Pathological mechanisms and therapeutic strategies for Alzheimer’s disease

Introduction
Alzheimer’s disease (AD) is the most common form of dementia, and is estimated to affect 131.5 million by 2050 if no effective therapies are available (Cummings et al., 2016). The only 4 available Food and Drug Administration (FDA) approved agents for AD treatment offered limited effects on cognitive improvement. Though considerable efforts have been directed to tackle this disease, AD remains inexorable and incurable. The high failure rate of AD drug development was thought to be mainly due to our poor knowledge about the complex pathological mechanism of this disease (Cao et al., 2018). There are numerous factors playing a role in the prognosis of AD. A number of hypotheses concerning the root cause of AD reveal the complexity of the disease. Cholinergic deficiency (Ferreira-Vieira et al., 2016), amyloid beta (Aβ) toxicity (Selkoe and Hardy, 2016), tau protein hyperphosphorylation (Lewis and Dickson, 2016), synaptic dysfunction (Briggs et al., 2016), oxidative-stress (Kumar and Singh, 2015), and neuroinflammation (Calzolari and Edison, 2016) were proposed to be responsible for AD development. Regardless what the root cause of AD is, all these factors intensify the progression of disease. For decades, the “one drug for one target” strategy has been dominant, but is still unable to conquer this multifactorial disease. It is hypothesized that the multifunctional strategy, which could simultaneously modify different pathological pathways, would be helpful to treat this multifaceted disease (Savelieff et al., 2019).

In this review, we will describe the pathological mechanisms of the multiple etiologies of AD, establish the associations with some potential therapeutic targets and follow with an outline of the treatments currently under clinical evaluations for tackling these therapeutic targets. Finally we will briefly highlight some studies using multi-target drug development for AD treatment.

Search Strategy and Selection Criteria
Studies cited in this review published from 1990 to 2020 were searched on PubMed or Google Scholar database using the following keywords: Alzheimer’s disease, neurodegenerative disease, therapeutic, choline, amyloid, tau, synapse, antioxidant, neuroinflammation, multifunction, synaptic plasticity, glutamatergic, GABA, dopaminergic, adrenergic, antioxidant, neuroinflammation, multifunction, synaptic plasticity, glutamatergic, GABA, dopaminergic, adrenergic.

Abstract
Alzheimer’s disease is a rather complex neurodegenerative disease, which is attributed to a combination of multiple factors. Among the many pathological pathways, synaptic dysfunctions, such as synapses loss and deficits in synaptic plasticity, were thought to be strongly associated with cognitive decline. The deficiencies in various sorts of neurotransmissions are responsible for the multifarious neurodegenerative symptoms in Alzheimer’s disease, for example, the cholinergic and glutamatergic deficits for cognitive decline, the excitatory and inhibitory neurotransmission dyshomeostasis for synaptic plasticity deficits and epileptiform symptoms, and the monoamine neurotransmission for neuropsychiatric symptoms. Amyloid cascade hypothesis is the most popular pathological theory to explain Alzheimer’s disease pathogenesis and attracts considerable attention. Multiple lines of genetic and pathological evidence support the predominant role of amyloid beta in Alzheimer’s disease pathology. Neurofibrillary tangles assembled by microtubule-associated protein tau are other important histopathological characteristics in Alzheimer’s disease brains. Cascade of tau toxicity was proved to lead to neuron damage, neuroinflammation and oxidative stress in brain. Ageing is the main risk factor of neurodegenerative diseases, and is associated with inflammation, oxidative stress, reduced metabolism, endocrine insufficiencies and organ failures. These aging related risk factors were also proved to be some of the risk factors contributing to Alzheimer’s disease. In Alzheimer’s disease drug development, many good therapeutic strategies have been investigated in clinical evaluations. However, complex mechanism of Alzheimer’s disease and the interplay among different pathological factors call for the come out of all-powerful therapies with multiple curing functions. This review seeks to summarize some of the representative treatments targeting different pathological pathways currently under clinical evaluations. Multi-target therapies as an emerging strategy for Alzheimer’s disease treatment will be highlighted.

Key Words: Alzheimer’s disease, pathological pathways, drug development, multiple pathologies

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**Synaptic Dysfunctions in Alzheimer’s Disease**

The most common symptom of AD is learning and memory decline. Synaptic connectivity between neurons is dynamic and plastic, which is fundamental in learning and memory (Stuchlik, 2014). Compared with other biochemical indices [e.g., senile plaques, neurofibrillary tangles (NFTs)], synaptic loss was reported to be strongly correlated with cognitive impairment in AD (Terry et al., 1991). Synapse loss decreased the efficiency of neural signal transmission and disintegrated the neuronal network leading to cognitive dysfunctions in AD transgenic mice (Kashyap et al., 2019). Multiple studies have demonstrated that alteration of synaptic protein expression and synaptic plasticity were early events during AD progression in human and AD mouse brains samples (Mango et al., 2019). “Synaptic plasticity” regulates the number, structure, and strength of the synaptic connections between neurons. Long term synaptic plasticity mainly consists of long-term potentiation (LTP) and long-term depression (LTD), in which potentiation and depression demonstrate the increase and decrease of synaptic signal strength. The inhibition of LTP and enhancement of LTD were found to be associated with the progressive memory impairment in AD (Jang and Chung, 2016).

The most extensively studied forms of synaptic plasticity are the LTP and LTD in CA1 region of the hippocampus. The predominant hypothesis is that the postsynaptic calcium signal within dendritic spines dictates whether LTP or LTD triggered, with LTP requiring a calcium increase beyond a threshold and LTD requiring a modest calcium increase (Malenka and Nicoll, 1993; Citri and Malenka, 2008). Specifically, LTP involves preferential activation of protein kinases such as the calcium/calmodulin (CaM)-dependent protein kinase II (CaMKII), the cyclic adenosine monophosphate-dependent protein kinase (PKA), the extracellular signal-regulated kinase (Erk)/mitogen-activated protein kinase (MAPK), Src kinase and protein kinase C (PKC). LTD involves activation of phosphatases such as the calcium/calmodulin-dependent phosphatase calcineurin, protein phosphatase 1 or dephosphorylation of PKA and protein kinase C substrates. Following LTD, there is enhanced α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors (AMPARs) exocytosis and incorporation of AMPARs into postsynaptic density involving the phosphorylation by CaMKII, accompanied by growth of new dendritic spines; while following LTD, there is enhanced endocytosis and dissociation of AMPARs from postsynaptic density regulated by calcium-dependent dephosphorylation, accompanied by shrinkage in the size of dendritic spines. The increase of synapse size in LTP is dependent upon dendritic protein synthesis, thus transcription factors such as cAMP response element-binding protein presumably to supply critical proteins, are required for maintaining synapse strength.

To ameliorate synaptic dysfunctions in AD, drug development strategies to improve synaptic plasticity and neural regeneration have been tested in clinical trials (Additional Table 1). Four clinical trials have been undertaken on 3 phosphodiesterase inhibitors to improve synaptic functions in AD. Among these four clinical trials, cilostazol has been advanced in phase 3, indicating phosphodiesterase inhibitors could be promising in AD treatment. Moreover, sigma-1 receptor agonists have been investigated in AD drug development in 7 ongoing clinical trials with 6 of which in phase 3. Interestingly stem cell therapies have attracted considerable attention in recent years. All these therapies are currently in phase 1 or phase 2.

**Neurotransmission in Alzheimer’s Disease**

**Cholinergic hypothesis of AD**

The nucleus basalis of Meynert in the basal forebrain is a major source of cortical acetylcholine, which was reported with significant neuronal loss in AD patients (Doucette et al., 1986). In cholinergic presynaptic neurons, acetylcholine is synthesized by the enzyme choline acetyltransferase (ChAT) from choline and acetyl-coenzyme A, and transported to synaptic vesicles by vesicular acetylcholine transporter. Following the depolarization of neurons, acetylcholine is released into the synaptic cleft. Acetylcholine binds to acetylcholine receptors (namely the ligand-gated channel nicotinic acetylcholine receptors, and the G-protein coupled muscarinic acetylcholine receptors) to enable neurotransmission. The acetylcholine presented at the synaptic cleft is rapidly decomposed by acetylcholinesterase into choline and acetate. The extracellular free choline can be uptake by choline transporters into the presynaptic neurons for acetylcholine synthesis.

