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Two decades of new drug discovery and development for Alzheimer's disease†

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Alzheimer's disease is a progressive and irreversible neurodegenerative disease, associated with a decreased cognitive function and severe behavioral abnormalities. Due to its complex pathophysiological characteristics, complicated interactions with a large number of genes and proteins, there is still no effective drug treatment of the disease. Amyloid cascade aggregation of senile plaques and hyperphosphorylation of Tau protein to form neurofibrillary tangles are the main pathological features of Alzheimer's disease, other mechanisms, such as oxidative stress, lack of central cholinergic neurotransmitters, inflammatory reaction and toxic metal ions have also been involved. The purpose of this review is to briefly introduce the progress of the development of the therapeutic agents based on their main mechanisms of action.

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1. Introduction

Alzheimer's disease (AD), a progressive and irreversible neurodegenerative disease and complicated multi-factorial disorder, is one of the most common forms of dementia.¹ It is estimated that there are more than 35 million AD patients worldwide. AD has been recognized as one of the most difficult medical problems with heavy social and economic costs.² So far, there has been no effective drug or treatment to prevent or reverse the progression of the disease. AD is characterized by progressive memory loss, cognitive impairment and severe behavioral abnormalities. The major pathological features of AD are extracellular aggregation of amyloid β peptide ($A\beta$) forming senile plaques (SP), the deposits of intracellular neurofibrillary tangles (NFTs).³ Although there is extensive study on the pathogenesis of AD, the exact mechanism of AD is still unknown due to its complex pathophysiological characteristics. Several mechanisms have been proposed attempting to explain the pathogenesis of the disease, the amyloid β ($A\beta$) cascade,⁴ the hyperphosphorylated Tau protein,⁵ oxidative stress,⁶ deficiency of central cholinergic neurotransmitter,⁷ inflammation⁸ and toxic metal ions⁹ have been involved. Although the pathogenesis of the disease is not clear, new agents associated with the pathological changes of the disease are significantly developed.

The purpose of this review is to briefly introduce the progress of the development on the therapeutic agents based on their main mechanisms of action, which hoping to promote the development of the next generation of AD therapeutic drugs.

2. The pathogenesis

2.1. Amyloid cascade hypothesis

One of the main pathological features of AD is the formation of senile plaques (SP), which caused by amyloid beta ($A\beta$) deposition. Normally, $A\beta$ is soluble small peptides, which is produced by cleavage of the amyloid precursor protein (APP) by the action of α -secretase, β -secretase and γ -secretase.¹⁰ The imbalance between β -amyloid ($A\beta$) production and clearance leads to various types of toxic oligomeric, namely protofibrils, fibrils and plaques depending upon the extent of oligomerization.¹¹ The reason of the formation of $A\beta$ is still unknown, but the sequence, concentration and conditions of stability of $A\beta$ are important factors.¹² Some studies suggested that neurotoxicity required assembly of the peptide into oligomers,¹³ and other evidences suggested that soluble oligomers forms of $A\beta$ could produce more neurotoxicity.¹⁴ A thorough study shows that amyloid toxicity associated with both protein-specific and conditional-determined by the function of vascular endothelial growth factor receptor 2 (VEGFR2) loss, which is essential for target protein in a biological context.¹⁵ A recent work systematic reviewed of the 25 years of the development and latest findings of the amyloid hypothesis, and elaborated the features of its cell biology and genetics. Suggesting that amyloid dyshomeostasis has emerged as the most extensively validated and compelling therapeutic target.¹⁶

2.2. Hyperphosphorylated Tau protein

The neurofibrillary tangles (NFTs) formed by hyperphosphorylated Tau protein are another major pathological

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feature of AD. Tau protein belongs to the family of microtubule-associated proteins, mainly existing in the axon,¹⁷ and its main function is to maintain the stability of microtubules.¹⁸ There are several phosphorylation sites on Tau protein, compared with the mature nerve cells, the Tau protein in the growing nerve cells is more likely to be phosphorylated.¹⁹ Normally, the phosphorylation and dephosphorylation of Tau protein maintain a dynamic balance, but when the hyperphosphorylated Tau protein aggregates to form a double-helix fiber, it loses the function of connecting and stabilizing microtubules, which leads to the death of neurons.^{20,21} There has been a long-standing debate over the temporal mechanistic relationship between the two major pathological features of AD, and evidence reports that soluble amyloid beta protein dimers induces Tau protein hyperphosphorylation and neurodegeneration.²²

2.3. Oxidative stress hypothesis

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced in many normal and abnormal processes in humans, they play a dual role as both beneficial function of numbers of cellular signaling pathways and deleterious process that can lead to damage of cellular structures (including cell membrane, lipid, protein, and DNA).²³ The high oxygen consumption of the brain, which utilizes 20% more oxygen than other mitochondrial respiratory tissues, means that the brain is more vulnerable to oxidative stress.²⁴ Neuron is the basic function unit of the brain, which contains a large number of polyunsaturated fatty acids. It can interact with ROS, leading to the lipid peroxidation reaction and molecular apoptosis,²⁵ in addition, less glutathione in neurons is also one of the causes of oxidative stress injury.²⁶

