



Chinese Pharmaceutical Association
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Acta Pharmaceutica Sinica B

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REVIEW

Comprehensive investigation of multiple targets in the development of newer drugs for the Alzheimer's disease

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Received 6 March 2024; received in revised form 20 August 2024; accepted 6 September 2024

KEY WORDS

Alzheimer's disease;
Amyloid hypothesis;
Tau targeting therapy;
Trem2;
BIN1;
Kinase;
Notum inhibitors

Abstract Alzheimer's disease, a significant contributor to dementia, is rapidly becoming a serious healthcare concern in the 21st century. The alarming number of patients with Alzheimer's disease is steadily increasing, which is contributed by the dearth of treatment options. The current treatment for Alzheimer's disease is heavily dependent on symptomatic treatment that has failed to cure the disease despite huge investments in the development of drugs. The clinical treatment of Alzheimer's disease with limited drugs is generally targeted towards the inhibition of *N*-methyl-D-aspartate receptor and acetylcholine esterase, which only elevate cognition levels for a limited period. Beyond the aforementioned molecular targets, β -amyloid was much explored with little success and thus created a feel and palpable growing emphasis on discovering new putative and novel targets for AD. This has inspired medicinal chemists to explore new targets, including microglia, triggering receptors expressed on myeloid cells 2 (Trem-2), and notum carboxylesterase, to discover new lead compounds. This review explores the functions, pathophysiological roles, and importance of all AD-related targets that address therapeutic and preventive approaches for the treatment and protection of Alzheimer's disease.

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Peer review under the responsibility of Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences.

<https://doi.org/10.1016/j.apsb.2024.11.016>

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1. Introduction

Neurologist Dr. Alois Alzheimer discovered the prevailing type of dementia in 1907, which was later named Alzheimer's disease (AD). The degenerative condition of the illness begins with mild memory impairment and progresses to a loss of capacity to communicate with the surroundings. AD is characterized by memory loss, linguistic problems, and impulsive or unpredictable behavior, it is defined as intracellular neurofibrillary tangles and deposition of external amyloid plaques that contribute to illness¹. AD is classified into two subtypes based on heritability: 1) Familial (FAD) and 2) Sporadic (SAD). Furthermore, AD varieties are expressed based on the onset period as early onset (EOAD) or late onset (LOAD). Although the symptoms are mild initially, they become more severe over time².

AD is further described by the destruction of the cerebral cortex and neuronal death in the cortical and subcortical areas. Pathological features of AD include senile plaques, masses of the β -amyloid protein associated with neurodegenerative illnesses, and masses of tau proteins and paired helical filaments containing neurofibrillary tangles. The affected hippocampus and associative cortex area are most abundant in advanced AD. In contrast, the motor and visual cortices are relatively spared, which matches the clinical symptoms of severe memory loss and the maintenance of eyesight and movement³.

Many risk factors such as autophagy defects, senescence, genetics apolipoprotein 4 (APOE4), Trem2, lifestyle choices, microbiota alteration, genetics, cardiovascular and traumatic brain injury, as well as environmental factors (pedagogical level, hypertension, and obesity), diametral disease, have been proposed as significant contributors to the onset of AD⁴.

According to current estimates, 25 to 30 million people are supposedly suffering from AD, which is projected to triple by 2040⁵. The treatment of AD has been attempted by using numerous drugs including inhibitors of acetylcholine esterase (AChE) exemplified by donepezil, rivastigmine, and galantamine, and antagonists of *N*-methyl-D-aspartate (NMDA), memantine (Fig. 1). The afore-listed drugs alleviate symptoms, but the condition may not be cured. These attributed medicinal chemists to drift towards exploring in deep. The pathology of various mechanisms exemplified by abnormal tau protein metabolism, β -amyloid, cardiovascular disease, inflammatory response and cholinergic can offer the development of viable medicines⁶ (Fig. 2).

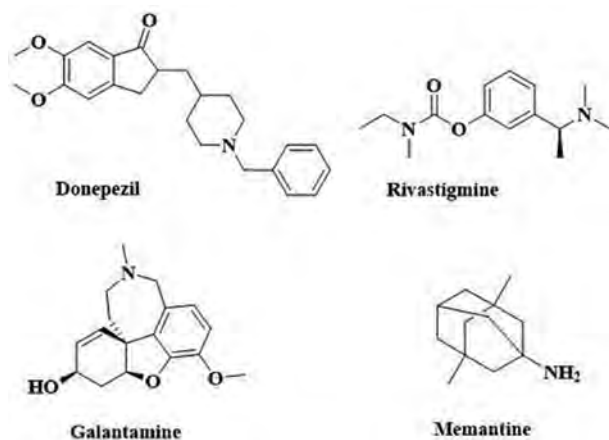


Figure 1 The two-dimensional structures of FDA-approved drugs used in the treatment of AD⁷.