Evidence shows that the brain cortical cerebrospinal fluid acetylcholine levels were significantly lower in AD patients, which was correlated with cognitive impairment (Jia et al., 2004). Significant ChAT depletions were observed in postmortem AD brains but the reduction of ChAT was reported to be correlated with the severity of dementia (Pappas et al., 2000). According to a longitudinal clinical study over 3 ± 1.5 years, the cholinergic basal forebrain atrophy rates were higher than the global brain shrinkage rates in the aging process, which was further increased in AD patients (Grothe et al., 2013). Significantly reduced nicotinic and muscarinic cholinergic receptors in nucleus basalis of Meynert of AD brains were observed according to the previous ligand binding studies in autopsied brains (Shimohama et al., 1986). The evidence of cholinergic innervation losses correlated with cognitive decline in AD patients formed the foundation of the “cholinergic hypothesis of Alzheimer’s disease”. Moreover, association between several strong anticholinergic drug exposure and increased risk of incident dementia were found in aged people (Coupland et al., 2019).

**Based on the “cholinergic hypothesis”, three acetylcholinesterase inhibitors, including donepezil, rivastigmine and galantamine were approved by US FDA for AD treatment. To modify the deficits of cholinergic neurotransmission in AD, more cholinesterase inhibitors were developed and some of them are undergoing clinical trials (Additional Table 2).** Nicotinic and muscarinic acetylcholine receptors agonists or positive allosteric modulators have entered clinical trials to evaluate the effects on enhancing cholinergic neurotransmission (Additional Table 2).

**Glutamatergic neurotransmission in AD**

Glutamate is the primary excitatory neurotransmitter in the brain. Glutamate can be produced from glutamine by glutaminase and is the precursor of gamma-aminobutyric acid (GABA), an inhibitory neurotransmitter. L-Glutamate is the most abundant free amino acid in brain and is the major excitatory neurotransmitter of the vertebrate central nervous system. Glutamatergic neurotransmission plays an important role in LTP, which is thought to be extremely important for learning and memory formation (Granger et al., 2013). Glutamate receptors are classified into two families: G protein-coupled metabotropic glutamate receptors (mGlURs) and the ligand-gated ionotropic glutamate receptors (iGlURs) (Reiner and Levitz, 2018). Glutamate binding to mGlURs leads to the production of inositol phosphate and second message signaling, affecting multiple signaling pathways within the cells. Glutamate binding to iGlURs which comprises three subfamilies: AMPA receptors, kainate receptors, and N-methyl-D-aspartate (NMDA) receptors) produces fast excitatory currents. AMPA receptors and kainate receptors are...
extremely fast receptors at high glutamate concentrations. AMPA receptors are permeable to Na⁺ and Ca²⁺ and kainate receptors are mainly permeable to Na⁺ and K⁺. NMDA receptors show slower activation and higher Ca²⁺ permeability than AMPA and kainate receptors. Glutamate, together with the receptor co-agonist (glycine or D-serine) binding to NMDA receptors, combined with a strong postsynaptic membrane depolarization to release the magnesium ions (Mg²⁺) block of the receptor channels. The opened NMDA receptors allow the flow of Na⁺, K⁺, and Ca²⁺ into the cell leading to excitatory postsynaptic current.

Synapse losses and glutamatergic dysfunctions with AMPA receptors and NMDA receptors downregulation in the hippocampus were observed in AD patients (Jacob et al., 2007). However, AD drug development targeting on glutamatergic neurotransmission has been mainly focused on reducing glutamatergic neurotransmission. The inappropriate activation of glutamatergic signaling (mainly through NMDA receptors activation) results in excitotoxicity. Amyloid deposition increased the activation of Fyn to phosphorylate GluN2B subunit of NMDA receptors (NMDARs), and subsequently to strengthen the activity of NMDARs, through which excessive harmful levels of calcium ions fluxed into postsynaptic neurons and impaired synaptic functions (Rudy et al., 2015). Based on this theory, memantine, a non-competitive NMDA receptor antagonist was developed and approved for moderate to severe AD treatment in clinic. There are agents in clinical trials for AD drug development to exert neuroprotective effect via the reduction of glutamate release (Additional Table 2).

**GABAergic neurotransmission in AD**

GABA is the principal inhibitory neurotransmitter in the mammalian central nervous system. It plays an important role in maintaining excitatory and inhibitory balance in the brain (Smart and Stephenson, 2019). Literature evidence suggested that GABAergic remodeling contributed to the pathogenesis of Alzheimer’s disease (Govindpani et al., 2017). GABA is generated via α-decarboxylation of L-glutamate by the glutamic acid decarboxylase (GAD) with pyridoxal-5’-phosphate as cofactor to converse the inactive apo-GAD into Gαi and Gβγ, and subsequently to strengthen the activity of Gαi and Gβγ, and subsequently to reduce the amount of the coupled G protein into Gαi and Gβγ, and subsequently to reduce the activity of adenylate cyclase. The cAMP signaling regulates the excitatory glutamatergic and cholinergic synaptic plasticity. Gβγ suppresses the influx of Ca²⁺ and triggers the release of the transmitter. Gβγ also promotes the K⁺ efflux through the K⁺ channel resulting in hyperpolarization and inhibition (Terunuma, 2018).

Up to 22% of AD patients experienced seizures (Mendez and Lim, 2003). Epileptiform discharge was observed in 22% of AD patients with no history or risk factors for epilepsy (Lam et al., 2020). Excitatory and inhibitory balance was found to be essential for brain oscillations, and disruption of functional oscillation contributed to memory deficits (Missionnier et al., 2020). GABAergic dysfunction has long been suggested to involve in the development of epilepsy and status epilepticus (Jones-Davis and Macdonald, 2003). Therefore, it has been hypothesized that targeting the enhanced GABAergic inhibition might be a valuable therapeutic option for AD treatment (Xu et al., 2020). Agents with anti-epilepsy efficacy (Additional Table 2) have been in clinical trials for AD treatment.

**Monoaminergic neurotransmission in AD**

Deficits in monoaminergic neurotransmission such as dopaminergic, noradrenergic and serotonergic neurotransmission were reported to be involved in AD. The monoamine neurotransmitters, which are synthesized and released from their presynaptic neurons, bind to the corresponding receptors on the postsynaptic membrane to exert functions. The excessive amount of monoamine neurotransmitters in the synaptic cleft is then degraded by monoamine oxidase or catechol-O-methyltransferase, and subsequently to undergo reuptake into the presynaptic terminal by monoamine transporters.

Dopaminergic deficits were most seen and investigated in Parkinson’s disease, a movement disorder characterized by rigidity, resting tremor, and bradykinesia (Cacabelos, 2017). More than 50% of patients with mild cognitive impairment or mild or mild AD were diagnosed with concomitant Parkinsonism with rigidity, resting tremor, and DA transporter reduction in the basal ganglia (Sasaki, 2018). Dopaminergic deficits were associated with cognitive dysfunctions in AD patients, and restoration of dopaminergic neurotransmission rescued the pathologies and cognitive deficits in AD patients and AD mouse models (Koch et al., 2014; Cordella et al., 2018). Dopaminergic stimulation is identified as a potential therapeutic strategy for AD. However, the dopaminergic system is closely related to the brain reward. It has been suggested that dopaminergic dysfunction might account for neuropsychiatric symptoms in AD (Mitchell et al., 2011). Dopamine agonists and dopamine reuptake inhibitors have been in clinical trials (Additional Table 2) to improve neuropsychiatric symptoms in AD.

There have been reports of significant Locus coeruleus (LC) noradrenergic neurodegeneration, such as neuron loss and astrocytic loss, associated with the severity of cognitive dysfunction in AD (Bondareff et al., 1987; Theofilas et al., 2017). Cognitive impairment exhibited correlations with LC tauopathy in aging, mild cognitive impairment and AD (Grudzien et al., 2007). Enhancing brain NE levels can reverse AD dysfunction, such as long-term potentiation deficits, cognitive decline, and neuroinflammation in AD animal models (Ardestani et al., 2017). Thus, the LC noradrenergic system is essential for maintaining cognitive function and could be targeted to improve cognition in AD. Agents aimed at maintaining normal noradrenergic neurotransmission are in clinical trials for AD treatment (Additional Table 2).