2.4. Cholinergic hypothesis

Bartus *et al.* proposed the cholinergic hypothesis that dysfunction of cholinergic activity in the brain of healthy elderly and dementia patients may play a role in the loss of memory and related cognitive impairment, so reconstruction of cholinergic function may be able to reduce the serious lack of cognitive function.²⁷ The activity of the cholinergic system was evaluated by choline acetyl transferase (ChAT) and acetylcholinesterase (AChE) *etc.*²⁸ The study showed that the Ach, ChAT and AChE in the brain of AD patients showed a continuous decline.²⁹ Acetylcholine (ACh) is neurotransmitters regulating cognitive performance and learning and memory process,³⁰ synthesized by acetyl CoA and choline under the catalysis of ChAT, ACh and its receptor (AChR) combined with transfer nerve impulses, AChE hydrolyzed into acetic acid and choline. Acetylcholine established synaptic contacts in networks of brain cells to remodelling of the cerebral cortical circuits, which will subserve complex cognitive functions.³¹

2.5. Inflammatory hypothesis

Inflammatory responses in the brain is another pathological characteristic in AD, usually chronic inflammation, main characteristics for a large numbers of mononuclear leucocytes and macrophages in the central nervous system, such as small

glial cells.³² Compared with normal subjects, acute phase proteins and proinflammatory cytokines over expression in AD patients brain tissues.^{33,34} Microglia and astrocytes are the main causes of the inflammatory response, the activated cells produce proinflammatory mediators, such as interleukin 1 beta (IL-1) and interleukin 6 (IL-6) and tumor necrosis factor (TNF alpha), chemotactic factor interleukin 8 (IL-8), macrophage inflammatory protein-1, prostaglandins, leukotrienes, coagulation factor, protease, protease inhibitors;^{35,36} The production of these substances can kill the neighboring neurons.³⁷

2.6. Metal ion hypothesis

Metal ions play an important role in the maintenance of homeostasis,³⁸ and the relationship between metal ions and neurodegenerative diseases has attracted much attention in recent years.^{39,40} The brain is rich in metals that act as essential cofactors in metalloproteins to participates in the process of metabolism,⁴¹ the concentration of metal ions in the brain is tightly regulated through the blood brain barrier, when the blood brain barrier of metal ion regulation system degradation,⁴² metal ion transport dysfunction, metal ions (iron, copper, manganese, aluminum, zinc, *etc.*) begin to affect the oxidative stress response of mitochondria and the wrong folding proteins, and ultimately lead to neurodegeneration.^{43,44} Studies have indicated that aluminum, zinc, copper and iron can lead to changes in the conformation of the A β protein.⁴⁵ Aluminum can lead to the accumulation of A β and Tau protein,⁴⁶ aluminum and copper are involved in the process of the development of nerve inflammation.⁴⁷ The increased levels of iron, aluminum and copper in the aged human brain may reflect the relationship between age and neurodegenerative diseases.⁴⁸

3. Cholinergic drugs

Drachman and Leavitt suggested that memory was associated with the cholinergic system and was age dependent,⁴⁹ and Bartus²⁷ proposed Alzheimer's cholinergic hypothesis on which the development of cholinergic inhibitors is mainly based.⁵⁰ Study suggested that the acetylcholine (ACh), neurotransmitters regulating cognitive performance and learning and memory process,⁵¹ in the brain of AD patients showed a continuous decline.⁵² The loss of cholinergic function is related to cognitive impairment and behavioral disorder, and these symptoms can be improved by acetylcholinesterase (AChE) inhibitors or by modulating other cholinergic receptors, such as muscarinic and nicotinic ACh receptors. In 1993, tacrine was first approved by FDA to treat with mild to moderate AD,^{53,54} in addition three cholinesterase inhibitors were followed: donepezil (1996),^{55,56} rivastigmine (2000)⁵⁷ and galantamine (2001).⁵⁸ However, other drugs have not been approved yet, including AChE inhibitors velnacrine, physostigmine, eptastigmine, metrifonate *etc.*,⁵⁹ muscarinic receptor agonists cevimeline (AF102B), milameline, sabcomeline (SB 202026), talsaclidine, xanomeline and alvameline (LU 25-109).⁶⁰ In addition to the cholinesterase inhibitor, memantine, a *N*-methyl *D*-aspartate (NMDA) receptor



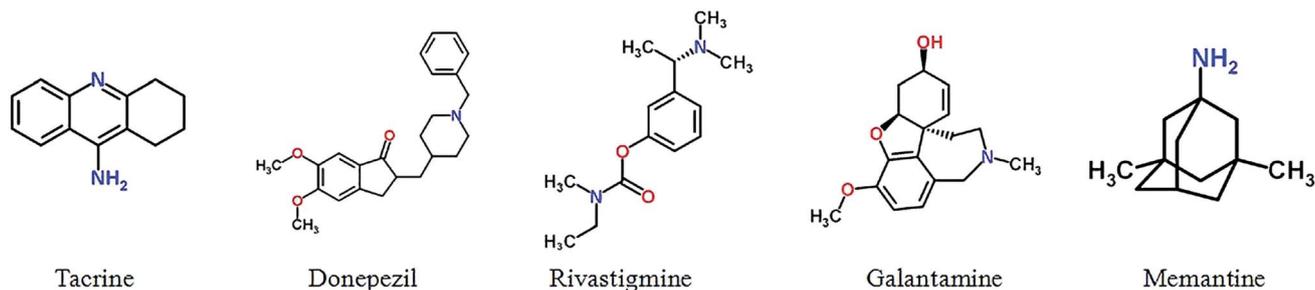


Fig. 1 Five medicines approved by the US FDA for treatment in Alzheimer's disease.

