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Emerging therapeutics and future insights into the pathobiology of Alzheimer's disease

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Abstract

Alzheimer's disease (AD) is a complex neurodegenerative disorder that involves progressive memory loss and brain atrophy due to deregulated neurobiological networks. Despite decades of intense research, therapies for AD are still in development, and the results of several ongoing pivotal clinical trials are anticipated. Because many recent amyloid-β (Aβ)-targeting therapies have failed, the amyloid hypothesis and alternative clinical strategies need to be reinvestigated. In addition to Aβ inhibition, the inhibition of the hyper-phosphorylation of tau, which is a downstream target of kinases and signaling cascades, is a potential therapeutic strategy. In this review, we discuss current AD treatment strategies that utilize small-molecule therapies that aim to inhibit Aβ accumulation or tau phosphorylation. We then present a comprehensive and balanced overview of recently discovered immunological pathways that make this disease more complex. Targeting these potential pathways will shape future therapeutic approaches in AD.

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Introduction

Alzheimer's disease (AD) is a devastating neurodegenerative disorder that is characterized by progressive dementia, which is a condition involving neuronal loss, disorientation, difficulty speaking and/or learning, and aberrant functioning(Huang *et al.*, 2012). Although extensive research has been done on AD, the underlying pathophysiological mechanisms remain unclear. Like other common chronic diseases, AD does not result from a single physiological factor but rather from multiple factors that may begin over10 years before disease symptoms appear (Kumar *et al.*, 2015). Currently, AD is the 6th leading cause of death in the United States, and it is expected to become a major unmet medical need in the next decade(Rafii, 2013). In 2016, about 5.2 million Americans with various ages of onset were diagnosed with AD, and this number is expected to grow to about 7.7 million by 2030. With the exponential increase in the aging population worldwide, AD is expected to be a severe global socioeconomic burden if effective therapies are not developed(Karran *et al.*, 2011).In continuation to underlying problem associate with AD, the aim of this review was to report newly investigated therapeutic targets for future drug development to cure AD.

Clinically, AD is categorized based on the age of onset. Early-onset familial AD results from mutations in genes that encode one of the following proteins:β-amyloid precursor protein (APP), presenilin-1, and presenilin-2. Late-onset, or sporadic, AD is thought to result from a combination of environmental and genetic risk factors(Burden, 2011). Histopathologically, AD is characterized by three major hallmarks: amyloid plaques, neurofibrillary tangles (NFTs) and neuronal degeneration. Amyloid plaques are insoluble extracellular deposits of amyloid-β (Aβ) protein that are formed by the sequential cleavage of APP, while NFTs are intracellular aggregates of the microtubule-binding protein tau (Haass *et al.*, 2012). Both of these structures induce extensive neuronal degeneration and cell death in the last stage of the disease(Mucke *et al.*, 2012).

Among the many approaches used to treat AD, the most advanced focuses on inhibiting Aβ peptide production and/or clearing it from the brain (Jia *et al.*, 2014). Combination therapies that target different causal or modifying factors, including tau, ApolipoproteinE4, secretases, Aβ aggregation, and/or neuro-inflammation, will soon be available (Fig. 1).

α-secretase-targeting therapies

Therapeutic strategies that aim to slow or prevent AD progression mainly targetγ-secretase, β-secretase, and the Aβ peptide. Initially, α-secretase was not considered a possible therapeutic target until a recent study reported that mice with catalytically inactive disintegrin and metalloprotease (ADAM10), which exhibits α-secretase activity, hadhigh amounts of Aβ deposition in the brain(Kuhn *et al.*, 2010). These results indicated that this enzyme is involved in the pathogenesis of AD. Interestingly, blocking the pathogenic features of ADAM10 did not affect its substrate Notch receptor, which showed that the activation of ADAM10 is therapeutically acceptable. Many animal studies and clinical trials have tested therapies to normalize the regulation of α-secretase activity (Table 1). The initial trial of SGS-742 (Novartis International AG, Basel, Switzerland), which was the first gamma-aminobutyric acid (GABA) receptor agonist, showed that SGS-742 enhanced cognition by regulating the activity of α-secretase and up regulating GABA receptors in the frontal cortex and hippocampus in a rat model of AD. Furthermore, the oral administration of SGS-742 significantly improved attention, but it did not exhibit therapeutic efficacy in elderly human volunteers with mild cognitive impairment (MCI)(Froestl *et al.*, 2004). Another pharmacologic modulator of the GABA receptor, EHT 0202 (Diaxonhit, Paris, France), has gained increasing attention due to clinical trials showing that EHT 0202 stimulates the production of secreted APPα (sAPPα), but additional information is not available (Vellas *et al.*, 2011). The roles of other α-secretase modulator/regulators,such as ADAM9 and ADAM17,have not been explored *invivo,* and their therapeutic potential should be evaluated. Other than the ADAM family, signaling pathways involving protein kinase C, mitogen-activated protein kinase,

and tyrosine kinase also regulate α-secretase. Therefore, targeting these pathways to up regulate α-secretase might be a novel therapeutic strategy for AD.