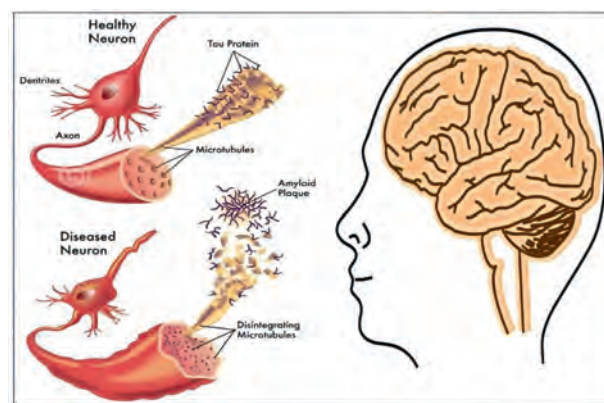


Figure 2 Pathophysiology of Alzheimer's disease diagnosis⁷.

Apart from numerous mechanisms involved in AD, depressive symptoms in AD may also be caused by genetic, neuroanatomic, vascular, and neurotransmitter imbalances. Inflammatory pathways, neurotrophic deficiency, and hypothalamic, pituitary, and adrenal axis deregulation are the possible biological mechanisms linked to depression and AD that their modulators could treat⁸. Neurotransmitters also play an important role in AD; there is a notable and disproportionate deficiency of acetylcholine (ACh) in subcortical cholinergic neurons, notably those within the basal forebrain, which give cholinergic transmission to the entire cerebral cortex. The deficiency of neurotransmitters in AD is even more intricate, involving many neurotransmitter systems, viz serotonin, glutamate, and neuropeptides as well as the cholinergic neurons, cortical, and hippocampal targets are affected. Cholinesterase inhibitors act by inhibiting ACh's breakdown, resulting in an increased concentration of ACh³. NMDA competitive antagonists decrease glutamate action by binding directly to the glutamate region of the NMDA receptor, which reduces the calcium concentration and results in minimal damage to the nerve cells⁹. There is strong evidence that oxidative brain injury has been implicated early in AD. Hence, antioxidants generally 'clear up' excess free radicals. Peroxisome proliferators activated receptor- γ (PPAR γ) agonists improve cognitive performance and decrease $A\beta$ initiation of microglia^{10,11}. γ -Secretase plays a pivotal part in the breakdown of the APP; thus, using γ -secretase inhibitors can avert the imminent process¹⁰. Patients suffering from AD have a persistent immune response and inflammation in their brain, and some experts believe that inflammation stands at 3rd in the major pathologic feature of AD¹¹. Pro-inflammatory cytokines such as TNF α and IL-1 β play a crucial role in neuro-inflammation progression by activating signaling systems involving p38 MAP kinase, nuclear factor κ B, nitric oxide, COX, and Akt/mTOR¹². The cellular prion protein (PrPC) was discovered as a key mediator in the $A\beta$ oligomers toxicity, which causes synapse loss and intellectual decline in AD. As a result, directing PrPC and its relationship with $A\beta$ oligomers or downstream mediators could be recognized as the next therapeutic link option for AD management. AD has a direct association with prion mechanisms. PrPC misfolds to form pathogenic prions, which further affects other prion proteins to misfold, eventually causing a significant elevation in abnormal protein levels, which causes brain harm¹³.

2. Mechanism of inception and progression of AD

The two pathological attributes of AD are:

- Deposition extracellular β -amyloid senile plaques
- Intracellular neurofibrillary tangles

The deposition of β -amyloid and neurofibrillary tangles initiates the loss of synapses and neurons, leading to gross atrophy of the affected part of the brain, which is a platform for onsets in the mesial lobe. It is unknown how exactly β -amyloid peptides and neurofibrillary tangles generate such devastation. As per the amyloid hypothesis, the cumulative deposition of β -amyloid inside the brain results in neuronal necrobiosis, loss of neuronal connections, and progressive neurotransmitter deficiencies, all of the facts above lead to the behavioural symptoms of dementia^{3,14}. In AD, β -amyloid in the cerebral area of the brain is deposited and tau in neurofibrillary tangles is believed to possess prion like properties for self replication. $A\beta$ principal binding region and helix-1 epitopes could prevent $A\beta$ binding and the $A\beta$ -facilitated interruption of synaptic plasticity¹³. Chung and colleagues administered the monoclonal anti-PrPC antibody 6D11 intraperitoneally to APP/PS1 transgenic (Tg) mice and discovered that antibody treatment entirely rescued the Tg animals' behavioural and cognitive deficits¹⁵. Also, $A\beta$ mainly accumulates in the mitochondria of Alzheimer's brain cells and hinders the activity of certain enzymes such as pyruvate dehydrogenase, isocitrate dehydrogenase, and α -ketoglutarate dehydrogenase complexes¹⁶.