antagonist which acts on the glutamatergic system, is another FDA approved for treatment of moderate-to-severe AD drugs.^{61,62} Besides the drugs approved by FDA, there has still been progress in development of cholinergic drugs in clinical trials as well as patented lead compounds. Fig. 1 and Table S1† show five drug approved by the US FDA for treatment in AD.

Huperzine A, an alkaloid derived from the Chinese herb *Huperzia serrata*, acts as a selective inhibitor of acetylcholinesterase, which has a mechanism of action similar to donepezil.⁶³ Huperzine A has shown promising effects on the treatment of Alzheimer's disease,⁶⁴ including improvement of cognitive function, daily living activity, and global clinical assessment.⁶⁵ However, one trial demonstrated no significant change in cognitive function as measured by Alzheimer's disease Assessment Scale-Cognitive Subscale (ADAS-Cog)⁶⁶ and clinical data are limited by poor methodological quality.⁶⁷ Pro-drug of huperzine A, named ZT-1, derived from natural product, is a potent and selective AChE inhibitor. The results from the Phase I clinical trials showed that ZT-1 has an admirable pharmacokinetic with a rapid absorption and a wide distribution in human.⁶⁸

Physostigmine, originally having been extracted from calabar beans, is an AChE inhibitor, but it has limited treatment effects and serious side effects.⁶⁹ The (–)-phenserine, a derivative of physostigmine, is an AChE inhibitor that has an effect on cognitive improvement. It also can reduce the translation of APP to reduce A β concentrations, suggesting (–)-phenserine may be a promising multitarget drug of AD.⁷⁰

Memogain (Gln-1062), an inactive pro-drug of galantamine, liberates galantamine on cleavage by a carboxylesterase in the brain. Memogain has more than 15-fold higher bioavailability in the brain than the same doses of galantamine due to the

more hydrophobic characteristics. Memogain may represent a valuable drug with higher potency in enhancing cognition for AD treatment, a significantly lower plaque density in the brain, and much lesser gastrointestinal side effects.^{71,72}

Ladostigil is a novel multitarget drug combined with acetylcholine–butyrylcholinesterase cholinesterase inhibitor and brain selective monoamine oxidase A and B inhibitor. It can relieve scopolamine-induced impairment in spatial memory, and increase brain cholinergic activity in rat. Furthermore, it was proved to possess anti-apoptotic and neuroprotective including the regulation of APP process, activation of protein kinase C and mitogen-activated protein kinase signaling pathways.⁷³ NGX267 (AF267B), as M1-selective muscarinic agonists, can enhance the cognitive ability.⁷⁴ In AD transgenic mice, it also reduced A β 1-42 and Tau hyperphosphorylation in the cortex and hippocampus, presenting an unique beneficial effects on therapy in AD.⁷⁵ EVP-6124 is a partial, selective agonist of the α -7 nicotinic acetylcholine receptor (α 7-nAChR) with highly CNS-penetrant. It can improve cognitive deficits by boosting the ACh response of α 7 nAChRs. EVP-6124 moved into Phase III for AD, supporting a new therapeutic strategy for the treatment of cognitive impairment.⁷⁶ Additionally, GTS-21 is a selective agonist of the α 7 nicotinic receptor, showed promising characteristics during Phase II clinical trial.⁷⁷ Fig. 2 and Table S2† show cholinergic inhibitors in clinical trials.

4. Amyloid-targeted therapies

4.1. Decreasing A β production

4.1.1. β -Secretase inhibitors. LY2811376 is the first orally non-peptidic small-molecule BACE1 inhibitor with satisfactory pharmacokinetic and pharmacodynamic properties from

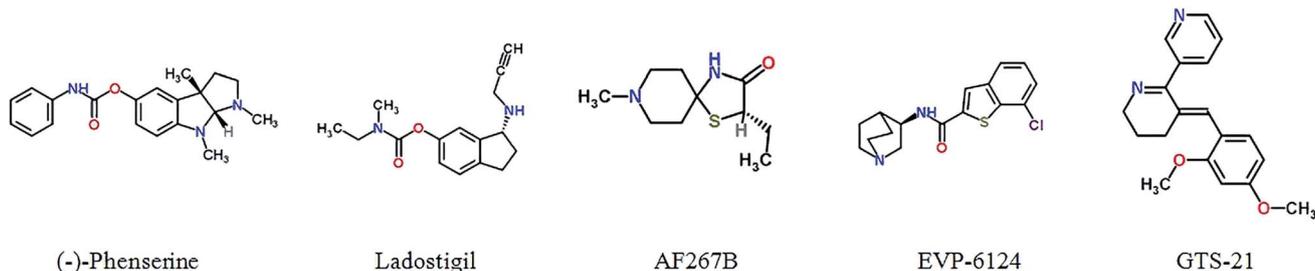


Fig. 2 Cholinergic inhibitors in clinical trials.