β-secretase (BACE)-targeting therapies

BACE, which is a member of the pepsin family, is theleading initiator of APP cleavage, and it generates toxic Aβ (Cole *et al.*, 2008). Because of its critical role in AD etiology, many pharmaceutical companies have focused on designing novel inhibitors of this enzyme (Table 1). Among the many clinically tested inhibitors,GSK188909 (GlaxoSmithKline plc, Brentford, UK) was the first BACE1 inhibitor tested that efficiently reduced Aβ levels in transgenic mice, but its use was later limited because of its low brain penetration(Hussain *et al.*, 2007). The new drug LY-2811376, which was designed by the Eli Lilly and Company research group (Indianapolis, IN, USA), has shown good bioavailability and high efficacy in dose-dependently reducing Aβ levels in a mouse model of AD, and its use appeared safe in healthy volunteers(May *et al.*, 2011). The same group also introduced LY-2886721, which specifically inhibits BACE1. The oral administration of this drug remarkably inhibits Aβ, sAPPβ, and a cell membrane-bound fragment (C99) in a dose-dependent manner. However, the use of this compound was abandoned during Phase-II studies because it produced liver abnormalities(Hung *et al.*, 2017). Eli Lilly and Company introduced another BACE1 inhibitor, LY2886721, but its use was later terminated during Phase-II studies because liver toxicity was observed in 4 of the 45 patients taking it.

Various compounds developed by Merck researchers (MSD, Darmstadt, Germany) have shown promising effects both *in vitro* and *in vivo*. Verubecestat (MK-8931), which had successful Phase-II clinical studies, is likely the most advanced inhibitor. It is well tolerated and has been shown to down regulate the levels of Aβ in both brain and cerebrospinal fluid (CSF) upto 92%. Furthermore, the optimal half-life of MK-8931 makes this inhibitor ideal for once a day dosing(Kennedy *et al.*, 2016).

A Phase-III study is recruiting 1,500 participants and is to finish in mid-2018.AZD3839, which is another drug specific to BACE1 and which was developed by AstraZeneca plc (Cambridge, UK), showed efficacy in mice, guinea pigs, and nonhuman primates, and remarkably reduced the levels of Aβ and sAPPβ in brain, CSF, and plasma. Currently, this drug is in Phase-Iclinical trials, and it is expected to be a valid and promising drug candidate(Jeppsson *et al.*, 2012). Noncompetitive inhibitorsthat indirectly target BACE1are anotheroption for the development of drugs thattreat AD. Anovel drug called TAK-070 promotes APP processing by modulating α-secretase and specifically disrupting BACE1 interactions with APP. The administration of this drug improves memory and cognition and decreases the amyloid burden up to 60% in the brain of transgenic mice. Clinical trials are needed to confirm its efficacy.

Recently, many other clinically important therapeutic options that target the regulation of the activity ofβ-secretase have been explored.

The pro-neurotrophin receptor sortilin regulates Aβ processing by increasing the BACE1-mediated cleavage of APPand Aβ generation(Finan *et al.*, 2011).Therefore, targeting sortilin might provide another way to slow BACE1-mediated pathogenesis. Another target, SNX12, which is a member of the phospholipid-binding sorting nexin family,is widely expressed in the brain tissue of patients with AD. SNX12 interacts with BACE1and regulates its endocytosis. Thus, the inhibition of the BACE1-mediated Aβ-processing of APP by targeting SNX12 might serve as an alternative strategy in AD interventions (Zhao *et al.*, 2012). Recently, Rheb has been shown to be involved in the progression of AD by regulating BACE1 activity. The exact mechanism underlying how it binds to BACE1and alters secretase levelsis not known(Shahani *et al.*, 2013). Studies have shown that Rheb levels are significantly decreased in the autopsied brains of patients with AD. These findings suggest that increasing the levels of Rheb might reverse the buildup of amyloid plaques. However, more research needs to be done beforedrug candidates can be developed.

Therapies targeting γ-secretase

γ-secretase is another important protease that binds to its substrate, APP,to generate Aβ peptides. In the last decade, this enzyme was considered an attractive drug candidate to target the Aβ pathway. To date, many drugs have been developed to either inhibit or modulate γ-secretase activity (Table 1).

Table 1. Secretase-targeting drugs in Alzheimer's disease clinical trials.

Drug	Target type	Clinical assessment	Current status	ID
SGS742	α-Secretase	Regulates α-Secretase and enhances cognition	Phase II (Discontinued)	NCT00093951
EHT0202	α-Secretase	Stimulates sAPPα production	Phase II (completed)	NCT00880412
LY2811376	β-Secretase	Reduces Aβ levels	Phase I (Discontinued)	NCT00838084
LY2886721	β-Secretase	Inhibits BACE1 activity	Phase II (Discontinued)	NCT01561430
MK-8931	β-Secretase	Improves cognition and behavior	Phase III (ongoing)	NCT01496170
AZD3839	β-Secretase	Reduces CSF and plasma Aβ levels	Phase I (Discontinued)	NCT01348737
LY-2811376	β-Secretase	Dose-dependent decrease of Aβ	Phase I (completed)	NCT00838084
Semagacestat	γ-Secretase	Reduces Aβ burden	Phase III (failed)	NCT01035138
Avagacestat	γ-Secretase	Inhibits γ-Secretase activity	Phase II (completed)	NCT00810147
CHF-5074	γ-Secretase	Promotes axonal growth and astrocyte plasticity	Phase II (completed)	NCT01723670
Ibuprofen	γ-Secretase	Modulates γ-Secretase activity	Phase II (Discontinued)	NCT00007189
Tarenflurbil	γ-Secretase	Generates nontoxic Aβ	Phase III (terminated)	NCT00322036

sAPPα, Soluble amyloid precursor protein-α; Aβ, Amyloid-β; BACE1, β-secretase; CSF, cerebrospinal fluid.