3. New targets in AD

The present treatment for AD is only a symptomatic approach. Tacrine gained the attention of medical practitioners in AD, but due to hepatotoxicity, it was taken off the market. Four FDA-approved drugs, namely, galantamine, donepezil, rivastigmine as AChE inhibitors, and memantine NMDA receptor antagonists, listed in Fig. 1, are available for treating AD. Even though inhibitors of cholinesterase and NMDA receptors have well established efficiency levels, clinical results of patients receiving these medications are restricted, and many authors see them as "symptomatic" therapy³. Several targets are being explored for drug discovery anti-Alzheimer treatment; with a few of them, therapeutic targets are previously well identified with inhibitors, whereas some are now being analysed critically for identifying or creating effective small molecules. Table 1¹⁷⁻³⁹ lists the targets with their origins and known functions.

3.1. The amyloid hypothesis

The amyloid cascade concept was first pointed out a couple of decades ago and is based on the $A\beta$ peptide, a member of the intrinsically disordered proteins class (IDPs), a significant plaque element. The $A\beta$ and CT (APP's carboxy-terminal peptide) peptides are amyloid proteins that play a pivotal part in the progress of AD⁴⁰. The formation of the $A\beta$ peptide from the APP molecule by the action of two proteolytic enzymes called β - and γ -secretase, which hydrolyse at the amino-terminal and carboxy-terminal of the peptide, respectively. Decreased metabolic ability to break down $A\beta$ is evidenced in $A\beta$ peptide aggregation in the elderly or diseased people. $A\beta_{42}$ amplifies the development of β -amyloid fibrils from senile plaques, subsequently causing tau pathology and neurotoxicity, resulting in cell apoptosis and neurodegeneration⁷.

Two metabolic pathways are involved in processing APP: i) amyloidogenic path and (ii) non-amyloidogenic pathway. The amyloidogenic pathway of APP processing is represented by the

APP amino-terminus (NT) by β -secretase and the cleavage of the APP CT by γ -secretase. Between M671 and D672, β -secretase cleaves APP, thus releasing the C99 fragment and an APP soluble peptide (APP). Near residue 712 of the CT, γ -secretase can cleave the CT region in V711 or I713 to form the short peptide $A\beta$, $A\beta_{40}$ or the long peptide $A\beta_{42}$. A CT peptide of about 50 residues from APP, sometimes called the APP intracellular domain, is also released with the $A\beta$ peptide (AICD). In the nonamyloidogenic pathway, APP can also be processed by α -secretase (TACE), which dissects the sAPP fragment between K687 and L688, thus resulting in the formation of soluble APP α and cell membrane bound C-fragment 83 (CTF83) at residue 711 or 713. The generated CTF83 is cleaved by γ -secretase to produce AICD and a small p3 fragment. This non-amyloidogenic pathway does not make the $A\beta$ peptide. The β -secretase produces sAPP and a 12 kDa protein segment (C99 or CTF), which is then parted by the β -secretase, resulting in $A\beta$. The " $A\beta$ cascade theory" describes the association between $A\beta$ histopathologic abnormalities, neural apoptosis, and cognitive deficit in AD^{41,42}.

Numerous mutations have been reported in the APP. These pathogenic APP mutations are related to a surge in the formation of $A\beta_{42}$ and an alteration in the ratio of $A\beta_{42}$ production. Remarkably, in Down's syndrome, individuals with trisomy 21 show symptoms which was assumed to be related to elevated APP and $A\beta$ levels in the brain. Autosomal dominant EOAD with cerebral amyloid angiopathy and massive $A\beta$ peptide accumulation is caused by APP locus duplication⁴³. The PS1 or PS2 γ -secretase components are associated with familial AD mutations (Fig. 3). These APP and PS mutations are tightly connected to $A\beta$ production, proving $A\beta$ production and amyloid fibril development. It was previously thought that the release from proteolysis of the β -amyloid peptide from the transmembrane region of its ample precursor protein was an abnormal process that necessitated neuronal damage⁴⁴. The rise in $A\beta$ levels in the brain is a critical event in the amyloid hypothesis and causes synaptic impairment, thus leading to early deficiency in cognition. Synaptic failure in the olfactory bulb can cause olfactory impairments in many Alzheimer's patients. In aging APP/PS1 mice, $A\beta$ deposition leads to functional and morphological modifications in the synapses of the olfactory processing sites⁴⁵.