preclinical animal models to man.⁷⁸ It can penetrate the blood brain barrier, and showed long-lasting effect on reducing the level of A β in healthy volunteers. However, clinical development was stopped according to a chronic non-target-associated toxicology. LY2886721, next-generation orally available BACE1 inhibitor with agreeable drug properties, reduce the concentrations of cerebral A β 40, A β 42 and sAPP- β with safety and good tolerability.^{79,80} Unfortunately, it was terminated because of abnormal liver biochemical tests. MK-8931 developed by the pharmaceutical company Merck, is a BACE1 inhibitor tested for the treatment of AD in Phase I clinical trial. It can significantly reduce the levels of CSF A β in a dose-dependent and sustained way.⁸¹ MK-8931 also reduces the concentration of CSF A β in patients with mild-to-moderate AD.⁸² And further research shows that MK-8931 elicits few adverse effects previously ascribed to BACE inhibition, different doses are well tolerated and reduce CNS β -amyloid in both healthy human subjects and AD patients. The human data are suitable for the amyloid pathway model and provide a meaningful guidance for further experiments.⁸³ E2609, an orally available BACE1 inhibitor, showed dose-dependent reductions of A β concentrations in CSF and/or plasma in a single oral ascending dose study and multiple oral ascending dose study respectively. Phase 2 clinical trial of E2609 is planned by Eisai.^{84,85} Fig. 3 and Table S3† show β -secretase inhibitors in clinical trials.

4.1.2. γ -Secretase inhibitors and modulators. Semagacestat (LY-450139) is a γ -secretase inhibitor in the aim to treat AD. It can lower A β concentrations in the plasma and cerebrospinal fluid with a dose-dependent manner.⁸⁶ However, the trial was terminated owing to severe adverse effects and worsen cognition performance compared to placebo group.⁸⁷ It is believed that inhibiting γ -secretase may disturb Notch signaling proteins and other cell surface receptors.⁸⁸ Avagacestat (BMS-708163) is also a γ -secretase inhibitor with Notch-sparing effect. Nevertheless, avagacestat did not demonstrate obvious efficacy from Phase II trials in MIC.⁸⁹ Begacestat (GSI-953) is a thiophene sulfonamide γ -secretase selectively inhibitor which inhibits cleavage of APP over Notch.⁹⁰ The compound has shown promise in recent Phase I clinical trials.⁹¹

NIC5-15 is a natural compound acted as a Notch-sparing γ -secretase inhibitor and an insulin sensitizer. The compound can improve cognitive function through multiple mechanisms including reduce A β production by modulating γ -secretase.⁹² The result shows that NIC5-15 is safe and has good tolerability

and further feasibility trials are needed.⁹³ CHF5074, a new microglial modulator, reduces brain A β burden to enhance spatial memory cognitive in transgenic mice of AD model.⁹⁴ CHF5074 shows dose-dependent effects in central nervous system and well tolerated and safety in mild-to-moderate patients.⁹⁵ E2012 is also a novel γ -secretase modulator which decreases the concentration of A β 40 and A β 42 in rat in a dose-dependent manner, and without affecting Notch cleavage.⁹⁶ Fig. 4 and Table S4† show γ -secretase inhibitors and modulators in clinical trials.

4.2. Promoting A β clearance

4.2.1. Active AD immunotherapy. AN-1792 was the first full-length A β 1-42 active vaccine used in clinical trial. Although the trial was terminated after 6% of the participants developed severe side effect, it provided an important proof of concept.⁹⁷ CAD106 is the second-generation active immunotherapy vaccine that comprises A β 1-6 peptide. The study suggests that CAD106 can reduce A β accumulation and lead to acceptable antibody response with a safe and tolerance manner in Phase II trials.⁹⁸ ACI-24 is a A β 1-15 liposome-based vaccine that can restore the memory defect and reduce plaque in transgenic mice. A phase I/II clinical trial is currently ongoing for investigating the safety and efficacy of mild-to-moderate AD patients.⁹⁹ UB-311 is synthetic peptides, consisting of UB1h helper T-cell epitopes and coupled to the A β 1-14 peptide.¹⁰⁰ A phase 1 clinical trial of UB-311 has successfully completed, illustrating safety and tolerability.¹⁰¹ In addition, Phase 2 clinical trials are being prepared.