Semagacestat was the first γ-secretase inhibitor patented by Eli Lilly and Company that entered Phase-III studies. Although semagacestat decreased plasma Aβ levels,it unfortunately increased the risk of skin cancer and infection in patients with AD in Phase-III clinical trials(Doody *et al.*, 2013).Eli Lilly andCompany launched another γ-secretase inhibitor called DAPT, which significantly decreases the toxic Aβ burden in APP transgenic mouse model(Branca *et al.*, 2014). However, the chronic administration of this inhibitor was later found to cause thymus, spleen, and skin abnormalities in patients with AD. A Phase-II clinical trial of the γ-secretase inhibitor avagacestat, which was developed by the same group, was recently terminated due to it worsening cognition(Coric *et al.*, 2012).

Table 2. Aβ and tau-targeting drugs in Alzheimer's disease clinical trials.

Drug	Target	Clinical assessment	Current status	ID
Alzhemed	Anti-Aβ fibrils	Reduces Aβ burden and improves cognition	Phase III (inactive)	NCT00314912
AZD-103	Anti-Aβ fibrils	Reduces LTP and Aβ pathology	Phase II (completed)	NCT00934050
PBT2	Anti-Aβ aggregation	Improves cognition	Phase II (completed)	NCT00471211
PQ912	Anti-Aβ aggregation	Inhibits pyroglutamate activity	Phase II (completed)	NCT02389413
Lithium	GSK-3 inhibition	Stabilizes tau phosphorylation	Phase II (completed)	NCT01055392
Tideglusib	Anti-tau	Improves cognition and learning	Phase II (completed)	NCT01350362
AL-108	MT stabilization	Reduces pTau and improves memory	Phase II (completed)	NCT00404014
BMS-241027	MT stabilization	Increases axonal MT density	Phase I (completed)	NCT01492374
Davunetide	MT stabilization	Reduces soluble and insoluble pTau	Phase II (completed)	NCT00422981

Aβ: Amyloid-β, LTP: long-term potentiation, GSK-3: Glycogen synthase kinase-3, MT: Microtubules, pTau: Phosphorylated Tau.

The unfavorable results of these inhibitors might be due to the inhibition of the Notch cleavage. Another inhibitor, MW167, has been shown to inhibit γ-secretase activity and amyloid plaque formation in βAPP-transfected cell cultures. However, because it also damaged the Notch intracellular domain, it has not been tested in humans(Zhou *et al.*, 2015).

Non-inhibitory modulators of γ-secretase have also been developed to ameliorate AD. A study on 6-

month-old AD mice receiving CHF-5074 (Chiesi Farmaceutici S.p.A., Parma, Italy) showed that the drug attenuated spatial memory deficits, amyloid burden, and Aβ levels both in brain and plasma. The prolonged treatment of patients with MCI significantly improved plaque clearance and cognitive abilities without effecting Notch signaling(Sivilia *et al.*, 2013).However, due to notable side effects, such as diarrhea, the Food and Drug Administration (FDA)did not approve this drug for patients with AD. Non-steroidal anti-inflammatory drugs have also been associated with decreasing AD risk by modulating γ-secretase.

Table 3. Immunotherapy and neuroinflammation drugs in Alzheimer's disease clinical trials.

Drug	Target	Clinical assessment	Current status	ID
Bapineuzumab	Passive immunotherapy	Binds to pathogenic Aβ and improves learning	Phase III (completed)	NCT00937352
Solanezumab	Passive immunotherapy	Reduces memory deficits	Phase III (completed)	NCT01900665
Ponezumab	Passive immunotherapy	Reduces cerebral Aβ	Phase II (completed)	NCT00722046
Crenezumab	Passive immunotherapy	Clears access to aggregated Aβ	Phase II (ongoing)	NCT01998841
AN1792	Active immunotherapy	Decreases Aβ	Phase II (completed)	NCT00021723
CAD106	Active immunotherapy	Decreases serum Aβ levels	Phase I (completed)	NCT01097096
Ustekinumab	Targeting inflammation	Inhibits IL-12/IL-23 signaling	Phase I (recruiting)	NCT02835716
Ratanasampil	Targeting inflammation	Decreases TNFα, IL-6, and Aβ	Phase III (completed)	NCT00105547

Aβ: Amyloid-β, IL: interleukin, TNF, tumor necrosis factor.

Ibuprofen shifts the γ-secretase cleaving activity from longer to shorter Aβ species without affecting Notch signaling(Wilkinson *et al.*, 2012). It was later confirmed that ibuprofen caused nausea and vomiting and didnot significantly affect cognition in AD. Another putative γ-secretase modulator, tarenflurbil (Myrexis, Inc. Salt Lake City, UT, USA), attenuates ADsymptoms by producing shorter peptides that are nontoxic. However, the patients who were treatedin the Phase-II clinical trial unfortunately exhibited abnormalities in their daily living and global function(Wilcock *et al.*, 2008). Although γ-secretase inhibitors and modulators were thought to be promising, most were discontinued due to side effects. Many patients who were treated with γ-secretase inhibitors had skin problems, weight loss, and vomiting(De Strooper, 2014).

Recently, γ-secretase activating protein (GSAP) was shown to promote the interaction between γ-secretase and APP, which indicated that this protein cleaved APP. In addition, GSAP-knockout transgenic mice showed selective decreases in the generation of the APP intracellular domain, which improved learning without affecting the normal physiology of γ-secretase (He *et al.*, 2010). G-protein-coupled receptors (GPCRs) are also thought to be active modulators of Aβ pathogenesis. APP processing that is mediated by BACE1 and γ-secretase can be regulated by various GPCRs, including the β2-adrenergic receptor (AR), δ-opioid receptor, and orphan GPR3 (Ni *et al.*, 2006; Thathiah *et al.*, 2009). Additionally, the activation of β (2)-AR has been shown to enhance γ-secretase activity after interacting with presenilin-1, which results in Aβ production, whereas chronic treatment with β (2)-AR agonists has been shown to increase cerebral amyloid plaques in an AD mouse model. These findings suggest that β (2)-AR is a key effector in AD that could be a promising new target for future drug development. Recent findings that β-arrestin-2, which is a scaffolding protein, interacts with GPCRs and γ-secretase and modulates AD progression have suggested new topics to explore AD-related research(Thathiah *et al.*, 2013).