Extensive serotonergic denervation and serotonergic alteration were observed in both AD patients and AD animal models, and were suggested to be associated with AD
pathogenesis (Ouchi et al., 2009; Ramos-Rodriguez et al., 2013). Restoration of serotonergic function by selective serotonin reuptake inhibitors, or serotonin receptor agonists or antagonists was proven to modulate behavioral and cognitive symptoms (Bianco et al., 2016; Bostanci kloğlu, 2020). Thus, targeting the serotonergic system would be a promising approach for treating AD symptoms. Agents targeting serotonergic system in clinical trials for AD treatment are listed in Additional Table 2.

Other neurotransmission in AD

Other neurotransmissions such as the cannabinoid neurotransmission and orexergic neurotransmission are also involved in motor learning and neuropsychiatric aspects. In the endogenous cannabinoid system, the endocannabinoids anandamide, for example, was generated firstly by transacylase to catalyze the conversion of phosphatidylethanolamine to N-acylphosphatidylethanolamine and then by phospholipase D cleavage. CB1 receptor activation was found to regulate intracellular Ca2+ concentration, glutamate release, neurotrophin expression and neurogenesis. CB2 activation was involved in the release of cytokine in microglia and had been suggested to play a role in the inflammatory pathology of AD (Talarico et al., 2019). Orexin is a hypothalamic neurotransmitter with functions to regulate wakefulness, appetite and mood. Investigations suggested that orexinergic signaling activation altered the sleep-wake cycle and induced Aβ and tau pathology mediated neurodegeneration (Liguori, 2017). Thus, there are cannabinoid and orexin related agents in clinical trials to modulate the symptoms in AD (Additional Table 2).

The agents targeting neurotransmission system in AD drug clinical trials are mainly for modulating AD symptoms, such as cognitive decline, epileptiform symptoms, insomnia and agitation. As shown in Additional Table 2, many agents are repurposed drugs specifically for these symptoms. It would be helpful to modulate AD symptoms based on the treatments developed for other neural disorders.

Amyloid Cascade Hypothesis in Alzheimer’s Disease

Senile plaques, which composed of Aβ peptides, are one of the most important pathological hallmarks in AD brains (Xiao et al., 2015). In pathological conditions, Aβ is the proteolytic product of amyloid precursor protein (APP) by β-secretase and then γ-secretase via an amyloidogenic pathway, while in physiological conditions, APP is catalyzed by α-secretase instead of β-secretase via a non-amyloidogenic pathway to form soluble APPα fragment (Soldano and Hassan, 2014). The strongest support for the initial role of Aβ in this disease comes from the genetic evidence clarifying the formation of AD. APP, presenilin 1 (PSEN1), and presenilin 2 (PSEN2) are mutation genes responsible for familial AD or early-onset AD. Aβ, the major subunit composed of amyloid plaques, is the cleavage product of APP, whose coding gene is located on human chromosome 21 (Masters et al., 1985). The AD-like anatomy characteristics, namely the senile plaques and NFTs, were formed and distributed in people with Down syndrome, even at young ages, indicating the importance of APP in AD pathology (Mann and Esiri, 1989). Missense mutations in APP genes were shown to alter Aβ metabolism with either aggregating or restraining effect on Aβ aggregation and cognitive decline in AD patients (Godbolt et al., 2006; Ian et al., 2014). AD symptoms formed in APP transgene mice models further verified the Aβ hypothesis (Hsiao et al., 1996). Mutations in PSEN1 and PSEN2 lead to an “aggressive forms of” Alzheimer’s disease by affecting γ-secretase activity to aggravate Aβ aggregation in AD patients (Bentahir et al., 2006). The strongest genetic risk factor for sporadic AD or late-onset AD is the apolipoprotein E (ApoE) (Musiek and Holtzman, 2015). ApoE isoforms influence AD by regulating Aβ clearance differently, with the APOE ε4 allele markedly increases AD risk while the APOE ε2 allele decreases Aβ accumulation (Castellano et al., 2011).

Another strong support for the crucial role of Aβ is the synergistic neurotoxic effects on other pathologies. It was shown that plaques formed by Aβ aggregation activated the microglia and ensued progressive neural changes and dysmorphic neurites in vivo models (Meyer-Luehmann et al., 2008). Aβ is considered a driving force for tau propagation. For instance, injection of Aβ1-42 into the brains of P301L mutant tau transgenic mice accelerated the AD symptom formation (Gott et al., 2001). This was further supported by the test of crossing rfgTauEC transgenic mice with APP/PS1 mice, in which the amyloid deposition dramatically increased tau propagation and spread, as well as the tau-induced neuron loss (Pooler et al., 2015). Amyloid cascade was also supposed to be a driving force of neuronal hyperexcitation in AD. A recent study revealed that Aβ induced hyperexcitation in sensitive neurons and sustained the vicious cycle of neuronal hyperactivation (Zott et al., 2019). Another study revealed that secreted APP (sAPP) specifically bound to GABAB1a and suppressed synaptic release, suggesting that secreted APP-GABAβ1a interaction might play a role in maintaining neural circuits homeostasis (Rice et al., 2019).

The amyloid cascade hypothesis has gained continuous support for nearly 30 years. Moreover, targeting amyloid transport, APP secretase enzyme, and amyloid aggregation and clearance were suggested as viable therapeutic strategies (Kumar et al., 2015). Therapies targeting Aβ have been studied extensively and intensively. The on-going anti-amyloid clinical trial studies are summarized in Additional Table 3. In these anti-amyloid strategies, targeting amyloid clearance seems to be rather popular. There are 10 immunotherapies in 18 clinical trials aiming to remove Aβ monomers, oligomers and plaques. Amongst them, 4 immunotherapies are currently in 10 phase 3 studies.

Tau Toxicity Cascade in Alzheimer’s Disease

NFTs are another important histopathological characteristics in AD brains (Lewis and Dickson, 2016). The NFTs comprise of paired helical filaments, which assembled by microtubule-associated protein known as tau. Tau protein assembles tubulin into microtubules and stabilizes microtubules (Goodson and Jonasson, 2018). As major cytoskeletal components of the neuron, microtubules play a fundamental role in neuronal development and function (Kapitein and Hoogenraad, 2015). The dissociation of microtubule stabilizer tau protein in AD induces depolymerization of microtubules and then further destroys neural functions. Tau phosphorylation is a normal metabolic process in physiological conditions. In contrast, in some pathological conditions, Aβ toxicity, neuroinflammation, and other stress conditions lead to aberrant tau phosphorylation (Gao et al., 2018). In particular, dysequilibrium of tau kinase and phosphatase activities leads to abnormal tau phosphorylation, thereby contributing to tau aggregation. A variety of tau kinases, such as CK1/2, glycogen synthase kinase-3 (GSK-3), PKA, p38MAPK, Erk1/2, JNK1/3, CDK5, TTBK1/2, and CaMKII, have been summarized elsewhere (Martin et al., 2013). The hyperphosphorylated tau is prone to dissociation from microtubules and aggregation to form NFTs (Wang et al., 2013). The existence of NFTs and the dissociation of microtubules then lead to axonal transport impairment, mitochondrial and cytoskeletal dysfunction, neuroinflammation, oxidative stress, and synapses loss (Hoover et al., 2010). Messing et al. (2013) reported that in...
the tau toxicity cascade, dendritic spine loss was observed before aggregation and cell death in an early stage, and tau aggregation and cell death in the later stage, were found to be accompanied by caspase-3 activation. These authors also proved that a tau aggregation inhibitor could prevent the phosphorylation, aggregation, and dendritic spine loss in tau pathology. The repeat domain located in paired helical filaments showed a high binding affinity to truncated tau and was responsible for tau-tau binding. These have led to the study of inhibitors targeting this repeat domain to stop tau aggregation.

In clinical trials of AD drug development, strategies targeting microtubule stability, tau protein aggregation, tau production and clearance were adopted to treat tau toxicity. **Additional Table 4** summarizes the agents to modulate tauopathy in AD clinical trials. Among these agents, therapies targeting tau protein clearance occupied most of the seats. However, none of these immunotherapies for tau protein clearance has entered phase 3 study yet. Tau aggregation inhibitor, TRX0237 (LMXT), is the only anti-tau agent currently in phase 3 study for AD treatment.

### Ageing Related Risk Factors in Alzheimer’s Disease

Ageing facilitates and accelerates cognitive impairment and is the most predominant risk factor for neurodegenerative diseases, including AD (Hou et al., 2019). In aged population, there are dysregulations of the immune system and decreased metabolism levels with higher risk of neuroinflammation, oxidative stress and vascular diseases as well as diabetes (Donato et al., 2018; Rea et al., 2018; Luo et al., 2020). These ageing related risk factors are supposed to involve in AD pathologies.