Other ongoing trails include ACC-001, V950, Lu AF20513 and AD02 as well. ACC-001 (Vanutide cridificar), an A β 1-7/Qs21 adjuvant immunotherapeutic vaccine, was evaluated in phase 2a and results came out with safety profile in mild-to-moderate AD patients.¹⁰² V950, multivalent A β peptide/ISCOMATRIX™ adjuvant, aimed to produce A β antibodies to recognize pyroglutamate-modified and other N-terminally truncated A β fragments. A phase 1 has been completed and further studies were discontinued.¹⁰³ Lu AF20513 was developed by Lundbeck A/S (Valby, Denmark) according to A β 1-12 peptide replaced with two foreign T-helper epitopes from tetanus toxoid. It is currently being tested in preclinical trail.¹⁰⁴ Additionally, AD02 is an amyloid-beta (A β)-targeting vaccine to elicit anti-A β antibodies, and phase II study was finished.¹⁰⁵ DNA amyloid-beta protein immunotherapy is currently being investigated in

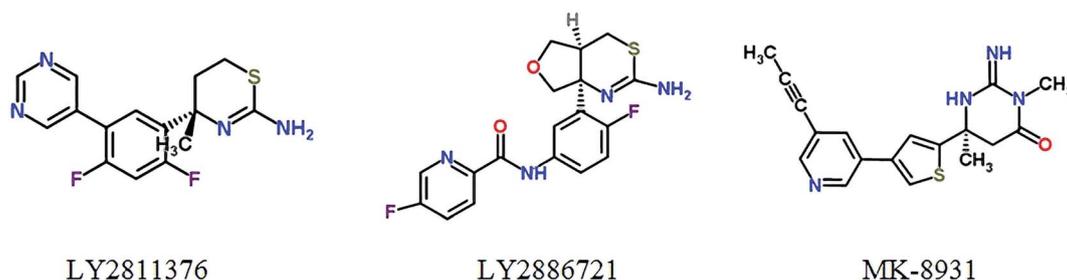


Fig. 3 β -Secretase inhibitors in clinical trials.



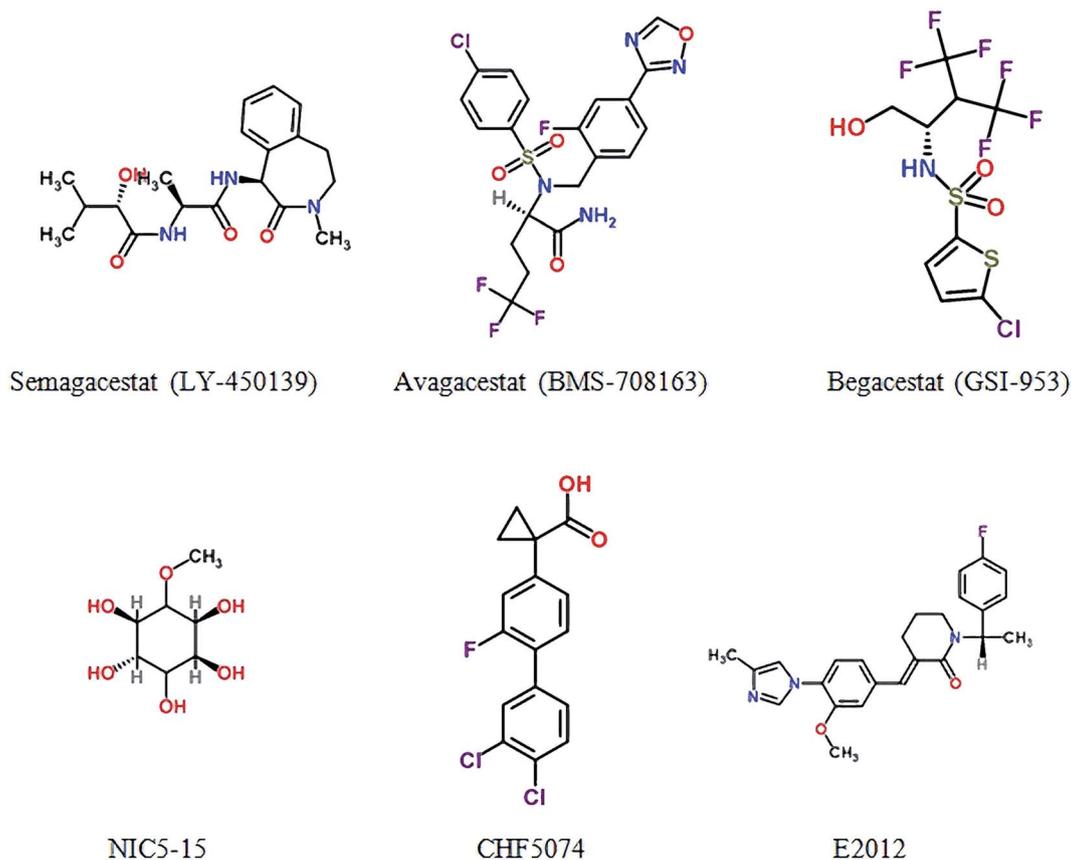


Fig. 4 γ -Secretase inhibitors and modulators in clinical trials.

preclinical studies.¹⁰⁶ Tau immunotherapy AADvac1 and ACI-35 are also under preclinical.¹⁰⁷