The molecular mechanisms involving these proteins in the γ-secretes complex formation are still unclear.

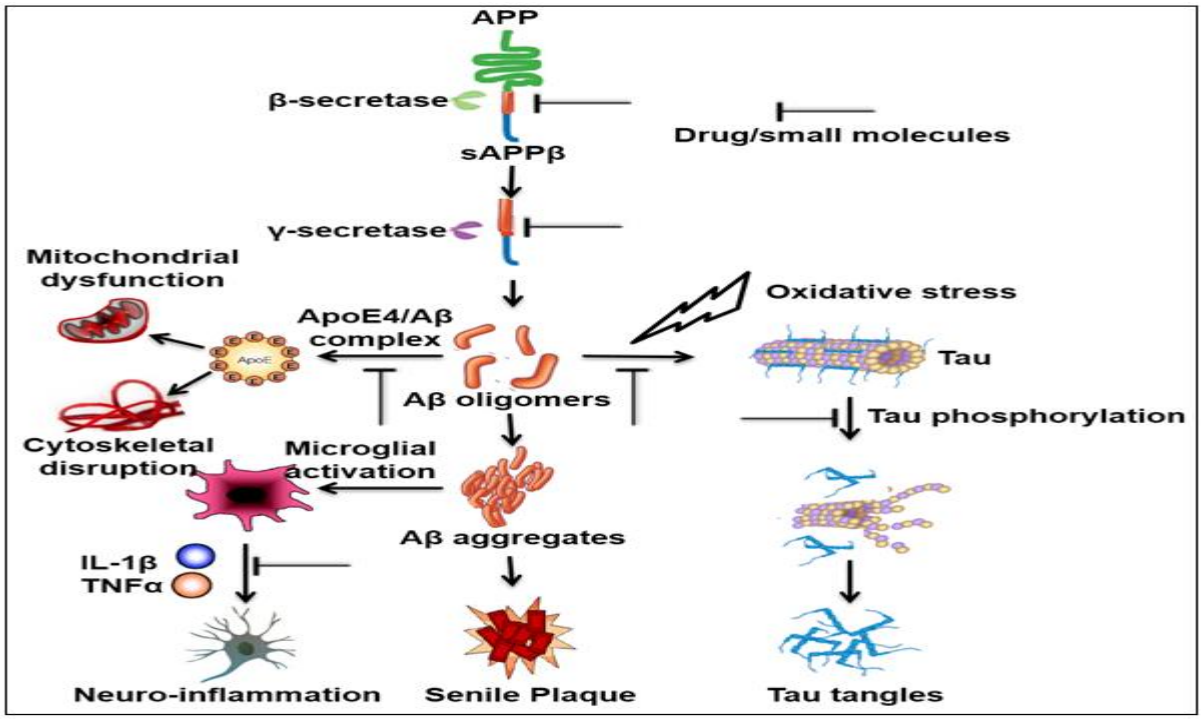

Fig. 1. Potential therapeutic targets in Alzheimer's disease (AD).
Amyloid-β (Aβ) aggregates are generated when β-secretase cleaves β-amyloid precursor protein (APP) to produce the membrane-bound carboxyl-terminal fragment β (CTFβ). CTFβ is then cleaved by γ-secretase to form the C-terminus of the Aβ proteins Aβ40 or Aβ42. A higher percentage of Aβ42 results in AD. The major therapeutic strategies for AD currently being investigated aim to decrease or prevent Aβ-, tau-, Apolipoprotein E4 (ApoE4)-, and inflammation-mediated toxicity. Abbreviations: IL-1β, Interleukin-1β; TNFα, Tumor necrosis factor α.

Another dysfunctional protein in the arresting family, β-arrestin-1, is also involved in AD pathogenesis. Like β-arrestin-2, the levels of expression of this protein are also upregulated in patients with AD. Therapeutically silencing and the genetic deletion of both β-arrestin-2 and β-arrestin-1 has been shown to significantly improve memory deficits and reduce Aβ production in an AD mouse model (Xiaosong Liu *et al.*, 2013; Thathiah *et al.*, 2013).

Thus, inhibiting these newly investigated targetswith small molecules might provide a safe and more selective approach for treating AD symptoms.

Therapies targeting Aβ fibrils

In the past decade, attention has focused on the role of amyloid fibrils and their early intermediates in AD(Chiti *et al.*, 2006). Recent evidence suggests that aromatic peptide fragments that are as short as penta- and tetra-peptides form typically toxic amyloid fibrils that lead to neurodegeneration (Makin *et al.*, 2005).

Currently, Aβ peptide aggregation in toxic fibrils is considered the key pathogenic event in the onset of AD. These pathogenic oligomers directly induce synaptic dysfunction, which weakensintra-neuronal connectivity and causes neuronal death. Drugs that target fibrillary aggregated Aβ are being explored (Table 2). Alzhemed (Bellus Health, Inc., Laval, QC, CAN) is ananti-amyloid dietary supplement. Phase-IIand -III clinical trials of Alzhemed have been conducted in 58 patients with mild to moderate AD who have shown dose-dependent reductions in Aβ levels in the CSF. Alzhemedis well tolerated and improves cognitive function with no serious side effects(Aisen *et al.*, 2007). Unfortunately, the FDA stopped the use of this drug because of its variability among the clinical sites in the Phase-III trials conducted on 1,052 North American patients with AD. Another anti-Aβ fibril agent, AZD-103 (Elan Corporation, Dublin, IR), rescues Aβ-inhibited long-term potentiation in TgCRND8 transgenic mice and

produces dose-dependent decreases in insoluble Aβ40 and Aβ42 and subsequent plaque accumulation. A Phase-II clinical trial involving patients with mild to moderate AD has demonstrated that AZD-103 is acceptably safe, but the primary endpoint of cognitive or functional improvement was not shown(Salloway *et al.*, 2011).Another important metal-protein-attenuating compound that targets Aβ,PBT-2 (Prana Biotechnology Limited, Parkville, VIC, AUS), effectively prevents Aβ accumulation by reducing metal-mediated Aβ aggregation in APP-transgenic mice (Crouch *et al.*, 2011).