Multiple studies have shown that there were elevated inflammatory cytokines and chemokines and accumulated activated microglial at the damage region in AD brains (Calsolaro and Edison, 2016). In recent years, genome-wide association studies have identified several AD-risk single nucleotide polymorphisms associated with or related to microglial function, including TREM2, CD33, CR1, CLU, CD2AP, EPHA1, ABCA7, and INP5SD (Spangenberg and Green, 2017), indicating that microglia played a critical role in the development of AD. An updated meta-analysis from the cohort of the year 1995 to 2016 demonstrated that the use of non-steroidal anti-inflammatory drugs was significantly associated with the reduced risk of AD (Zhang et al., 2018). Anti-inflammatory agents for AD treatment currently in clinical trials are listed in **Additional Table 5**.

Oxidative stress, an imbalance between reactive oxygen species and antioxidants in biological system, is related to aging and involved in AD pathology to induce tau phosphorylation and synapse dysfunction in the brain (Kumar and Singh, 2015). Glutathione redox imbalance in the brain was found to contribute to the pathology of neurodegenerative diseases, suggesting that therapies aimed at improving the anti-oxidant level could be promising approaches for AD treatment (Gu et al., 2015). Natural products could provide many antioxidant agents, and have proved beneficial to AD patients. Polyphenols, such as curcumin, resveratrol and epigallocatechin-3-gallate, were suggested to have good potential for AD treatment with low frequency of adverse events (Syarifah-Noratiqah et al., 2018). The currently antioxidant agent in clinical trials for AD treatment are summarized in **Additional Table 5**.

Certain vascular lesions such as cerebral amyloid angiopathy, microvascular degeneration, and periventricular white matter lesions are evident in almost all cases of AD (Kalaria and Ballard, 1999). In the two-hit vascular hypothesis of AD etiology, on one hand, the disrupted BBB leads to a reduced clearance of neurotoxins including Aβ; on the other hand, brain oligemia leads to overexpression and enhanced processing of APP, and brain hypoperfusion (Nelson et al., 2016). According to a meta-analysis, treatment of vascular risk factors with antihypertensives and statins reduced the incidence of dementia and AD (Larsson and Markus, 2018). Thus, vascular risk factors treatment might be a potential strategy to slow cognitive decline in AD. To restore vascular function in AD, some vascular protection agents, such as angiotensin receptor blockers, angiotensin converting enzyme inhibitor, calcium channel blocker, cholesterol agent, omega-3 fatty acid, and direct thrombin inhibitor are now in clinical evaluations for AD treatment (**Additional Table 5**).

Diabetes has been implicated as a major risk factor of AD development (Vignini et al., 2013; Baglietto-Vargas et al., 2016). The pathological features of diabetes, such as insulin/insulin-like growth factor resistance, hyperglycemia and glucose metabolism dysfunction, were observed to induce AD pathologies in Aβ production, tauopathy, neuroinflammation and cognitive impairment. Anti-diabetic agent or agent regulating metabolism are thought to be helpful against AD. Sex steroid hormones, such as estrogen and androgen, exert neuroprotective benefit in adult brains (Pike, 2017). Insufficiency of sex hormones in male and female both enhance the vulnerability to AD. In **Additional Table 5**, agents in AD drug development clinical trials with potential to modulate metabolism and endocrine related risk factors are summarized.

Although the mechanisms of how these aging related risk factors play roles in AD etiology are still poorly understood, considerable research effort has been undertaken to tackle these factors for AD treatment. As shown in **Additional Table 5**, there are 50 agents (including 21 anti-inflammatory agents, 6 anti-oxidation agents, 9 vascular modifying agents, 12 metabolism modifying agents and 2 endocrine modifying agents) and 53 clinical trials to modulate the ageing related risk factors for AD treatment.

### Conclusions

AD is a complex neurodegenerative disease with various pathological factors. Although a number of promising therapeutic strategies have been evaluated, more extensive and intensive fundamental studies are still needed. To date, there is still no effective drug that can cure AD patients. Therapies developed based on cholinergic deficiency offered only limited cognitive improvement. The up-to-now disease modifying drugs failed to improve cognition in clinical trials. What the previous failures indicating is that targeting on single factor alone may not necessarily work well on disease caused by multiple factors. Consequently, the disease’s complex mechanisms and the interplay between the multiple factors call for the come out of all-powerful therapies with multiple curing functions.

Indeed, multitarget strategy has already been put into practice in the clinic and clinical trials. A combination of one of the cholinesterases inhibitors (donepezil) with memantine is the fifth FDA approved prescription for moderate-to-severe Alzheimer’s patients (Bennett et al., 2019). Blarcamesine, a multifunctional drug as the sigma-1 and muscarinic dual agonist and GSK-3β inhibitor, is currently in phase 3 clinical trial for AD treatment. Multitarget therapies, mainly the combination of several agents with different aspects of anti-AD functions, and multitarget agents currently in AD drug clinical trials are summarized in **Additional Table 6**. 13 multitargeting agents and 22 clinical trials are on-going for AD treatment, including 6 agents in phase 3, 6 agents in phase 2 and 1 agent in phase 1 clinical studies. Among these therapies, ANAVEX2-73 is expected to modulate synaptic dysfunction,
cholinergic neurotransmission, tauopathy by regulating the sigma-1 receptor, muscarinic receptors and GSK-3β.

It is noted that many therapeutic targets play roles in multiple pathological pathways. Thus, therapeutically modulations of these targets could be beneficial in AD treatment via multiple mechanisms of action. For example, apart from metabolic function, GSK-1915308 agents were observed to modulate neuroinflammation (Yun et al., 2018) and neurovascular functions (Zhao et al., 2020) in neurodegenerative disease models. Moreover, sigma-1 receptor (Jin et al., 2015) and GSK-3β (Lauretti et al., 2020) are regarded as multi-functional therapeutic targets. These therapeutic targets with multiple mechanisms of action could offer great potential in multitarget AD drug development.

Considering the complexity of AD pathology, multifunctional agents designed with multitarget potential could lead to a breakthrough in AD therapeutic development. Preclinical studies on different pathologies and multitarget treatments (Wang et al., 2019; Ju et al., 2020; Ju and Tam, 2020) may provide a pool of lead compounds for future clinical investigations. There is no royal road to overcome AD, but multifunctional drug is likely to give hope for AD treatment.

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Additional Table 1: Agents modifying synaptic dysfunction of AD in clinical trials.

Additional Table 2: Neurotransmission modifying agents for AD drug development in clinical trials.

Additional Table 3: Anti-amyloid agents for AD treatment in clinical trials.

Additional Table 4: Anti-tau agents for AD treatment in clinical trials.

Additional Table 5: Neuroprotective agents for AD treatment in clinical trials.

Additional Table 6: Multitarget therapies for AD treatment in clinical trials.

References

Ardestani PM, Evans AK, Yi B, Nguyen T, Coutellier L, Shamloo M (2017) Modulation of neuroinflammation and pathology in the 5XFAD mouse model of Alzheimer’s disease using a biased and selective beta-1 adrenergic receptor partial agonist. Neuropharmacology 116:371-386.

Baglietto-Vargas D, Shi J, Yaeger DM, Ager R, LaFerla FM (2016) Diabetes and Alzheimer’s disease. Alzheimer Dis Assoc Disord 1:256-262.

Bianco OA, Manzine PR, Nascimento CM, Vale FA, Pavarini SC, Cominetti MR (2016) Baglietto-Vargas D, Shi J, Yaeger DM, Ager R, LaFerla FM (2016) Diabetes and Alzheimer’s disease. Alzheimer Dis Assoc Disord 1:256-262.

