSI-953). ACS Chem Neurosci 2012; 3: 3-4.
23. Huovila AP, Turner AJ, Pelto-Huikko M, Kärkkäinen I, Ortiz RM. Shedding light on ADAM metalloproteinases. Trends Biochem Sci 2005; 30: 413-422.
24. Iliff JJ, Wang M, Liao Y, Plogg BA, Peng W, Gundersen GA, Benveniste H, Vates GE, Deane R, Goldman SA, Nagelhus EA, Nedergaard M. A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid β. Sci Transl Med 2012; 4: 147ra111.
25. Janssens J, Hermans B, Vandermeeren M, Barale-Thomas E, Borgers M, Willems R, Meulders G, Wintmolders C, Van den Bulck D, Bottelbergs A, Ver Donck L, Larsen P, Moechars D, Edwards W, Mercken M, Van Broeck B. Passive immunotherapy with a novel antibody against 3pE-modified Aβ demonstrates potential for enhanced efficacy and favorable safety in combination with BACE inhibitor treatment in plaque-depositing mice. Neurobiol Dis 2021; 154: 105365.
26. Kidd M. Paired helical filaments in electron microscopy of Alzheimer's disease. Nature 1963; 197: 192-193.
27. Kimberly WT, LaVoie MJ, Ostaszewski BL, Ye W, Wolfe MS, Selkoe DJ. Gamma-secretase is a membrane protein complex comprised of presenilin, nicastrin, Aph-1, and Pen-2. Proc Natl Acad Sci U S A 2003; 100: 6382-6387.
28. Koran MEI, Wagener M, Hohman TJ. Sex differences in the association between AD biomarkers and cognitive decline. Brain Imaging Behav 2017; 11: 205-213.
29. Kritis AA, Stamoula EG, Paniskaki KA, Vavilis TD. Researching glutamate-induced cytotoxicity in different cell lines: a comparative/collective analysis/study. Front Cell Neurosci 2015; 9: 91.
30. Lacosta AM, Pascual-Lucas M, Pesini P, Casabona D, Pérez-Grijalba V, Marcos-Campos I, Sarasa L, Canudas J, Badi H, Monleón I, San-José I, Munuera J, Rodríguez-Gómez O, Abdelnour C, Lafuente A, Buendía M, Boada M, Tárraga L, Ruiz A, Sarasa M. Safety, tolerability and immunogenicity of an active anti-Aβ(40) vaccine (ABvac40) in patients with Alzheimer's disease: a randomised, double-blind, placebo-controlled, phase I trial. Alzheimer's Res Ther 2018; 10: 12.
31. Lauer AA, Janitschke D, Dos Santos Guilherme M, Nguyen VTT, Bachmann CM, Qiao S, Schrul B, Boehm U, Grimm HS, Hartmann T, Endres K, Grimm MOW. Shotgun lipidomics of liver and brain tissue of Alzheimer's disease model mice treated with acitretin. Sci Rep 2021; 11: 15301-15301.
32. Lauriola M, Paroni G, Ciccone F, G DO, Cascavilla L, Paris F, Gravina C, Urbano M, Seripa D, Greco A. Erythrocyte associated amyloid-β as potential biomarker to diagnose dementia. Curr Alzheimer Res 2018; 15: 381-385.
33. Li H, Wang J, Li Y, Chen X, Zhang W, Zhao Y, Liu G, Pan J. Detection of Aβ oligomers in early Alzheimer's disease diagnose by in vivo NIR-II fluorescence imaging. Sens Actuators B Chem 2022; 358: 131481.
34. Liu CC, Hu J, Zhao N, Wang J, Wang N, Cirrito JR, Kanekiyo T, Holtzman DM, Bu G. Astrocytic LRP1 mediates brain Aβ clearance and impacts amyloid deposition. J Neurosci 2017; 37: 4023-4031.
35. Marcade M, Bourdin J, Loiseau N, Peillon H, Rayer A, Drouin D, Schweighoffer F, Désiré L. Etazolate, a neuroprotective drug

linking GABA(A) receptor pharmacology to amyloid precursor protein processing. J Neurochem 2008; 106: 392-404.
36. Marcello E, Borroni B, Pelucchi S, Gardoni F, Di Luca M. ADAM10 as a therapeutic target for brain diseases: from developmental disorders to Alzheimer's disease. Expert Opin Ther Targets 2017; 21: 1017-1026.
37. Martone RL, Zhou H, Atchison K, Comery T, Xu JZ, Huang X, Gong X, Jin M, Kreft A, Harrison B, Mayer SC, Aschmies S, Gonzales C, Zaleska MM, Riddell DR, Wagner E, Lu P, Sun SC, Sonnenberg-Reines J, Oganesian A, Adkins K, Leach MW, Clarke DW, Huryn D, Abou-Gharbia M, Magolda R, Bard J, Frick G, Raje S, Forlow SB, Balliet C, Burczynski ME, Reinhart PH, Wan HI, Pangalos MN, Jacobsen JS. Begacestat (GSI-953): a novel, selective thiophene sulfonamide inhibitor of amyloid precursor protein gamma-secretase for the treatment of Alzheimer's disease. J Pharmacol Exp Ther 2009; 331: 598-608.