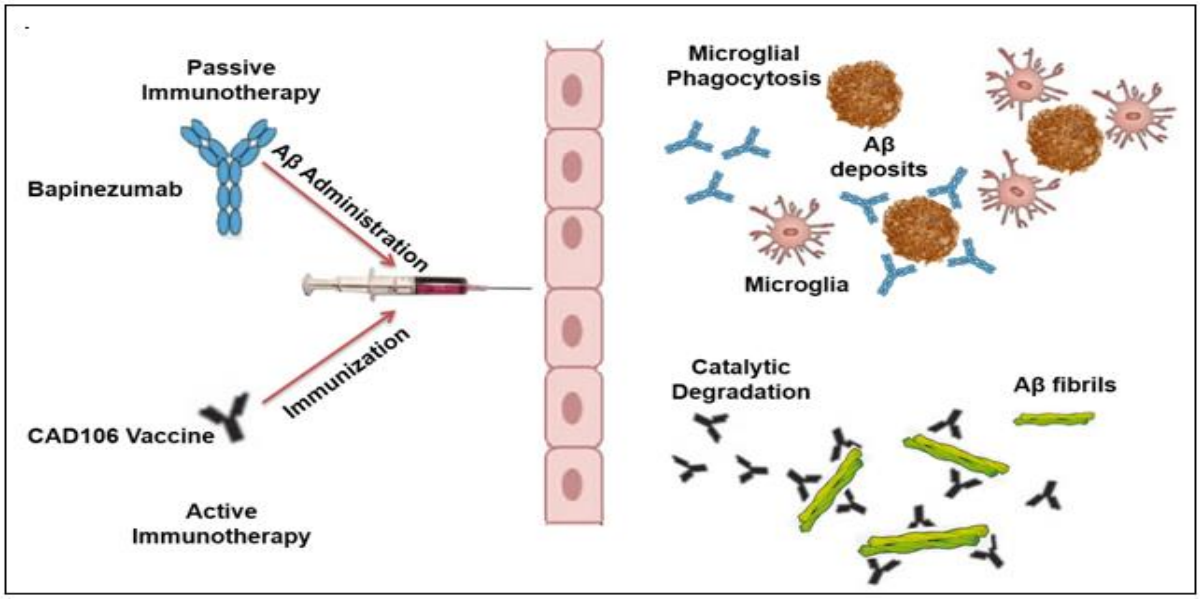

Fig. 2. Immunotherapy for Alzheimer's disease.
Anti-amyloid antibodies, such as bapineuzumab and CAD-106, specifically bind to Aβ oligomers/fibrils and prevent their subsequent deposition into plaques. During microglia phagocytosis, accumulated Aβ is degraded by recruiting activated microglia to opsonize them. Antibodies can also catalytically disaggregate Aβ fibrils and efflux them out of the central nervous system.

The Phase-II studies that were conducted on 78 patients with early AD and 42 patients with mild AD exhibited decreased levels of Aβ in the CSF only and not in plasma(Lannfelt *et al.*, 2008). This drug was later shown to result inin-significant differences between the treatment and placebo groups. Pyroglutamate, which is present in Aβ aggregates, has a critical role in the pathology of AD. Glutaminyl cyclase (QC) catalyzesthe conversion of glutamate into pyroglutamate by altering its biochemical properties with severe pathological consequences. A clinical study has reported that theoral administration of the QC inhibitor PQ912 (Probiodrug AG, Halle, Germany) improved memory and learning and reduced the levels of pyroglutamate-modified Aβ and conventional Aβ, which suggested that QC therapy will be beneficial for patients with AD(Schilling *et al.*, 2008).

The early outcomes of Phase-II trials conducted on 120 patients with MCI and mild dementia have shown that PQ912 inhibited ~92% of the QC activity in the CSF with minimal skin and gastrointestinal side effects. Recent studies have discussed several other proteins and receptors that interact with Aβ and disrupt neural connections, resulting in severe adverse effects on synaptic plasticity and memory. The recent pairing of the immunoglobulin-like receptor Band its orthologimmunoglobulin-like receptor B2 has been shown totrigger the onset of AD(Benilova *et al.*, 2013; Djurisic *et al.*, 2013). The results of cell culture and animal studies have suggested that treatments that block these targets will delay the development of or even prevent this disease. Thus, designing potent drugs that selectively block these receptors may produce promising future treatments for patients with AD, even those in the prodromal stage.