Bostancioglu M (2020) Optogenetic stimulation of serotonin nuclei retrieve the lost memory in Alzheimer’s disease. J Cell Physiol 235:836-847.
| Therapeutic purpose | Agent | ClinicalTrials.gov ID/Phase/Status | Mechanism of action |
|---------------------|-------|---------------------------------|---------------------|
| To improve synaptic plasticity (By enhancing LTP and decreasing LTD) | Tacrolimus | NCT04263519/2/N | Calcineurin inhibitor Tacrolimus inhibits calcineurin-dependent LTD which may mediate synaptic loss in the AD brain. |
| DAOI | NCT03752463/2/U | D-amino acid oxidase inhibitor DAOI increase the level of D-serine, a co-agonist of NMDARs. |
| L-serine | NCT03062449/2/R | L-serine is the precursor of D-serine, a co-agonist of NMDARs. |
| SAGE718 | NCT04602624/2/N | Positive allosteric modulator of NMDARs |
| Bryostatin 1 | NCT04538066/2/R | PKC modulator |
| AR1001 | NCT03625622/2/AN | PDE inhibitors |
| BPN14770 | NCT03817684/2/AN | PDEs are responsible for hydrolysis of cAMP and cGMP. The inhibition of PDEs increase brain cAMP and cGMP concentrations, which activate PKA orPKG and subsequent CREB phosphorylation in brain tissue. |
| Cilostazol | NCT02491268/2/AN |  |
| NCT03451591/3/R |  |  |
| To improve synaptic plasticity (By enhancing LTP and decreasing LTD) | AMX0035 (sodium phenylbutyrate and tauroursodeoxycholic acid combination) | NCT03533257/2/AN | Sodium phenylbutyrate induces astrocytic BDNF and NT-3 expression via PKC-CREB pathway. |
| Therapeutic purpose | Agent | ClinicalTrials.gov ID/Phase/Status | Mechanism of action |
|---------------------|-------|-----------------------------------|---------------------|
| ANAVEX2-73 (blarcamesine) | NCT03790709/3/R | Sigma-1 receptor agonist | Sigma-1 receptor agonist, Blarcamesine is a sigma-1 receptor agonist (high affinity), M1 receptor agonist and M2 receptor antagonist (low affinity). Sigma receptor locates in endoplasmic reticulum. Agents regulate endoplasmic reticulum functions to improve synaptic plasticity by activating sigma-1 receptor. |
| | NCT04314934/3/R | | |
| | NCT02756858/2/AN | | |
| AVP786 (combination of dextromethorphan and quinidine) | NCT03393520/3/R | Sigma-1 receptor agonist | Dextromethorphan is a Sigma-1 receptor agonist. |
| | NCT02446132/3/R | | |
| | NCT04464564/3/R | | |
| | NCT04408755/3/R | | |
| T-817MA (endonerpic) | NCT04191486/2/R | Sigma receptor activator | |
| CT1812 | NCT03507790/2/AN | Sigma-2 receptor antagonist | |
| | NCT03493282/2/AN | | |
| | NCT03522129/1/R | | |
| Neflamapimod (VX-745) | NCT03435861/2/R | Inhibitor of p38 MAPKα (p38α) | Endosome-associated protein Rab5 plays critical role in dysregulation of the endo-lysosomal system in early pathogenesis of AD and is mainly regulated by p38α. Inhibition of p38α reduced synaptic dysfunction by normalizing dysregulated Rab5 activity. |
| Therapeutic purpose | Agent | ClinicalTrials.gov ID/Phase/Status | Mechanism of action |
|---------------------|-------|-----------------------------------|---------------------|
| ORY-2001 (vafidemstat) | NCT03867253/2/AN | LSD1 inhibitor | To restore transcription equilibrium in neurodegenerative disorders. |
| Nilotinib | NCT02947893/2/AN | Tyrosine kinase inhibitor | Tyrosine kinase inhibition increases functional parkin-Beclin-1 interaction and enhances amyloid clearance and cognitive performance. |
| To reduce synapses loss (By promoting regeneration and reducing apoptosis) | Allogenic human MSCs | NCT02833792/2/R | Stem cell therapy |
| | Allogenic human MSCs | NCT04040348/1/R | Cells derived from stem cells can be differentiated into normal neurons, which may integrate into neuronal circuits and improve their functions. |
| | Allogenic human MSCs | NCT02600130/1/AN | |
| | Astrostem | NCT04482413/2/N | Secretions from stem cells regulate the microenvironment to resist neurodegeneration and promote neuro-regeneration. |
| | Autologous adipose-derived MSCs | NCT04228666/2/AN | |
| | Placenta-derived MSCs (CB-AC-02) | NCT02899091/2/R | |
| | Human umbilical cord blood-derived MSCs | NCT03172117/2/R | |
| | Human umbilical cord blood-derived MSCs | NCT02672306/2/U | |
| | MSCs-Exos | NCT04388982/2/R | Stem cell-derived exosome |
| | | | MSCs-Exos include active cargos such as proteins (Aβ degradation enzymes, anti-oxidative enzymes, neuron-supporting proteins, anti-inflammatory cytokines), lipid raft, nucleic acid (mRNA and miRNA). |
| Therapeutic purpose | Agent | ClinicalTrials.gov ID/Phase/Status | Mechanism of action |
|---------------------|-------|-----------------------------------|---------------------|
|                     | ATH-1017 (NDX-1017) | NCT04488419/2/R | Neurotrophic factors  |
|                     | ATH-1017 (NDX-1017) | NCT04491006/2/R | Neurotrophic factors regulate proliferation and survival of different types of cells and promote synaptic plasticity and cognition. ATH-1017 activates the HGF. |
|                     | Allopregnanolone | NCT03748303/1/R | Growth hormones  |
|                     | Allopregnanolone | NCT03748303/1/R | Allopregnanolone promotes neurogenesis. |
|                     | NNI-362 | NCT04074387/1/R | NNI-362 selectively activate neural progenitor cells to neurons. |
|                     | GV1001 | NCT03959553/2/N | A 16-amino-acid peptide comprising a sequence from the hTERT  |
|                     | GV1001 | NCT03959553/2/N | GV1001 mimics TERT’s functions.  |
|                     | AAV-hTERT | NCT04133454/1/R | hTERT delivered by AAV transduction  |

AD: Alzheimer’s disease; BDNF: brain-derived neurotrophic factor; cAMP: cyclic adenosine monophosphate; cGMP: cyclic guanosine monophosphate; CREB: cAMP-response element binding protein; HGF: hepatocyte growth factor; hTERT: human enzyme telomerase reverse transcriptase; LSD1: lysine-specific demethylase 1; LTD: long-term depression; LTP: long-term potentiation; MSCs: mesenchymal stem cells; NMDAR: N-methyl-D-aspartate receptor; PDE: phosphodiesterase; PKA: cAMP-dependent protein kinase; PKC: protein kinase C; PKG: cGMP-dependent protein kinase. Status: AN: Active, not recruiting; N: not yet recruiting; R: recruiting; U: unknown. There are 22 agents and 30 clinical trials related to modification of synaptic plasticity and reduction of synapses loss for AD treatment.
# Additional Table 2 Neurotransmission modifying agents for AD drug development in clinical trials (ClinicalTrials.gov on November 22, 2020)