3.1.1. Inhibition of amyloid aggregation

Some IDP proteins tend to aggregate non-covalently to form oligomers, and $A\beta$ can also undergo oligomerization. Amyloid aggregation in fibrils, oligomers, and plaques is thought to cause synaptic functional impairment and nerve cell death in AD. Albeit, amyloid plaques are the principal pathogenic species, and the most neurotoxic form of $A\beta$ is soluble oligomers. Agents, which hinder aggregation of $A\beta$ monomers by binding with the monomers, are thus a rational way of containing neurotoxicity and AD⁴⁶. Two inhibitors of such aggregation, scyllo-inositol (ELND005) and tramiprosate (Fig. 4) are explored in phase II clinical trials. In a recent trial, scylla-inositol was safe at low doses (250 mg) but did not show encouraging efficacy. In the higher doses, it was associated with severe side effects like infection and fatalities; thus, further detailed research investigations are needed to determine and measure the efficacy⁴⁷. Phase III studies of mild to moderate patients with AD tramiprosate did not affect cognitive scores. Re-examining trial data revealed therapeutic advantages among ApoE4 homozygotic subjects of ALZ-801, a tramiprosate prodrug⁴⁸.

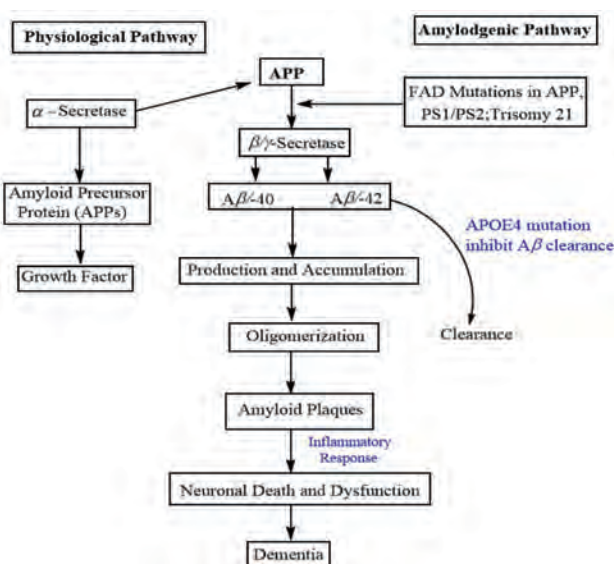
Table 1 Summary of new targets in AD along with source and functions of targets.

Sr. No.	Target	Location	Function	Ref.
1	β -secretase (BACE1)	Activity of β -secretase was found in most of the tissues, especially neural tissue and neuronal cell line. Highest activity was observed in trans-Golgi network and endosomes	Generation of A β through BACE1 cleavage of APP	17
2	Butyryl cholinesterase	Highly active in heart, lung, liver, kidney and intestine	Regulates brain ACh level and cholinergic transmission in absence of AChE	18
3	γ -secretase: Presenilin 1	Found in endoplasmic reticulum	Catalyses intramembranous proteolysis of the notch receptor and generates A β by the sequential cleavage of the substrate C99	19
4	Calcitonin gene-regulated peptide (CGRP)	α CGRP is predominant in CNS and endocrine tissues. β CGRP is located in brain, thyroid gland and sensory ganglia	At the transcriptional level, CGRP receptors reduce acetylcholinesterase availability and elevate cAMP levels	20
5	Phosphodiesterase (PDE)	Different isoforms of PDE are found in CNS	A key function in neurons is to regulate intracellular signalling cascades initiated by neurotransmitters and control the intracellular levels of cAMP and/or cGMP	21
6	Muscarinic acetylcholine receptor	mAChRs located on detrusor smooth muscle and cholinergic nerve terminal of the bladder	It modulates ACh release, which can improve voiding efficiency with increased nerve traffic	22
7	Dopamine-2 receptor	Found in the olfactory tubercle, striatum and nucleus accumbent in large amounts and to a small amount in the amygdala, hippocampus and cortical region	Regulates various brain functions, playing vital role in regulating locomotor activity, motivation and cognition	23
8	γ -aminobutyric acid receptor (GABA)	Widely spread in the brain cortex, frontal, occipital, and temporal lobes	It controls cortical excitability	24
9	Nuclear factor-erythroid 2 related factor-2 (Nrf2)	Expressed in both the cytoplasm and nucleus of neuron hippocampi	Retaining redox balance, antioxidant and remove damaged proteins	25
10	Metabotropic glutamate receptor (mGluRs)	mGluRs are primarily found at the excitatory synapse, however their positions may vary depending on the type of various isoforms. It is found in the cerebellum, amygdala, dorsal striatum, olfactory bulb, nucleus accumbens and hippocampus	Long-term effects of mGluRs control neuronal plasticity, maintain CNS function, and contribute to pathological situations such as anxiety, fear extinction, and spatial working memory	26
11	Parkinson's disease protein (DJ-1/PARK7)	It is abundantly expressed in astrocytes in the frontal cortex and substantia nigra of idiopathic PD brains	Protection from oxidative stress	27
12	N-myc downstream-regulated gene	Astrocytes, epithelial tissue and glia cells	Act as an endogenous neuroprotectant in AD	28
13	Protein tyrosine phosphatase 1B (PTP1B)	Hippocampus, microglia	It modulates several CNS processes relevant to the physiopathology of AD and acts as a positive controller of neuroinflammation	29
14	Monoamine oxidase-B (MAO-B)	Expression of MAO-B is increased in the cerebral cortex and hippocampus of AD brain	Deactivates trace amines like 2-phenylethylamine (PEA), neurotransmitters like dopamine, and potentially other neuro-modulatory amines	30
15	NAD(P)H quinone oxidoreductase	Found in neurons of hippocampus and astrocytes of AD brain	Protects cell from oxidative injury	31
16	Amyloid protein precursor (APP)	Found in brain	In transfected cell lines, APP regulates cell proliferation, motility, neurite outgrowth, and survival. The soluble ectodomain, generated by APP cleavage, can replicate these actions	32
17	Peroxisome proliferator activated receptor- γ (PPAR- γ)	Detected in higher quantities in the brains of Alzheimer's patients	It has helpful effects on memory and learning are specifically caused by inflammation inhibition, insulin action enhancement, or mitochondrial function within the brain	33
18	C-C chemokine receptor type-5 (CCR5)	Macrophages, dendritic cells, and memory T cells in the immune; in CNS	Potent inhibitor of learning and memory by inhibiting hippocampus and cortical neuronal plasticity	34
19	Nicotinic acetylcholine receptor (nAChR)	nAChRs are found in both neurons and glia in the central nervous system	It plays role in the development of locomotion, memory, anxiety and attention	35