4.2.2. Passive AD immunotherapy. AAB-001 (Bapineuzumab) is the first humanized monoclonal antibody targeting the A β N-terminus (A β 1–5). The antibody binds strongly to deposit amyloid plaques to reduce the A β plaque burden and induces Fc-mediated microglial phagocytosis of A β plaques in mouse.¹⁰⁸ Two phase 3 trials involved patients with mild-to-moderate Alzheimer disease were conducted, however, bapineuzumab failed to improve primary clinical outcomes.¹⁰⁹ LY-2062430 (Solanezumab) is a humanized monoclonal antibody to the mid-domain of A β 16–24, which binds to the soluble A β .¹¹⁰ The compound has been proved safety in Phase 2 findings, nevertheless, neither clinical trial in phase 3, showing significant cognition improvement nor functional ability in patients with mild-to-moderate AD.¹¹¹ Two previously phase 3 clinical trial results were renewed in some mild patients, and the secondary outcomes suggested that Solanezumab can slow the cognitive decline of 34% according to the ADAS-Cog and Mini-Mental State Examination (MMSE) ($P < 0.05$).¹¹² PF-04360365 (Ponezumab) is a humanized IgG2 δ A monoclonal antibody aiming to reduce immune effector. PF-04360365 showed accepted safety and well tolerated findings without antibody-induced side effects.¹¹³ And another clinical trial also showed similar results.¹¹⁴ GSK-933776 is a humanized Fc-attenuated/inactivated anti-A β monoclonal antibody. GSK933776 showed pharmacological activity and

engaged target in plasma and CSF without causing brain amyloid-related imaging abnormalities-edema (ARIA-E/H) in patients with mild AD or MCI.^{115,116} MABT5102A was a humanized A β 1–15 monoclonal antibody with IgG4 isotype. It can inhibit A β aggregation and promote its disaggregation without vasogenic edema and cerebral microhemorrhage induced by overactivation of microglial cells.¹¹⁷ Aducanumab (BIIB037) is a human monoclonal antibody that selectively targets misfolded A β peptides. Aducanumab restores calcium homeostasis in Tg2576 mice,¹¹⁸ and also reduce soluble and insoluble A β in a dose-dependent manner.¹¹⁹ In a recent study, Sevigny *et al.* reports beneficial effects on the amyloid pathology and the cognitive status in patients with prodromal or mild AD.¹²⁰ The phase 1b clinical test had revitalizes the “amyloid cascade hypothesis” and bring mononuclear phagocytes to the center stage of AD treatment.

4.3. Preventing A β aggregation

Tramiprosate (3-amino-1-propanesulfonic acid, 3APS) is an orally-administered amyloid antagonist, which binds to soluble A β peptide and designs to reduce A β aggregation and prevent fibril formation.¹²¹ Tramiprosate produces cytoprotective effects against A β -induced neurotoxicity, and exerts significant reduction of soluble and insoluble A β in the brain of transgenic mice.¹²² Clinical trials show that tramiprosate can slow



hippocampal atrophy,¹²³ and have some benefit on cognition.¹²⁴ However, the further Phase III trial has been terminated due to its unsuccessful in demonstrating efficacy.^{125,126} Scyllo-inositol, an endogenous inositol stereoisomer, is another anti-aggregation compound, exerting specific health-promoting effects for Alzheimer disease.¹²⁷ It stabilized a small conformer of A β 42 *in vitro*, and neutralized cell-derived A β oligomers *in vivo*. Moreover, scyllo-inositol can decrease neuronal toxicity and abate cognitive deficits in multiple mouse models of AD.¹²⁸ A Phase II clinical trial demonstrated acceptable safety, however, primary clinical efficacy outcomes were not significant.¹²⁹ Epigallo-catechin-3-gallate (EGCG), a natural flavanol derived from green tea which shows multiple neuro-protective activities,¹³⁰ bind to unfolded peptide to prevent the formation of A β toxic oligomers.¹³¹ It can also modulate cell signalling and reverse superoxide dismutase activity and the damage effects of AlCl₃ neurotoxicity, which improves mitochondrial and cholinergic synaptic functions.^{132,133} PBT1 is a metal chelator that promotes the solubilisation by disturbing the chelation between A β and metal ions *in vitro* or mouse model studies. However, Phase II clinical suggested that there was no significant positive clinical benefit for patients with AD. Unfortunately, phase III trial was abandoned.¹³⁴

The second-generation metal-protein attenuating compound PBT2, was developed as a metal chaperone which affected the metal-induced A β oligomerisation.¹³⁵ It has greater blood-brain barrier permeability. PBT2 can obstruct A β oligomerization, decrease soluble and insoluble A β and promote the clearance of A β oligomer.¹³⁶ Phase II study showed that PBT2 can reduce the concentrations of A β 42 in CSF and improved cognitive function with safety and tolerance.¹³⁷ TTP488 (PF-04494700) is a small-molecule oral antagonist of the receptor for advanced glycation

end products. The low dose (5 mg) shows good safety profile but associated with conclusive results in Phase II trial with mild to moderate AD.¹³⁸ Another clinical trial demonstrates that low-dose (5 mg) could be a benefit dose in further Phase 3 trials in patients with mild AD.¹³⁹

A recent study provided a new framework for the rational identification of a series of drug candidates for neurodegenerative diseases, chemical kinetics approach was applied to study the effect of small molecules on the deposition rate of A β 42 which was quantitatively analyzed. An anticancer drug, bexarotene, was reported to suppress A β 42 deposition by targeting the primary nucleation step in the aggregation of A β 42 and delaying the formation of toxic species in neuroblastoma cells.¹⁴⁰ Fig. 5 and Table S5† show drugs in clinical trials to prevent A β aggregation.