| Therapeutic purpose | Agent | ClinicalTrials.gov ID/Phase/Status | Mechanism of action |
|---------------------|-------|-----------------------------------|---------------------|
| To improve cholinergic neurotransmission in AD patients | AD-35 | NCT03625401/2/AN | AChE inhibitor |
| | Octohydroaminoacridine succinate | NCT03283059/3/R | Inhibit AChE activity to enhance acetylcholine level |
| | Nicotine Transdermal Patch | NCT02720445/2/R | Nicotinic acetylcholine receptors agonists |
| | Nicotine | NCT01778946/2/R | Nicotinic acetylcholine receptors agonists |
| | ANAVEX-73 (blarcamesine) | NCT03790709/3/R | Muscarinic acetylcholine receptors |
| | | NCT04314934/3/R | Blarcamesine is a sigma-1 receptor agonist (high affinity), M1 receptor agonist and M2 receptor antagonist (low affinity) |
| | | NCT02756858/2/AN | |
| To restore E/I balance (By reducing glutamatergic excitotoxicity) | AVP786 (combination of dextromethorphan and quinidine) | NCT03393520/3/R | NMDA receptor antagonist |
| | | NCT02446132/3/R | Dextromethorphan is an NMDA receptor antagonist. |
| | | NCT04464564/3/R | |
| | | NCT04408755/3/R | |
| | BHV4157 (troriluzole, the prodrug of riluzole) | NCT03605667/3/AN | Sodium channel blockers |
| | | | Riluzole blocks sodium channel to reduce glutamate release. |
| To restore E/I balance (By enhancing inhibitory neurotransmission) | Allopregnanolone | NCT03748303/1/R | Positive allosteric GABA\_ARs modulators |
| Therapeutic purpose                        | Agent               | ClinicalTrials.gov ID/Phase/Status | Mechanism of action                                           |
|-------------------------------------------|---------------------|-----------------------------------|----------------------------------------------------------------|
| To restore E/I balance                    | Levetiracetam (AGB101) | NCT03486938/3/R                   | SV2A modulator                                                 |
| (By modulating neurotransmitter release)  |                     | NCT02002819/2/AN                   | Reduce hyperactivity and epileptiform symptoms                 |
|                                           |                     | NCT03461861/2/R                   | Reduce neurotoxins damage                                      |
|                                           |                     | NCT03489044/2/AN                   |                                                                |
|                                           |                     | NCT03875638/2/R                   |                                                                |
|                                           |                     | NCT04004702/2/N                   |                                                                |
| To enhance dopaminergic neurotransmission | Brexpiprazole       | NCT03620981/3/R                   | D2 receptor partial agonist                                    |
|                                           |                     | NCT03594123/3/R                   |                                                                |
|                                           |                     | NCT03548584/3/R                   |                                                                |
|                                           |                     | NCT03724942/3/R                   |                                                                |
|                                           | Bromocriptine       | NCT04413344/2/R                   | Dopamine receptor agonist                                      |
| To enhance adrenergic neurotransmission   | Guanfacine          | NCT03116126/3/R                   | Alpha-2 adrenergic agonist                                     |
|                                           | Dexmedetomidine     | NCT04205539/1/E                   | Alpha-2 adrenergic agonist                                     |
|                                           | Mirtazapine         | NCT03031184/3/AN                   | Alpha-2 adrenergic antagonist                                   |
|                                           |                     |                                   | Mirtazapine was supposed to exert an anti-agitation effect may by blocking the presynaptic α2 adrenergic receptor to improve central noradrenergic and serotonergic activity. |
| Therapeutic purpose                                      | Agent                          | ClinicalTrials.gov ID/Phase/Status | Mechanism of action                                                                 |
|--------------------------------------------------------|--------------------------------|-----------------------------------|-------------------------------------------------------------------------------------|
| To inhibit adrenergic activity                         | Prazosin                       | NCT03710642/2/R                   | Alpha-1 adrenergic receptor                                                        |
|                                                       |                                |                                   | Prazosin antagonizes NE effects at brain postsynaptic α1 adrenergic receptor and is  |
|                                                       |                                |                                   | primarily used to treat hypertension and benign prostatic hypertrophy.              |
| To enhance serotonergic neurotransmission              | Brexpiprazole                   | NCT03620981/3/R                   | 5-HT receptor agonist                                                             |
|                                                       |                                | NCT03594123/3/R                   | Brexpiprazole is a partial agonist of the serotonin 5-HT1A receptor.              |
|                                                       |                                | NCT03548584/3/R                   |                                                                                   |
|                                                       |                                | NCT03724942/3/R                   |                                                                                   |
| To modulate endocannabinoid neurotransmission          | Nabilone                        | NCT04516057/3/N                   | CB1 and CB2 endocannabinoid receptor agonist                                       |
|                                                       | THC-free CBD (Cannabidiol) oil  | NCT04436081/2/N                   | Phytocannabinoid targeting the endocannabinoid system                              |
|                                                       | Dronabinol                      | NCT02792257/2/R                   | CB1 and CB2 endocannabinoid receptor agonist                                       |
| To modulate orexinergic neurotransmission              | Suvorexant                      | NCT04629547/2/N                   | Dual antagonist of orexin receptor 5X1R and 5X2R                                  |
| To modulate adenosine neurotransmission                | Caffeine                        | NCT04570085/2/N                   | Adenosine receptors antagonist                                                     |
|                                                       |                                |                                   | AR antagonists inhibit PDEs, promote calcium release from intracellular stores,     |
|                                                       |                                |                                   | and interfere with GABA-A receptors. Caffeine affects brain functions such as       |
|                                                       |                                |                                   | sleep, cognition, learning, and memory, and modifies brain dysfunctions and         |
|                                                       |                                |                                   | diseases through antagonism of ARs.                                                |

5-HT: 5-Hydroxytryptamine; AChE: acetylcholine; AD: Alzheimer’s disease; AR: adenosine receptor; CB1: cannabinoid receptor type 1; CB2: cannabinoid receptor type 2; MAO-B: monoamine oxidase B; NMDA: N-methyl-D-aspartate; GABA, Rs: γ-aminobutyric acid type A receptors; NE: norepinephrine; OX1R: orexin receptor type 1; OX2R: orexin receptor type 2; SSRI: selective serotonin reuptake inhibitor; SV2A: synaptic vesicle glycoprotein 2A. Status: AN: Active, not recruiting; E: Enrolling by invitation; N: Not yet recruiting; R: Recruiting; U, Unknown. There are 23 agents and 39 clinical trials to modulate neurotransmission for AD treatment.
### Additional Table 3 Anti-amyloid agents for AD treatment in clinical trials (ClinicalTrials.gov on November 22, 2020)

| Therapeutic purpose                  | Agent                                           | ClinicalTrials.gov ID/Phase/Status | Mechanism of action                                                                                                                                 |
|--------------------------------------|------------------------------------------------|------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------|
| To reduce Aβ production              | APH-1105                                        | NCT03806478/2/N                    | Alpha-secretase modulator                                                                                                                                          |
|                                      | E2609 (Elenbecestat)                            | NCT03036280/3/AN                   | BACE inhibitor                                                                                                                                                  |
|                                      |                                                | NCT02956486/3/AN                   |                                                                                                                                                                  |
|                                      | Posiphen                                        | NCT02925650/2/R                    | APP inhibitor                                                                                                                                                  |
|                                      |                                                | NCT04524351/2/R                    | Posiphen selectively inhibit APP production                                                                                                                   |
|                                      | PQ912                                           | NCT03919162/2/N                    | Glutaminyl cyclase enzyme inhibitor                                                                                                                             |
|                                      |                                                | NCT04498650/2/R                    | Reduce production of pyroglutamate Aβ                                                                                                                          |
| To reduce Aβ aggregation toxicity    | PTI-125 (sumifilam)                            | NCT04388254/2/R                    | Filamin A (FLNA) protein inhibitor                                                                                                                                 |
|                                      | AMX0035 (combination of tauroursodeoxycholic acid and sodium phenylbutyrate) | NCT03533257/3/AN                   | Connective tissue growth factor (CTGF) inhibitor                                                                                                                                 |
|                                      | BEY2153                                         | NCT04476303/1/R                    | Aβ aggregation inhibitor                                                                                                                                                                                                 |
| To enhance Aβ clearance              | Thieethylperazine (TEP)                        | NCT03417986/2/AN                   | ABC1 transporter activator                                                                                                                                       |
|                                      |                                                |                                    | ABC1 was discovered to be a major β-amyloid-exporting molecule at the BBB.                                                                                                                                              |
| Therapeutic purpose | Agent | ClinicalTrials.gov ID/Phase/Status | Mechanism of action |
|---------------------|-------|-----------------------------------|---------------------|
| AAVrh.10hAPOE2 vector | NCT03634007/1/R | APOE | APOE4 and APOE2 alleles are associated with a higher and lower risk of Alzheimer’s dementia, respectively. AAVrh.10hAPOE2 covert ApoE4 homozygotes protein isoforms to ApoE2-ApoE4 isoforms in CSF. |
| Aducanumab | NCT04241068/3/E | Monoclonal antibody directed at plaques and oligomers |
| Gantenerumab | NCT02051608/3/AN, NCT03444870/3/R, NCT03443973/3/AN, NCT04339413/3/R, NCT04374253/3/N, NCT04592341/2/R | Monoclonal antibody directed at plaques and oligomers |
| RO7126209 | NCT04639050/2/N | Monoclonal antibody directed at plaques and oligomers | RO7126209 is a new version of gantenerumab, more easily crossing the blood-brain barrier. |
| Solanezumab | NCT02008357/3/AN | Monoclonal antibody directed at monomers |
| Gantenerumab and solanezumab | NCT01760005/3/R | Combination therapy |
| BAN2401 | NCT03887455/3/R, NCT04468659/3/R, NCT01767311/2/AN | Monoclonal antibody directed at protofibrils |
| Therapeutic purpose | Agent | ClinicalTrials.gov ID/Phase/Status | Mechanism of action |
|--------------------|-------|-----------------------------------|---------------------|
|                    | Crenezumab | NCT01998841/2/AN | Monoclonal antibody targeting soluble oligomers |
|                    | LY30028123 (donanemab) | NCT03367403/2/AN | Monoclonal antibody specific for pyroglutamic peptide fragment of Aβ |
|                    | LY3372993 | NCT04451408/1/R | Anti- Aβ monoclonal antibody |
|                    | ABvac40 | NCT03461276/2/R | Active immunotherapy |