38. Masters CL, Selkoe DJ. Biochemistry of amyloid β-protein and amyloid deposits in Alzheimer disease. Cold Spring Harb Perspect Med 2012; 2: a006262.
39. May PC, Dean RA, Lowe SL, Martenyi F, Sheehan SM, Boggs LN, Monk SA, Mathes BM, Mergott DJ, Watson BM, Stout SL, Timm DE, Smith Labell E, Gonzales CR, Nakano M, Jhee SS, Yen M, Ereshefsky L, Lindstrom TD, Calligaro DO, Cocke PJ, Greg Hall D, Friedrich S, Citron M, Audia JE. Robust central reduction of amyloid-β in humans with an orally available, non-peptidic β-secretase inhibitor. J Neurosci 2011; 31: 16507-16516.
40. Mckee AC, Carreras I, Hossain L, Ryu H, Klein WL, Oddo S, Laferla FM, Jenkins BG, Kowall NW, Dedeoglu A. Ibuprofen reduces Aβ, hyperphosphorylated tau and memory deficits in Alzheimer mice. Brain Res 2008; 1207: 225-236.
41. Mucke L, Selkoe DJ. Neurotoxicity of amyloid β-protein: synaptic and network dysfunction. Cold Spring Harb Perspect Med 2012; 2: a006338.
42. Pfeifer M, Boncristiano S, Bondolfi L, Stalder A, Deller T, Staufenbiel M, Mathews PM, Jucker M. Cerebral hemorrhage after passive anti-Abeta immunotherapy. Science 2002; 298: 1379.
43. Qosa H, Abuasal BS, Romero IA, Weksler B, Couraud PO, Keller JN, Kaddoumi A. Differences in amyloid-βclearance across mouse and human blood-brain barrier models: kinetic analysis and mechanistic modeling. Neuropharmacology 2014; 79: 668-678.
44. Rofo F, Buijs J, Falk R, Honek K, Lannfelt L, Lilja AM, Metzendorf NG, Gustavsson T, Sehlin D, Söderberg L, Hultqvist G. Novel multivalent design of a monoclonal antibody improves binding strength to soluble aggregates of amyloid beta. Transl Neurodegener 2021; 10: 38-38.
45. Sanabria-Castro A, Alvarado-Echeverría I, Monge-Bonilla C. Molecular pathogenesis of Alzheimer's disease: an update. Ann Neurosci 2017; 24: 46.
46. Sevigny J, Chiao P, Bussière T, Weinreb PH, Williams L, Maier M, Dunstan R, Salloway S, Chen T, Ling Y, O'Gorman J, Qian F, Arastu M, Li M, Chollate S, Brennan MS, Quintero-Monzon O, Scannevin RH, Arnold HM, Engber T, Rhodes K, Ferrero J, Hang Y, Mikulskis A, Grimm J, Hock C, Nitsch RM, Sandrock A. The antibody aducanumab reduces Aβ plaques in Alzheimer's disease. Nature 2016; 537: 50-56.
47. Solomon B, Koppel R, Frankel D, Hanan-Aharon E. Disaggregation of Alzheimer beta-amyloid by site-directed mAb. Proc Natl Acad Sci U S A 1997; 94: 4109-4112.
48. Sperling RA, Aisen PS, Beckett LA, Bennett DA, Craft S, Fagan AM, Iwatsubo T, Jack CR, Jr., Kaye J, Montine TJ, Park DC, Reiman EM, Rowe CC, Siemers E, Stern Y, Yaffe K, Carrillo MC, Thies B, Morrison-Bogorad M, Wagster MV, Phelps CH. Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 2011; 7: 280-292.
49. Sun ZT, Ma C, Li GJ, Zheng XY, Hao YT, Yang Y, Wang X. Application of antibody fragments against Aβ with emphasis on combined application with nanoparticles in Alzheimer's disease. Front Pharmacol 2021; 12: 654611.
50. Tackenberg C, Brandt R. Divergent pathways mediate spine alterations and cell death induced by amyloid-beta, wild-type tau, and R406W tau. J Neurosci 2009; 29: 14439-14450.
51. Tagawa K, Maruyama K, Ishiura S. Amyloid beta/A4 precursor protein (APP) processing in lysosomes. Ann N Y Acad Sci 1992; 674: 129-137.
52. Takamura R, Watamura N, Nikkuni M, Ohshima T. All-trans retinoic acid improved impaired proliferation of neural stem cells and suppressed microglial activation in the hippocampus in an Alzheimer's mouse model. J Neurosci Res 2017; 95: 897-906.