5. Other potential therapeutic strategies in AD

5.1. Drugs to target Tau protein

Leuco-methylthionium (LMTX), a selective Tau aggregation inhibitor by preventing the formation and spread of NFTs and reducing the Tau pathology in transgenic mouse models, is the reduced form of methylthionium (MT), which is the first substance that can degrade Tau protein.¹⁴¹ An exploratory phase 2 study in mild or moderate Alzheimer's disease was conducted, safety and effectiveness was determined as well.¹⁴² The main results of a broad participation of 115 academic centres in 15 months of randomised, controlled double-blind, parallel-group clinical trial is negative, the results do not suggest the benefit of LMTX as an additional treatment of mild to moderate Alzheimer's disease.¹⁴³

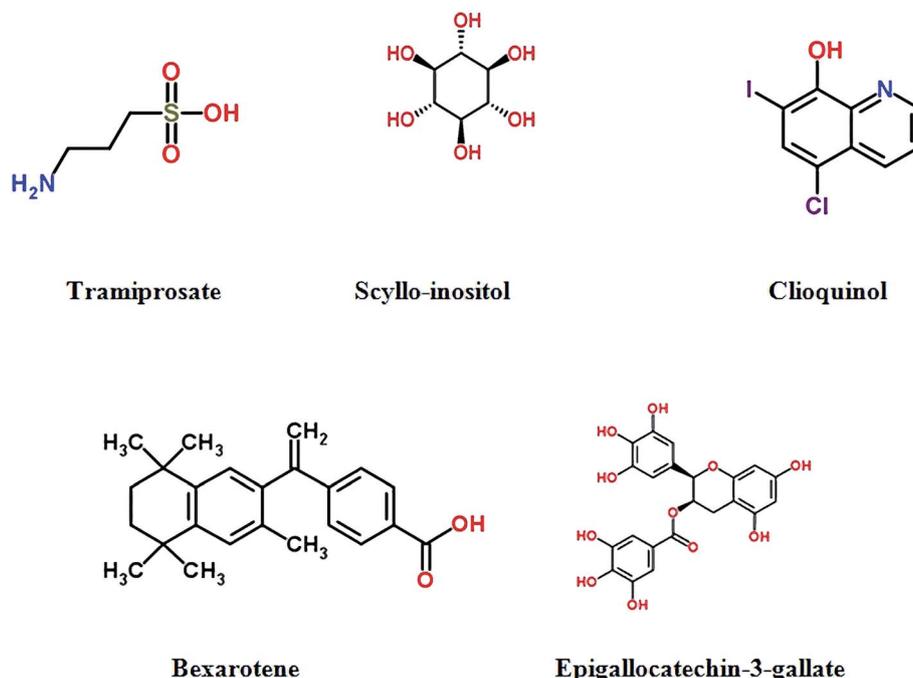


Fig. 5 Drugs in clinical trials to prevent A β aggregation.



Glycogen synthase kinase 3 β (GSK3 β) is a serine/threonine kinase which plays an important role in regulating Tau protein phosphorylation and involves in processing of amyloid-beta peptides.^{144,145} Tideglusib (NP-031112) is a small non-ATP competitive GSK-3 inhibitor, for the treatment of Alzheimer's disease in clinical trials. It can lower Tau protein hyperphosphorylation, reduce brain amyloid plaque levels, improve learning and memory and prevent the loss of neurons in some animal models.¹⁴⁶ Studies demonstrated valuable safety in clinical trials in AD patients.^{147,148}

5.2. Neurotrophins

Neurotrophins are dimeric peptide hormones. Nerve growth factor (NGF), the first neurotrophin, regulates many aspects of neuronal development and function, plays an important role in the survival and differentiation of neurons.¹⁴⁹ Recently, the study shows that BDNF exerts substantial protective effects on crucial neuronal circuitry involved in Alzheimer's disease, revealed the correlation between the decreased NGF and AD.¹⁵⁰ Thus, neurotrophins have been acted as an attractive target for treatment of AD. AAV2-NGF (CERE-110) is designed to deliver NGF by gene to cross the blood-brain barrier, which increases production of acetylcholine and enhances the function basal forebrain cholinergic neurons.^{151,152} A Phase I study has been completed and a multi-center, placebo-controlled Phase II clinical trial in the observation phase.¹⁵³ T-817MA [1-{3-[2-(1-benzothiophen-5-yl)ethoxy]propyl}azetidin-3-olmaleate] has both neuroprotective and neurotrophic effects and also has the ability to improve the cognitive impairment in transgenic mice,¹⁵⁴ The Phase II trial has been completed for its evaluations on safety and tolerability.¹⁵⁵