AAV: Adeno-associated virus; ABCC1: ATP Binding Cassette Subfamily C Member 1; AD: Alzheimer’s disease; APOE: apolipoprotein E; APP: amyloid precursor protein; Aβ: amyloid beta; BACE: beta-site APP cleaving enzyme; BBB: blood-brain barrier; CSF: cerebrospinal fluid; CTGF: connective tissue growth factor; FLNA: filamin A; nAChR: nicotinic acetylcholine receptor. Status: AN: Active, not recruiting; E: Enrolling by invitation; N: Not yet recruiting; R: Recruiting. There are 18 agents and 30 clinical trials on-going targeting Aβ pathology for AD treatment.
### Additional Table 4 Anti-tau agents for AD treatment in clinical trials (ClinicalTrials.gov on November 22, 2020)

| Therapeutic purpose | Agent | ClinicalTrials.gov ID/Phase/Status | Mechanism of action |
|---------------------|-------|-----------------------------------|---------------------|
| To maintain microtubule stability | Nicotinamide | NCT03061474/2/R | Histone deacetylase (HDAC) inhibitor |
|  | Vorinostat | NCT03056495/1/R | HDAC inhibitors reduce tau-induced microtubule depolymerization. |
| To inhibit tau protein aggregation | TRx0237 (LMXT) | NCT03446001/3/R | Tau aggregation inhibitor |
|  | BEY2153 | NCT04476303/1/R | Tau aggregation inhibitor |
|  | BDPP (bioactive dietary polyphenol preparation) | NCT02502253/1/R | Grape seed polyphenolic extract and resveratrol prevent tau aggregation. |
| To reduce tau production | IONIS MAPTRx (BIIB080) | NCT03186989/2/AN | Antisense oligonucleotide to reduce MAPT expression |
| To enhance tau protein clearance | ABBV-8E12 | NCT02880956/2/AN | Anti-tau antibody |
|  |  | NCT03712787/2/E | |
|  | BIB092 | NCT03352557/2/AN | Monoclonal antibody targeting truncated form of tau |
|  | LY3303560 (zagotenemab) | NCT03518073/2/AN | Monoclonal antibody targeting soluble tau |
|  | Semorinemab (RO07105705) | NCT03289143/2/AN | Monoclonal antibody targeting extracellular tau |
|  |  | NCT03828747/2/AN | |
|  |  | NCT04639050/2/N | |
|  | JNJ-63733657 | NCT04619420/2/N | Monoclonal antibody to recognize mid-region of tau |
|  | Lu AF87908 | NCT04149860/1/R | Monoclonal antibody to phosphorylated tau protein |
|  | ACI-35.030 | NCT04445831/1/R | Tau targeted vaccine |
|  | JACI-35.054 | NCT04445831/1/R | |

AD: Alzheimer’s disease; BDPP: bioactive dietary polyphenol preparation; HDAC: histone deacetylase; MAPT: microtubule associated protein tau. Status: AN: Active, not recruiting; E: Enrolling by invitation; N: Not yet recruiting; R: Recruiting. There are 14 agents and 17 clinical trials targeting tau protein pathology for AD treatment.
### Additional Table 5 Neuroprotective agents for AD treatment in clinical trials (ClinicalTrials.gov on November 22, 2020)

| Therapeutic purpose | Agent | ClinicalTrials.gov ID/Phase/Status | Mechanism of action |
|---------------------|-------|------------------------------------|---------------------|
| Anti-inflammation   | ALZT-OP1 (cromolyn + ibuprofen) | NCT02547818/3/AN, NCT04570644/2/R | Combination therapy (mast cell stabilizer + NSAID) Cromolyn is a mast cell stabilizer. Mast cells release proinflammatory mediators and regulate BBB’s permeability. Ibuprofen is an NSAID. |
|                     |       | NCT01872598/3/AN                  | Tyrosine kinase inhibitor Masitinib is a selective tyrosine kinase inhibitor target on c-kit on mast cells. Mast cells release proinflammatory mediators and regulate BBB’s permeability. |
|                     | Azeliragon | NCT03980730/3/R                  | RAGE inhibitor |
|                     | COR388  | NCT03823404/3/AN                  | Gingipain inhibitor COR388 inhibit *P. gingivalis* infection in AD. |
|                     | Curcumin + aerobic yoga | NCT01811381/2/AN                  | Herb extract with antioxidant and anti-inflammatory properties |
|                     | Daratumumab | NCT04070378/2/R                  | Monoclonal antibody targeting CD38 |
|                     | Dasatinib + Quercetin | NCT04063124/2/R                  | Combination therapy (tyrosine kinase inhibitor + flavonoid) |
|                     | GB301   | NCT03865017/2/N                   | Cell therapy |
|                     | Lenalidomide | NCT04032626/2/R                  | Regulatory T cells (CD4+CD25+CD127dimFOXP3+) with immunosuppressive functions. |
|                     | Montelukast | NCT03402503/2/R                  | Leukotriene antagonist |
|                     | Pepinemab | NCT04381468/2/R                  | Monoclonal antibody of SEMA4D (CD100) |
| Therapeutic purpose | Agent | ClinicalTrials.gov ID/Phase/Status | Mechanism of action |
|---------------------|-------|----------------------------------|---------------------|
|                     | Rapamycin | NCT04629495/2/N | mTOR inhibitor |
|                     |         | | Rapamycin inhibit T cells and B cells by reducing interleukin-2 (IL-2) through mTOR inhibition. |
|                     | Rifaximin | NCT03856359/2/AN | Antibiotic to reduce proinflammatory cytokines from the harmful gut bacterial. |
|                     | Valacyclovir | NCT03282916/2/R | Antiviral against HSV-1 and HSV-2 infection |
|                     | 3TC | NCT04552795/2/N | Antiretroviral therapy |
|                     | Emtriva | NCT04500847/1/N | Antiviral |
|                     |         | | Nucleoside reverse transcriptase inhibitors |
|                     | AL002 | NCT04592874/2/R | Monoclonal antibody targeting TREM2 receptors |
|                     | AL003 | NCT03822208/1/R | Monoclonal antibody targeting SIGLEC-3 (CD33) |
|                     | JNJ-40346527 | NCT04121208/1/N | CSF1R antagonist |
|                     |         | | CSF1R is also known as macrophage colony-stimulating factor receptor (M-CSFR), and CD115 (Cluster of Differentiation 115). It is a receptor for a cytokine called colony stimulating factor 1. |
|                     | Salsalate | NCT03277573/1/AN | NSAID |
|                     | XPro1595 | NCT03943264/1/R | TNF inhibitor |
| Anti-oxidation       | Ginkgo biloba | NCT03090516/3/R | Plant extract with antioxidant properties |
|                     | Curcumin + aerobic yoga | NCT01811381/2/AN | Herb extract with antioxidant and anti-inflammatory properties |
|                     | Grapeseed Extract | NCT02033941/2/R | Antioxidant polyphenolic compound |
|                     | BDPP (bioactive dietary polyphenol preparation) | NCT02502253/2/R | The antioxidant grapeseed polyphenolic extract and resveratrol |
| Therapeutic purpose          | Agent                                      | ClinicalTrials.gov ID/Phase/Status | Mechanism of action                                                                 |
|-----------------------------|--------------------------------------------|-----------------------------------|------------------------------------------------------------------------------------|
|                              | Grape Power                                | NCT03361410/2/R                   | Antioxidant polyphenolic components                                                 |
|                              | Deferiprone                                 | NCT03234686/3/R                   | Iron chelating agent                                                               |
| Reduce vascular risk         | Losartan + amlodipine + atorvastatin       | NCT02913664/3/AN                  | Angiotensin II receptor blocker: losartan                                           |
|                              |                                             |                                   | Calcium channel blocker: amlodipine                                                 |
|                              |                                             |                                   | Cholesterol agent: atorvastatin                                                    |
|                              | Icosapent ethyl (IPE)                      | NCT02719327/3/R                   | Omega-3 fatty acid                                                                 |
|                              |                                             |                                   | IPE is a purified form of omega-3 fatty acid EPA.                                   |
|                              | Omega-3 (DHA + EPA)                        | NCT03691519/3/R                   | Omega-3 fatty acid                                                                 |
|                              | Omega-3 PUFA                                | NCT01953705/2/AN                  | Omega-3 fatty acid                                                                 |
|                              | DHA                                        | NCT03613844/2/R                   | Omega-3 fatty acid                                                                 |
|                              | PMZ-1620 (sovatehlide)                     | NCT04052737/2/R                   | Endothelin-B receptor agonist                                                       |
|                              | Telmisartan+Perindopril                    | NCT02085265/2/R                   | Angiotensin II receptor blocker: telmisartan                                        |
|                              |                                             |                                   | Angiotensin converting enzyme inhibitor: perindopril                                |
|                              | Dabigatran                                  | NCT03752294/1/N                   | Direct thrombin inhibitor                                                           |
|                              | Telmisartan                                 | NCT02471833/1/R                   | Angiotensin II receptor blocker                                                    |
| Improve metabolism function | Azeliragon                                  | NCT03980730/3/R                   | RAGE inhibitor                                                                      |
|                              | Metformin                                   | NCT04098666/3/N                   | Insulin sensitizer                                                                  |
|                              | Ketones                                     | NCT04466735/R                     | Ketones to improve glucose use                                                      |
|                              | Tricapril                                   | NCT04187547/3/N                   | Ketone body stimulant; caprylic triglyceride                                        |
|                              |                                             |                                   | Improve glucose metabolism                                                          |
| Therapeutic purpose                      | Agent                      | ClinicalTrials.gov ID/Phase/Status | Mechanism of action                                                                 |
|-----------------------------------------|----------------------------|-----------------------------------|-------------------------------------------------------------------------------------|
|                                        | Benfotiamine               | NCT02292238/2/AN                  | Synthetic thiamine                                                                  |
|                                        |                            |                                   | Improve glucose use                                                                  |
|                                        | Dapagliflozin              | NCT03801642/2/R                   | SGLT2 inhibitor                                                                      |
|                                        |                            |                                   | Improve insulin sensitivity and glucose metabolism                                   |
|                                        | Empagliflozin              | NCT03852901/1/R                   | SGLT2 inhibitor                                                                      |
|                                        | Liraglutide                | NCT01843075/2/AN                  | Glucagon-like peptide 1 receptor agonist                                             |
|                                        | Metabolic cofactor         | NCT04044131/2/R                   | Mixture of N-acetylcysteine, L-carnitine, tartrate, nicotinamide riboside, and serine.|
|                                        | S-equol (AUS-131)          | NCT03101085/2/R                   | Agonist of non-hormonal estrogen receptor B located on mitochondrial                  |
|                                        |                            |                                   | Improve mitochondrial function                                                       |
|                                        | T3D-959                    | NCT04251182/2/S                   | Dual agonist of PPAR-σ and PPAR-γ                                                    |
|                                        |                            |                                   | Regulate glucose and lipid metabolism and reduce insulin resistance                   |
|                                        | Efavirenz                 | NCT03706885/1/R                   | Cytochrome P450 46A1 activator and the antiretroviral                                 |
| Enhance endocrine function             | Lupron (leuprolide acetate depot) | NCT03649724/2/R                   | GnRH receptor agonist                                                                |
|                                        |                            |                                   | Reduce negative effect of elevated GnRH and gonadotrophins in brain.                 |