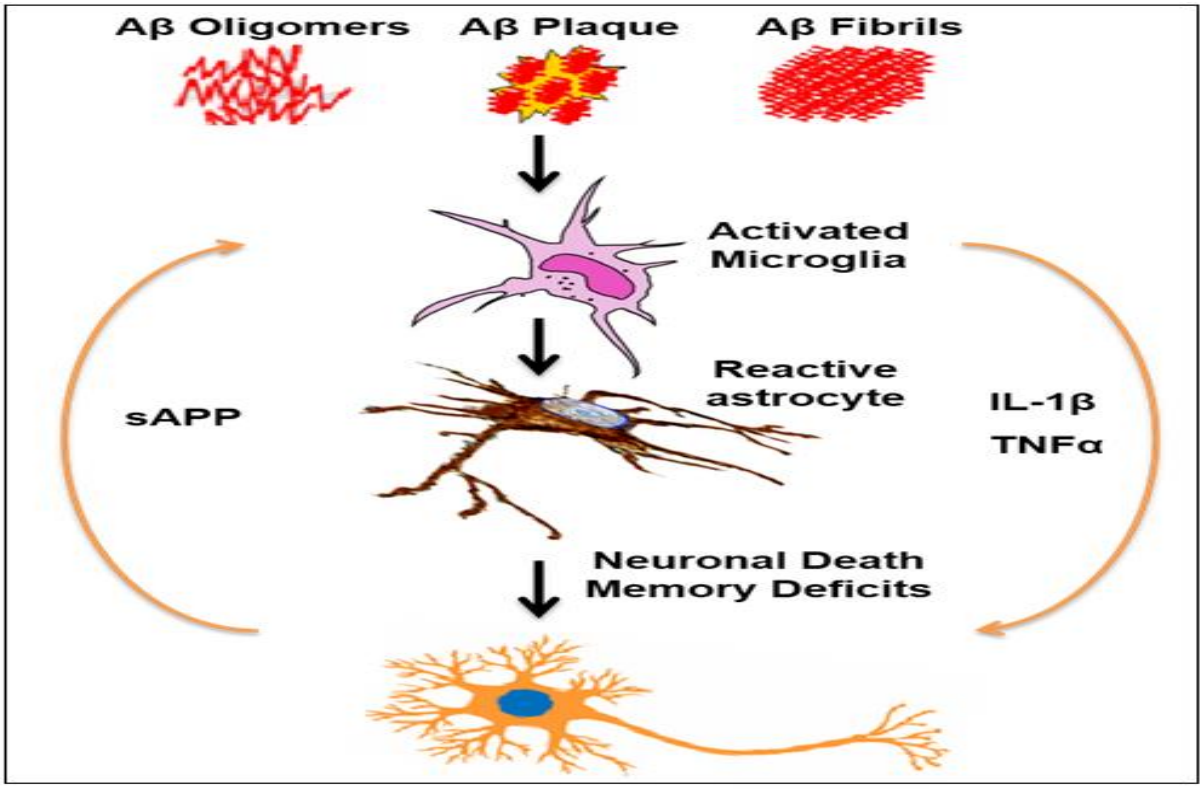

Fig. 3. Amyloid-β (Aβ)-mediated neuroinflammation in Alzheimer's disease.
Aβ accumulation activates microglia and induces the release of proinflammatory cytokines, such as interleukin (IL)-1β and tumor necrosis factor (TNF)α. The resulting action further increase neuronal β-amyloid precursor protein (APP) expression. This mechanism yields a continuous increase in the levels of Aβ, which increases Aβ plaque density and cognitive decline**.**

Therapies targeting tau phosphorylation

The phosphorylation of tau is another hallmark in the pathology of AD. The phosphorylation and de-phosphorylation of tau are normal processes in healthy nerve cells. In AD, tau proteins form NFTs following their overproduction or over phosphorylation (Konzack *et al.*, 2007). Various potential treatments that would correct tau-based neurodegeneration have been explored(Panza *et al.*, 2016). Discovering drugs that specifically target these pathways may prevent AD progression. After the failure of many of the anti-amyloid drugs in clinical studies, pharmaceutical companies have developed tau-based therapies, some of which target tau phosphorylating and dephosphorylating enzymes. A number of these drugs are being tested in clinical trials (Table 2).

Several kinases have recently been reported to phosphorylate tau protein at various phosphorylations sites that induce tau tangles. The two main protein kinases, glycogen synthase kinase 3 (GSK-3) and cyclin-dependent kinase 5 (Cdk5), are apparently involved in the abnormal hyper-phosphorylation of tau(Cruz *et al.*, 2004). Commonly used inhibitors, such as lithium, significantly reduce Aβ production both in cell culture and animal models(Tajes *et al.*, 2008). Treatments with micro-doses (300μg) of lithium stabilize cognitive impairments in patients with AD(Andrade Nunes *et al.*, 2013). In combination with Divalproex, lithium effectively improves dementia-related behavioral symptoms and decreases the levels of altered tau protein. AR-A014418, which is another selective GSK-3β drug patented by AstraZeneca Inc. Cambridge, UK, reduces tauphosphorylation at a GSK-3-specific site (Ser-396) and provides neuroprotection by blocking GSK-3β-mediated pathways. AR-A014418 also improves cognition, but its use is limited because its continuous administration is required to reduce plaque pathology (Bhat *et al.*, 2003).

Tideglusib (Pharma Mar, S.A., Madrid, Spain), which is an orally available small-molecule drug for the treatment of AD and tauopathy progression, inhibits GSK-3β, decreases tau hyper-phosphorylation, and lowers the brain amyloid plaque load in multiple animal models(del Ser *et al.*, 2013). Tideglusib missed its primary endpoints and some secondary endpoints in Phase-II clinical trials conducted on 308 patients with mild to moderate AD, and it was therefore discontinued by the FDA. The other kinase enzyme Cdk5 is also highly expressed in the central nervous system, and the inhibition of Cdk5 reduces Aβ-induced neurodegeneration in cortical neurons(Wen *et al.*, 2008), which suggests that targeting Cdk5 might be an effective future therapeutic strategy for neurodegenerative disorders. Many selective inhibitors of Cdk5 are now at preclinical status (Shu-Lei Liu *et al.*, 2016).