5.3. Targeting mitochondrial dysfunction

There is a growing body of evidence supporting that mitochondrial dysfunction has a significant influence on the process of AD.¹⁵⁶ Mitochondrial dysfunction also has connection with oxidative stress and Tau pathology.^{157,158} Due to the correlation between mitochondrial alterations and AD, strategies targeting decreases the related oxidative stress and removal of damaged mitochondria possess great promise in AD treatment. Latrepirdine (Dimebon®), a small molecule compound, is used for the treatment of AD. Dimebon can modify hippocampal APP/A β pathology,¹⁵⁹ and ameliorate mitochondrial membrane

potential and ATP production,¹⁶⁰ indicated the potential treatment for neurodegenerative diseases.¹⁶¹

5.4. PPAR- γ agonists

Pioglitazone is an insulin sensitizer of the thiazolidinedione class of peroxisome-proliferator activated receptor γ (PPAR- γ) agonists. Takeda developed pioglitazone as a once-daily treatment of type 2 diabetes. The PPAR- γ agonist improves cognition in AD mice, and mixed results in prior human trials.¹⁶² In August 2013, Takeda and Zinfandel Pharmaceuticals began 'Tomorrow', a Phase III trial that is to enroll 5800 cognitively-normal participants and run for 4 years. The study has two separate goals; one is to evaluate how accurately a diagnostic algorithm based on the genes ApoE and TOMM40, developed by Zinfandel, predicts a person's risk of developing mild cognitive impairment due to AD within 5 years. The other is to evaluate pioglitazone's ability to delay this diagnosis.

5.5. Computer simulation

Studies showed that molecular dynamics simulation can be used to design A β aggregation inhibitor and a structure-based drug discovery procedure has been explored to identify the binding pockets between small-molecule and A β peptide.^{163,164} In a study, a total 11 compounds were identified which reduce A β cytotoxicity by shifting the equilibrium of A β from oligomers to fibers by comprehensive performing these methods.¹⁶⁵ NQ-Trp (1,4-naphthoquinon-2-yl-L-tryptophan), a small molecular which has been reported to inhibit aggregation of A β .¹⁶⁶ NQ-trp was found to be the best binders of five small-molecule drugs with A β 17-42 by using a hierarchical computational procedure,¹⁶⁷ further more, an extensive atomistic replica exchange molecular dynamics simulations was used to explain the beneficial effect of NQTrp in reducing both the level of A β 1-42 aggregation and toxicity.¹⁶⁸ Aiming to investigate the molecular mechanism of NQ-Trp combined experimental and simulation studies were performed. The converging results explained its low inhibitory efficiency which due to the lack of specific "binding site"-type between NQ-Trp and A β , and suggested that another mechanism was involved in anti-AD activity of NQ-Trp-type molecules models *in vivo*.^{169,170} Yang Z. *et al.* found that graphite can inhibit A β peptide monomer fibrils and can clear the mature amyloid fibers through penetration and extraction of peptides. Experimental evidence such as molecular dynamics



Fig. 6 Other potential therapeutic strategies in AD.



simulations, atomic force microscopy images, thioflavin fluorescence assays, and cell viability and ROS assays confirmed the prediction results of computer simulation.¹⁷¹ The molecular mechanism and molecular dynamics of (–)-epigallocatechin gallate (EGCG)¹⁷² and CQ1-3 (ref. 173) inhibiting A β aggregation were studied respectively by computer simulation. Computer simulation provides new insights into the underlying molecular mechanisms that define drug–amyloid interaction and suggests the directions of further AD drug development.¹⁷⁴ Fig. 6 and Table S6† show other potential therapeutic strategies in AD.

6. Concluding remarks and future perspective

So far, the development of AD drugs has achieved some success in the improvement of symptoms, whereas it also has several failed aspects of disease modification. While many clinical and drug design studies are undergoing, we must recognize that it is quite difficult to successfully cure AD with single therapy, which is attributed to the complex pathophysiology of AD. It is believed not to be caused by single gene defects, but rather by a large number of genes, proteins and their complex interactions that ultimately lead to the change of this disease.¹⁷⁵ Multi-target drug discovery may be a more promising treatment strategy for AD.¹⁷⁶ It could overcome the deficiency of the poor development effects of one-target-one-compound. Several multi target compounds already have been designed, such as dual binding AChE and BACE1 inhibitors,¹⁷⁷ AChE inhibitors and antioxidants,¹⁷⁸ which provide better therapeutic effects on both symptomatic and disease modifying in AD. At this point, multiple-pharmacology natural products can be used as prototypes for the drugs design of AD treatment.¹⁷⁹ Herbal formulae such as Kai-Xin-San (consisting of *Ginseng Radix*, *Poria*, *Polygonae Radix*, and *Acori Tatarinowii Rhizoma*) has been used in the treatment of Parkinson's disease and Alzheimer's disease,^{180,181} also provide new insight for the treatment of AD. New methods, such as quantitative systems pharmacology,¹⁸² chemogenomics knowledgebase,¹⁸³ metabolomics^{184–189} and chinmedomics strategy^{190–195} will meet the challenge and provide a promising avenue for the discovery and clinical development of new-generation drugs for AD.

Competing financial interests

The authors declare no competing financial interests.

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