AD: Alzheimer’s disease; BBB: blood-brain barrier; CD115: cluster of differentiation 115; CSF1R: colony stimulating factor 1 receptor; EPA: eicosapentaenoic acid; GnRH: gonadotrophin-releasing hormones; M-CSFR: macrophage colony-stimulating factor receptor; mTOR: mammalian target of rapamycin; NSAID: nonsteroidal anti-inflammatory drug; PPAR: peroxisome proliferator-activated receptor; RAGE: receptor for advanced glycation end products; SEMA4D: semaphorin-4D; SGLT2: sodium-glucose co-transporter-2; SIGLEC-3: sialic acid binding Ig-like lectin 3. Status: AN: Active, not recruiting; N: Not yet recruiting; R: Recruiting; S, suspended. There are 50 agents and 53 clinical trials to modify the aging related risk factors for AD treatment.
| Therapies                                                                 | Mechanism of action on targets                                                                 | ClinicalTrials.gov ID/Phase/Status |
|--------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|------------------------------------|
| ALZT-OP1 (Combination of cromolyn and ibuprofen)                         | Mast cell stabilizer (cromolyn)                                                              | NCT02547818/3/AN                   |
|                                                                          | Anti-inflammation (ibuprofen)                                                                | NCT04570644/2/R                    |
| ANAVEX2-73 (blarcamesine)                                                | Sigma-1 receptor agonist                                                                     | NCT03790709/3/R                    |
|                                                                          | M1 receptor agonist and M2 receptor antagonist                                               | NCT04314934/3/R                    |
|                                                                          | GSK-3β inhibitor                                                                              | NCT02756858/2/AN                   |
| AVP786 (Combination of dextromethorphan and quinidine)                   | Sigma-1 receptor agonist (dextromethorphan)                                                 | NCT03393520/3/R                    |
|                                                                          | NMDA receptor antagonist (dextromethorphan)                                                 | NCT02446132/3/R                    |
|                                                                          |                                                                                               | NCT04464564/3/R                    |
|                                                                          |                                                                                               | NCT04408755/3/R                    |
| Brexpiprazole                                                            | D2 receptor agonist                                                                          | NCT03620981/3/R                    |
|                                                                          | 5-HT receptor agonist                                                                        | NCT03594123/3/R                    |
|                                                                          |                                                                                               | NCT03548584/3/R                    |
|                                                                          |                                                                                               | NCT03724942/3/R                    |
| Gantenerumab and solanezumab                                            | Monoclonal antibody directed at plaques and oligomers (ganenerumab)                          | NCT02008357/3/AN                   |
|                                                                          | Monoclonal antibody directed at monomers (solanezumab)                                       |                                    |
| Therapies                                                                 | Mechanism of action on targets                                                                 | ClinicalTrials.gov ID/Phase/Status                  |
|--------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|---------------------------------------------------|
| Losartan + amlodipine + atorvastatin                                     | Angiotensin II receptor blocker: losartan<br>Calcium channel blocker: amlodipine<br>Cholesterol agent: atorvastatin | NCT02913664/3/AN                                  |
| AMX0035 (sodium phenylbutyrate and tauroursodeoxycholic acid combination)| Chemical chaperone to inhibit endoplasmic reticulum stress responses. (Sodium phenylbutyrate)<br>Naturally occurring bile acid to tackle mitochondrial dysfunction. (tauroursodeoxycholic acid) | NCT03533257/2/AN                                  |
| Dasatinib + Quercetin (Combination therapy)                             | Tyrosine kinase inhibitor (Dasatinib)<br>Flavonoid with antioxidant and anti-Aβ fibrilization properties (Quercetin) | NCT04063124/2/R                                  |
| Grapeseed Extract                                                        | Polyphenolic compound with antioxidant property<br>Anti-oligomerization                          | NCT02033941/2/R                                  |
| L-serine                                                                 | Synthesis of sphingolipids and phosphatidylserine<br>The precursor of D-serine, a co-agonist of NMDARs. | NCT03062449/2/R                                  |
| ORY-2001 (vafidemstat)                                                   | LSD1 inhibitor<br>MAO-B inhibitor                                                             | NCT03867253/2/AN                                  |
| Telmisartan+Perindopril                                                 | Angiotensin II receptor blocker (telmisartan)<br>Angiotensin converting enzyme inhibitor (perindopril) | NCT02085265/2/R                                  |
| Allopregnanolone                                                         | Growth hormones to promote neurogenesis<br>Positive allosteric GABA<sub>A</sub>Rs modulators     | NCT03748303/1/R                                  |

5-HT: 5-hydroxytryptamine; AD: Alzheimer’s disease; GABA<sub>A</sub>Rs: γ-aminobutyric acid type A receptors; GSK-3β: glycogen synthase kinase 3; LSD1: Lysine-specific histone demethylase 1A; MAO-B: monoamine oxidase B; NMDA: N-methyl-D-aspartate; NMDARs: N-methyl-D-aspartate. Status: AN: Active, not recruiting; R: Recruiting. 13 multi-targeting agents are on-going in 22 clinical trials for AD treatment.