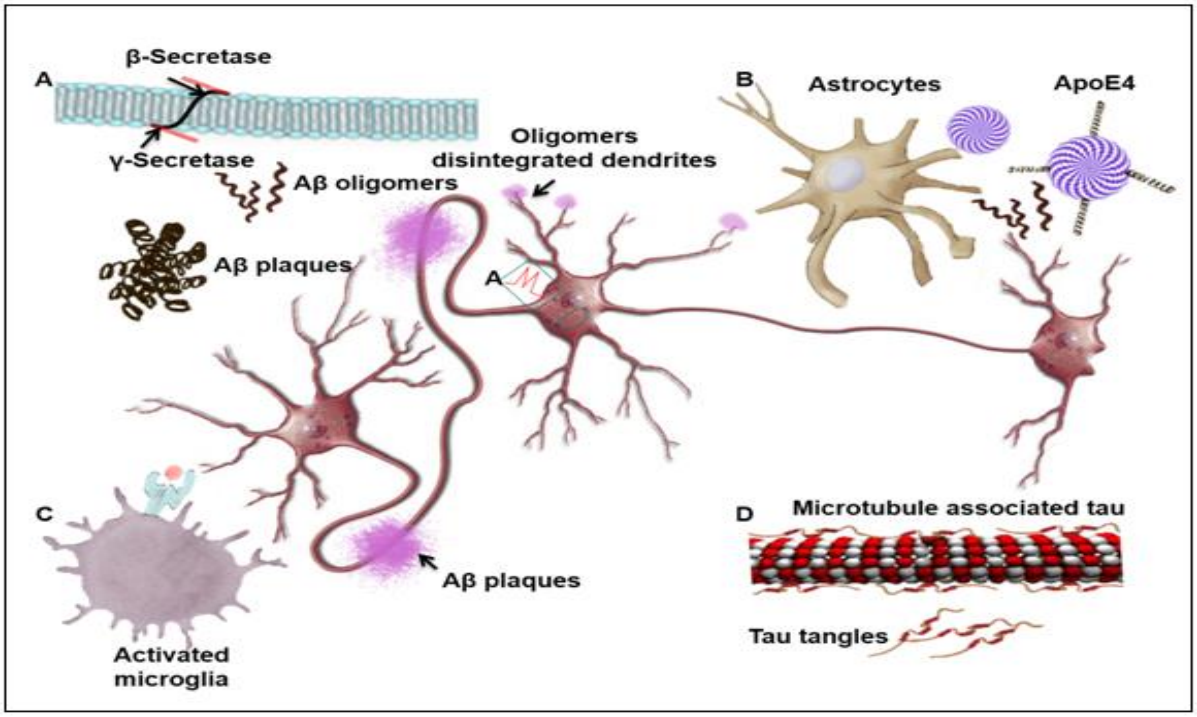

Fig. 4. Schematic representation of the pathway of Alzheimer's disease.
(A) Amyloid precursor protein undergoes amyloidogenic processing by β- and γ-secretase to yield the amyloid-β (Aβ) peptide followed by aggregation to generate plaques. (B) Apolipoprotein E4 (ApoE4), which is synthesized by astrocytes, is engaged by Aβ oligomers to enhance Aβ production, and cause mitochondrial dysfunction. (C) The Aβ deposits activate microglia and start neuro-inflammation by secreting proinflammatory cytokines. (D) The pathological aggregates of tau result in further neuronal loss.

Microtubule stabilization isa critical factor in the restoration of imbalanced tau phosphorylation.BMS-241027 is a small molecule that stabilizes microtubules, increases axonal microtubule density, and improves fast axonal transport and cognitive performance in tau-transgenic mice(Bin Zhang *et al.*, 2012). However, the drug was discontinued due to tolerability and pharmacology concerns in the Phase-I study. The neuropeptide Davunetide (AL-108), which was patented by Allon Therapeutics Inc. (Vancouver, BC, CAN),preferentially interacts with neuronal and glial tubulin and increases microtubule stabilization. The intranasal administration of this drug significantly reduces the level of soluble and insoluble hyper-phosphorylated au and improves cognitive functions in a mouse model of AD(Matsuoka *et al.*, 2008). Phase-II and -III clinical trials have been conducted using the nasal spray formulation on patients with MCI, and the tauopathy, progressive supranuclear palsy, was negative at all endpoints. Therefore, the FDA stopped the use of this peptide.

Dephosphorylating phosphatases have recently been investigated to directly reverse tau hyper-phosphorylation. Phosphatase 2A (PP2A) is one of the most important serine/threonine phosphatases involved in tau dephosphorylationin the mammalian brain(Xu *et al.*, 2008). Interestingly, the inhibition or knockdown of PP2A activity increases tau hyper-phosphorylation both in cultured cells and transgenic mice. One recent study reported that the levels of phosphatase and tensin homolog (PTEN) were depleted in patients with AD compared to control patients. Low levels of PTEN strongly affect tau phosphorylation(Xue Zhang *et al.*, 2006). Therefore, the upregulation of PP2A and PTEN might control tau-related pathology in AD.