Table 1 (continued)

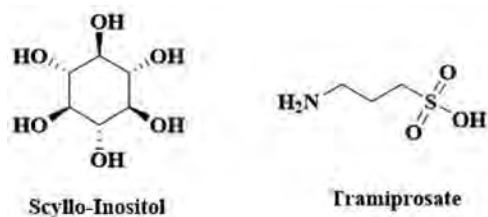
Sr. No.	Target	Location	Function	Ref.
20	Angiotensin receptor I and II (AT1 & II receptor)	Blood vessels, kidney, adrenal cortex, heart, lung and basal ganglia, circumventricular organs of brain, brainstem	Rise brain A β level <i>via</i> various mechanisms with increasing APP mRNA, β -secretase action, and presenilin expression	6
21	Triggering receptor expressed on myeloid cells 2 (TREM2)	Present in CSF and myeloid cells including microglia, monocytes, macrophages, neutrophils osteoclasts, and dendritic cells	Strongly increase the risk of AD development, mediates the role of AD pathogenesis	37
22	BDNF	Pyramidal and granule cells of the hippocampus and specific regions of the CNS	Essential for the life of adult cortical neurons in the entorhinal cortex, whose early failure causes the short-term memory loss in AD	38
23	GSK-3	In the brain of AD patient	Kinase dysregulation has been shown to affect both A β and tau metabolism and toxicity	39

AT1 & II receptor, angiotensin receptor I and II; (CP)-AMPA, (calcium permeable)- α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; cGMP, cyclic guanosine monophosphate; GABA, γ -aminobutyric acid receptor derived neurotropic; MAO-B, monoamine oxidase-B; mAChR, muscarinic acetylcholine receptor; DJ-1/PARK7, neurotrophin brain-Parkinson's disease protein; PTP1B, protein tyrosine phosphatase 1B; PDE, phosphodiesterase; TREM2, triggering receptor expressed on myeloid cells 2.

**Figure 3** Pathological pathway and amyloidogenic pathway.

3.1.2. Nonpeptidic anti-aggregates

The first class of specified agents in the suppression of aggregation are nonpeptidic anti-aggregates, of which tramiprosate is a primitive example generated from propionic acid. Subsequent phase III trials contradicted this agent's promising results in terms of safety and tolerance: the European trial avoided medical issues that could have resulted in a negative outcome in the trials of North America and proved feeble penetration of the drug in the brain and insufficient

**Figure 4** The structures of amyloid aggregation inhibitors which have extended clinical trials.

potency⁴⁹. New nonpeptidic anti-aggregates were supposed to address these drawbacks and accumulates are thought to dissociate faster when scyllo-inositol is present. As this molecule may cross the blood–brain barrier (BBB), it can reach significant concentrations in the CNS by peripheral administration⁴⁷. This drug also involved cell transduction modulation, cell survival and cell death regulation, and mitochondrial function. The numerous properties of this natural molecule have identified it as a potential contender, and a phase III trial with primary AD patients using epigallocatechin-3-gallate (EGCG) is now under screening consideration⁵⁰.