53. Tan X, Guan H, Yang Y, Luo S, Hou L, Chen H, Li J. Cu(II) disrupts autophagy-mediated lysosomal degradation of oligomeric Aβ in microglia via mTOR-TFEB pathway. Toxicol Appl Pharmacol 2020; 401: 115090.
54. Tarasoff-Conway JM, Carare RO, Osorio RS, Glodzik L, Butler T, Fieremans E, Axel L, Rusinek H, Nicholson C, Zlokovic BV, Frangione B, Blennow K, Ménard J, Zetterberg H, Wisniewski T, de Leon MJ. Clearance systems in the brain-implications for Alzheimer disease. Nat Rev Neurol 2015; 11: 457-470.
55. Thangudu S, Cheng FY, Su CH. Advancements in the blood-brain barrier penetrating nanoplatforms for brain related disease diagnostics and therapeutic applications. Polymers 2020; 12: 3055.
56. Tian Y, Crump CJ, Li YM. Dual role of alpha-secretase cleavage in the regulation of gamma-secretase activity for amyloid production. J Biol Chem 2010; 285: 32549-32556.
57. Tolar M, Abushakra S, Hey JA, Porsteinsson A, Sabbagh M. Aducanumab, gantenerumab, BAN2401, and ALZ-801-the first wave of amyloid-targeting drugs for Alzheimer's disease with potential for near term approval. Alzheimers Res Ther 2020; 12: 95.
58. Van Gool B, Storck SE, Reekmans SM, Lechat B, Gordts PLSM, Pradier L, Pietrzik CU, Roebroek AJM. LRP1 has a predominant role in production over clearance of Aβin a mouse model of Alzheimer's disease. Mol Neurobiol 2019; 56: 7234-7245.
59. Vassar R, Kuhn P-H, Haass C, Kennedy ME, Rajendran L, Wong PC, Lichtenthaler SF. Function, therapeutic potential and cell biology of BACE proteases: current status and future prospects. J Neurochem 2014; 130: 4-28.
60. Villemagne VL, Burnham S, Bourgeat P, Brown B, Ellis KA, Salvado O, Szoeke C, Macaulay SL, Martins R, Maruff P, Ames D, Rowe CC, Masters CL. Amyloid βdeposition, neurodegeneration, and cognitive decline in sporadic Alzheimer's disease: a prospective cohort study. Lancet Neurol 2013; 12: 357-367.
61. Vos SJB, van Boxtel MPJ, Schiepers OJG, Deckers K, de Vugt M, Carrière I, Dartigues JF, Peres K, Artero S, Ritchie K, Galluzzo L, Scafato E, Frisoni GB, Huisman M, Comijs HC, Sacuiu SF, Skoog I, Irving K, O'Donnell CA, Verhey FRJ, Visser PJ, Köhler S. Modifiable risk factors for prevention of dementia in midlife, late life and the oldest-old: validation of the LIBRA Index. J Alzheimers Dis 2017; 58: 537-547.
62. Walsh DM, Selkoe DJ. A beta oligomers – a decade of discovery. J Neurochem 2007; 101: 1172-1184.
63. Wang HQ, Liu M, Wang L, Lan F, Zhang YH, Xia JE, Xu ZD, Zhang H. Identification of a novel BACE1 inhibitor, timosaponin

A-III, for treatment of Alzheimer's disease by a cell extraction and chemogenomics target knowledgebase-guided method. Phytomedicine 2020; 75: 153244.
64. Wisniewski T, Goñi F. Immunotherapeutic approaches for Alzheimer's disease. Neuron 2015; 85: 1162-1176.
65. Xia X, Jiang Q, McDermott J, Han JJ. Aging and Alzheimer's disease: comparison and associations from molecular to system level. Aging Cell 2018; 17: e12802.
66. Xiong J, Kang SS, Wang Z, Liu X, Kuo TC, Korkmaz F, Padilla A, Miyashita S, Chan P, Zhang Z, Katsel P, Burgess J, Gumerova A, Ievleva K, Sant D, Yu SP, Muradova V, Frolinger T, Lizneva D, Iqbal J, Goosens KA, Gera S, Rosen CJ, Haroutunian V, Ryu V, Yuen T, Zaidi M, Ye K. FSH blockade improves cognition in mice with Alzheimer's disease. Nature 2022; 603: 470-476.
67. Yuan XZ, Sun S, Tan CC, Yu JT, Tan L. The role of ADAM10 in Alzheimer's disease. J Alzheimers Dis 2017; 58: 303-322.
68. Zhang W, Oya S, Kung M-P, Hou C, Maier DL, Kung HF. F-18 Stilbenes as PET imaging agents for detecting β-amyloid plaques in the brain. J Med Chem 2005; 48: 5980-5988.