Immunological approaches

Recent research has focused on promoting the immunological clearance of Aβ from the brain and disrupting polymerized tau tangles (Fig. 2). Several passive immunotherapeutic agents, including bapineuzumab, crenezumab, ponezumab, gantenerumab and solanezumab, that have a high affinity for Aβhave been evaluated for their potential to clear Aβ from the brain (Table 3). Bapineuzumab (AAB-001; Pfizer, Inc., New York, NY, USA)is a humanized form of the murine monoclonal antibody 3D6, which targets the N-terminal region of Aβand activates microglial phagocytosis that is followed by the clearance of Aβ.In Phase-II and Phase-III clinical studies conducted on patients with mild to moderate AD, patients treated with this drug did not exhibit significant reductions in Aβregardless of its specificity for the target(Salloway *et al.*, 2009). All subsequent trials were discontinued after two Phase-III studies showed no clinical benefits of the drug. Solanezumab (Eli Lilly and Company, Indianapolis, IN, USA) is another humanized monoclonal antibody that binds to the central region of the Aβmonomer that playsa key role in AD pathogenesis. Phase-II studies conducted on patients with AD have shown that solanezumab has a good safety profile and dose-dependently cleared Aβ, which suggested that it might have efficacy in treating AD(Dodel *et al.*, 2013). However, a Phase-III study involving patients with mild dementia who were treated with solanezumabdid not show statistically significant slowing of the cognitive decline compared to placebo, and the company therefore decided to not pursue FDA approval. The monoclonal antibody ponezumab (Pfizer, Inc. New York, NY, USA) improved behavior and cognitive dysfunction in an animal model by binding to Aβ. However, a subsequent phase-II trial did not confirm its clinical efficacy and reported increased plasma levels of Aβ40, which suggested a peripheral sink effect (Landen *et al.*, 2013). Crenezumab (Genentech, Inc., San Francisco, CA, USA)is another humanized antibody that was engineered to clear excess Aβ.This drug can recognize multiple forms of aggregated Aβ, including oligomeric and fibrillar species and amyloid plaques with high affinity and monomeric Aβ with low affinity(Panza *et al.*, 2014). This drug is now entering Phase-III studies that are expected to be completed soon.

Active immunotherapy for the removal of Aβ offers notable advantages. AN-1792, which was the first active vaccine and which was patented by Janssen Pharmaceutica NV (Beerse, Belgium), reached clinical studies but was unfortunately stopped during a phase-II study because it triggered the development of meningoencephalitis (Orgogozo *et al.*, 2003).

Although this Aβ vaccine appears safe andis well tolerated, additional studies are needed to assess its target engagement and clinical efficacy. Novartis International AG has designed another vaccine, CAD106, to react with monomer and oligomer Aβ and block Aβ toxicity. Immunization of APP-transgenic mice with CAD106 has decreased Aβaccumulation in the brain(Wiessner *et al.*, 2011). A Phase-II study on 58 patients with mild AD has shown positive outcomes, and a Phase-III study is expected to be completed in 2023.

Therapies targeting neuroinflammation

In AD, Aβ proteins, NFTs, and neuronal degeneration are the major contributors to inflammation(Walters *et al.*, 2016). In addition, findings of abundant reactive glial cells, including microglia and astrocytes,

at amyloid plaque sites have indicated that inflammation plays an important role in AD progression (Fig. 3). The two main proinflammatory cytokines of interest, interleukin (IL)-12 and -23, showed a robust upregulation in patients with AD(Griffin, 2013). The genetic ablation or therapeutic silencing of IL-12 and/or IL-23 or their shared receptor p40 strongly and significantly decreases the Aβ-plaque load(vom Berg *et al.*, 2012). The FDA has approved studies of the use of ustekinumab, which was previously used to treat psoriasis, to treat AD (Table 3). Another drug, Ratanasampil, which is traditionally used to treat cerebrovascular diseases, improves cognitive function in transgenic mice. Its administration to patients with mild to moderate AD at high doses significantly decreases the serum levels of Tumor Necrosis Factor-α, IL-6, and Aβ with no observable adverse effects (Zhu *et al.*, 2012).These results indicated that inhibiting the inflammatory signaling pathway quenches Aβ in the brain. Recent reports state that many receptors are associated with Aβ-induced microglial inflammatory activation and Aβ internalization, but the underlying mechanisms remain unclear. Toll-like receptors are considered a primary receptor for Aβfor triggering euro inflammation, and their deficiency in cultured microglia significantly reduces Aβ-triggered M1 activation(Lehnardt, 2010). Fcγ RIIb, which is another microglia receptor, interacts with Aβ and up regulates its expression in cortical neurons. Therefore, the use of RNA inte reference approaches or selective inhibitors that block the Fcγ RIIb and Aβ interaction while allowing FcγRIIb to interact as usual with immunoglobulin's might be a new therapeutic way to prevent neurotoxicity(Kam *et al.*, 2013).

Other therapeutic approaches

AD is a very complicated manifestation of multiple physiological abnormalities. Current research on therapies focuses on blocking pathologic signaling pathways that are initiated by Aβ and phosphorylated tau (Fig. 4). Fyn, a member of the Src enzyme family, is a causative indicator of the pathophysiology of AD. Thus, the blockage of Fyn kinase by highly specific inhibitors is a therapeutic intervention with much potential. Insulin plays a very important role in synaptic dysfunction by regulating glucose homeostasis in the AD brain. Evidence has shown that the insulin pathway is disrupted and the concentration of insulin is decreased in patients with AD. The intranasal administration of insulin to patients with AD improves cognitive function and delays clinical worsening (Craft *et al.*, 2012). A study of longer administration of insulin will be started soon.

Conclusion

The key finding in this review is that, till now no truly effective therapy to improves cognition or memory impairment associated with AD has been found. The lesson from failed AD therapies in clinics highlights the need to explore alternative therapeutic targets. In future, the use of combinational therapies that treat multiple targets that we reviewed will be more effective soon. In addition, a successful system to deliver small molecule drugs to the brain is urgently needed to understand AD pathobiology.

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