3.1.3. Metal chelators

Several studies link AD pathogenesis to neocortical amyloid aggregation; this could be facilitated by abnormal A β interaction and metal mediated oxidative stress; in the AD brain, aluminium, zinc, iron, and copper increase A β aggregation and neurotoxicity⁴⁰. Although the aluminium related hypothesis has some discrepancies, aluminium may play a cardinal part in developing neurofibrillary tangles and neurotic plaques in AD⁵¹. Using aluminium binding ligands (silicates) or deferoxamine (DFO, Fig. 5), the metal chelator, researchers have tried to slow or reverse A β accumulation^{52,53}. Two years' study by McLachlan found that DFO-treated groups had significantly lower neocortical aluminium concentrations than untreated groups, with behavioural improvement. Although DFO has an aluminium chelating effect, zinc or copper chelating effects are possible⁵³.

A β can bind with metals, including copper and zinc, which are present in high amounts in neurotic plaques in AD and promote *in vitro* A β aggregation and neurotoxicity⁵⁴. In a masked study of APP, nine weeks of treated Tg mice escalated soluble A β levels by 52%⁵⁵. This hinted that metal chelators like clioquinol (Fig. 5) could inhibit A β accumulation and thus be used to treat AD. Vital evidence indicated that soluble A β levels correlated with cognitive dysfunction in AD Tg mice and that soluble A β levels precede resulted in A β plaque deposition in AD Tg mice^{56,57}. It is more important to prevent both soluble and extracellular A β plaque accumulations⁵⁸.

3.2. Anti-amyloid immunotherapy

In vitro investigations were the first to show that antibodies contrary to the A β peptide might help reduce amyloid accumulation. Anti-A β antibodies with little stoichiometries prevented A β by

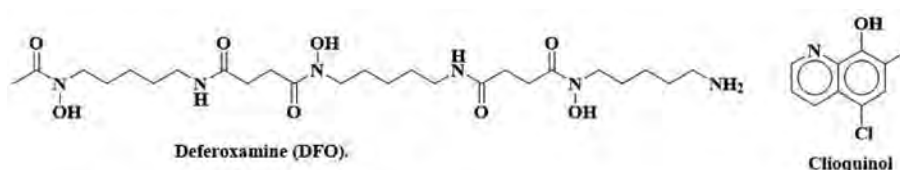


Figure 5 Structures of metal chelators.

developing fibrils *in vitro*⁵⁹. The study identified the EFRH epitope at 3–6 positions of A β as a crucial target for that antibody's catalytic action⁶⁰.

As prospective Alzheimer's immunotherapies, active immunization with A β peptide synthetic fragments hinted at a transporter protein, and passive immunization with monoclonal antibodies targeted against A β peptide is being investigated. In AD animal models, immunization with A β peptide provided protection and reversed pathology. Meningoencephalitis, vasogenic oedema, and microhaemorrhages have all been reported with AN1792. As of now, immunotherapy has not shown a substantial cognitive effect on Alzheimer's patients, but peptide immunotherapy is being studied against tau pathology as well⁶⁰.

3.2.1. Immunotherapy mechanism

Several hypotheses have been proposed to understand how antibodies engaged at A β enhance peptide clearance *in vivo*^{60,61}.

(i) Microglial phagocytosis:

According to the elementary hypotheses, whenever anti-A β antibodies bind to A β peptides, their Fc component finally hits Fc receptors on the cell membrane of microglial cells, where it is phagocytized and digested. According to this theory, antibodies of anti-A β can travel through the BBB and attach to A β inside the central nervous system. While evidence has been found to back this theory, additional research has unequivocally shown that Fc-promoted phagocytosis is not essential for A β clearance brought on by immunotherapy⁶¹.

(ii) Peripheral sink:

Monomeric β -amyloid could be removed from the brain by generating antigen–antibody complexes on the outside, preventing the formation of new plaques. Another idea is that A β could be evacuated from the brain straight into the blood by altering the A β brain blood balance to improve clearance of soluble A β , which is supported by a rise in serum A β , the majority of which is antibody bound⁶⁰.

(i) Deaggregators:

AD immunotherapy is being studied in many forms; the first direct immunization by using synthetic A β_{42} was tested in Tg mouse models and humans recently⁶¹. Passive immunization has been observed to prevent the production of new amyloid plaques and eliminate existing ones. Anti-A β monoclonal antibody treatment wholly and quickly restored the hippocampus Ach release and uptake of high-affinity choline in mice. The anti-A β antibody neutralizes cholinotoxic species upon binding the A β peptide, eventually reversing the deficiency of early memory^{62,63}.

3.2.2. Molecular understandings of active and passive immunization

Antigen-specific antibodies are produced *via* vaccination, *i.e.*, active immunization. Antigens employed in AD include whole A β or a portion of A β , which is conjugated to a foreign T cell epitope carrier protein. Antigen-presenting cells deliver T cell epitopes to naive T cells, resulting in a humoral immune response. Surface co-stimulatory molecule binding promotes T cell activation by providing a secondary signal to help activated T cells generate antibodies against the antigen. The soluble antigen attaches to receptors of β cell receptors through the β cell epitope-activated T cells to cause cellular immunity. Pro-inflammatory cytokines are released during a Type 1 T helper (Th1) reaction, while anti-inflammatory cytokines are released during a Type 2 T helper (Th2) response. Passive immunization eliminates the requirement to stimulate the immune system to manufacture antigen-specific antibodies. Anti-A β antibodies target the peptide for elimination in both active and passive A β vaccination (Fig. 6)⁶³.

Immunotherapy for AD has been met with mixed reactions. Unexpected adverse effects from the clinical trials dampened initial enthusiasm. The statistics on cognitive performance implied that some people benefited from the treatment despite its brevity. Many ways are being studied to avoid the adverse effects of active immunization trials. Managing anti-A β titres and stopping therapy with passive immunization is appealing. High anti-A β titres may increase cognophilic angiopathy and vascular outflow in mice, and immunotherapy trials are previously in process. One includes passive N-terminal antibody immunization, a second use of a shortened peptide vaccine to reduce T cell responses. The preceding trial's results will demand caution in future immunotherapy trials for AD. These trials may be the primary therapeutic tests of the amyloid hypothesis in AD if they are completed successfully⁶⁴.

Despite early failures, some phase II clinical trials have been conducted in the last decade with no serious adverse events. Merck (MK-8931), Novartis (CAD106), and Afiris (Aflitope AD02) funded such clinical trials; however, none of them reported their findings⁶⁵. Several passive immunization approaches are being investigated, including intravenous immunoglobulin, intravenous delivery of anti-A β_{42} monoclonal antibodies, and so on. An intravenous immunoglobulin clinical experiment with a small number of AD patients that was followed for six months discovered a substantial effect on cognitive performance and a drop in CSF A β_{42} levels following immunization (Fig. 6)⁶⁰.

Gantenerumab is the first anti-A β_{42} monoclonal antibody formulation. It appears to slow the growth of amyloid deposits in AD patients, although its efficacy has yet to be proven. Limited clinical data exist on the relationship between reduced amyloid deposit formation and clinical outcomes⁶⁶. Solanezumab is a well-tolerated anti-A β_{42} monoclonal antibody formulation, but its efficiency is still unknown. A study of 1000 mild to moderate AD patients who were administered intravenously 400 mg Solanezumab every four weeks for 80 weeks failed to show its efficacy⁶⁷.

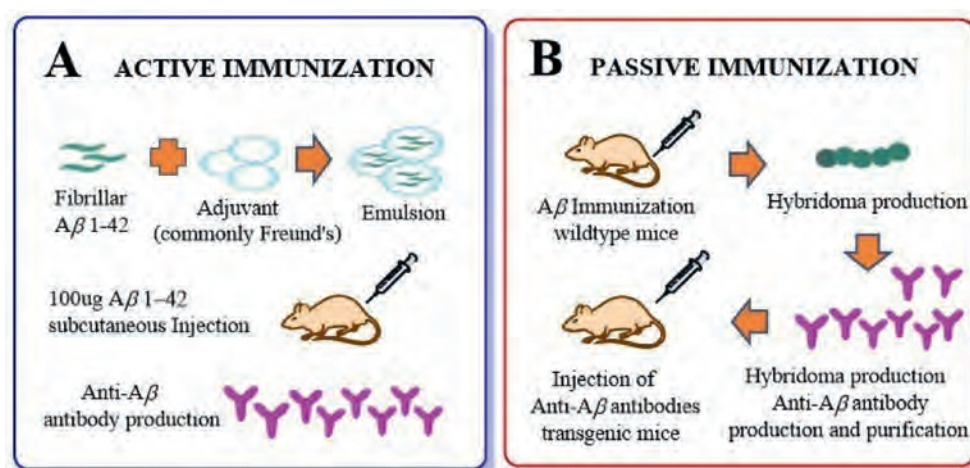


Figure 6 Molecular basis of active and passive immunisation⁶¹. (Reprinted with the permission from Bentham Ref. 61. Copyright © 2011 The authors).

Six well-designed randomized critical trials by Richard et al.⁶⁸ showed that anti-amyloid β immunotherapy does not affect AD. Second, unlike classical frequentist analysis, which merely allows us to conclude that there is no evidence, Bayes factor hypothesis testing will enable us to quantify the plausibility of six well-designed RCTs of the null hypothesis. Third, while the results of frequent meta-analysis may hint that further research is needed, the Bayesian analysis strongly suggests that anti-amyloid β immunotherapy is no longer effective; this opens up new avenues of research that may yield additional fruit⁶⁸.

3.3. Anti-amyloid strategies

$A\beta$ amyloid targets, *i.e.* secretases are implicated in APP metabolism, selective reduction of $A\beta_{42}$ production, amyloid aggregation prevention, and anti-amyloid immunotherapy help bring down $A\beta_{42}$ ⁶⁹. Although these drugs have serious side effects and should only be used in severe cases with serious care. Thus, current amyloid-based therapy research will pave the way for efficient medical care to address this deadly illness. In particular, genetic studies have assisted in strengthening the amyloid hypothesis during the previous two decades⁶⁹. Several therapeutic strategies have been developed to block $A\beta$ peptide effects, and the advancement of effective treatment strategies for reducing $A\beta$ production or increasing $A\beta$ clearance is currently a primary focus in research. Among such approaches include the metabolism of APP by the enzyme secretases, which are discussed below.

3.3.1. γ -Secretase inhibitors (GSIs)

The $A\beta$ peptide is formed from the APP molecule because of the action of two proteolytic enzymes β - and γ -secretase, which hydrolyze at the NT and CT of the peptide, respectively; thus, inhibitors of both secretases could benefit from the decreased levels of $A\beta$. Theoretically, inhibitors of either β - or γ -secretase could reduce $A\beta$ formation⁷⁰. γ -Secretase is a type of aspartyl protease with multiple subunits that hydrolyze APP and another type 1 transmembrane protein. Presenilin 1 (PS 1), nicastrin (NTC), anterior pharynx defective-1 (Aph-1), and presenilin enhancer-2 (Pen-2) are the four primary elements for enzyme activity in the γ -secretase complex (Fig. 7)⁷¹. PS is a protein that is required for β -APP transmembrane cleavage⁷² and further, Presenilin-1 (PS-1)

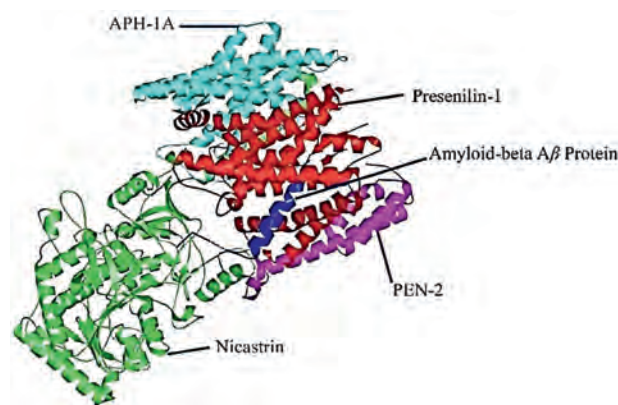


Figure 7 Human γ -secretase complex three-dimensional structure (PDB ID: 6IYC)⁷⁴. Reprinted with the permission from Ref. 74. Copyright © 2020 The authors.

is responsible for most $A\beta$ production, but Presenilin-2 (PS-2) can also change. PS1 and PS2 work together to form the catalytic core of γ -secretase⁷¹. Currently, over 200 missense mutations in the *PS-1* gene were discovered in destructive early onset FAD, with the bulk of them altering the $A\beta_{42}/A\beta_{40}$ ratio and meddling with APP and other γ -secretase substrate processing⁷³.

A small molecule has been observed that can inhibit β - and γ -secretase, which are essential for controlling the $A\beta$ synthesis. In preclinical studies, the use of specific inhibitors of γ -secretase has been shown to reduce levels of soluble $A\beta$ levels and accumulation of $A\beta^{70}$.

3.3.1.1. Crystal structure of human γ -secretase. The γ -secretase complex is a member of a family of intramembrane cleaving proteases (I-CLiPs) that hydrolyze substrates in the hydrophobic environment of lipid bilayers. Membrane encased proteases are also soluble proteases, such as site two protease metalloproteases, presenilin type aspartyl proteases, and rhomboid serine proteases. I-CLiPs are found in nearly all life forms with a broad range of vital roles in biology and cut within their substrates' transmembrane domain^{75,76}. Entirely several crystal structures of

γ -secretase are in the apo and inhibitors state have been identified^{73,77}. Crystal structures of human γ -secretase co-crystallized with APP85, notch85, and inhibitors have been solved⁷³. The catalytic subunit of the complex present in PS1 and PS2 in the aspartyl protease presenilin⁷⁸. Complexes of presenilin (bears the active site aspartate), nicastrin, Aph-1, and Pen-2 are found in the complex with γ -secretase. The complex structure has at least eighteen transmembrane domains, thus making crystallographic experiments more challenging. It also comprises two pores (apical and basal pores) that allow water molecules to enter and a low density interior chamber. These holes may explain this remarkable intramembrane cleavage (peptide bond hydrolysis) by γ -secretase for water molecules. Two pores could allow the release of A β and AICD into extracellular and cytosolic regions, respectively⁷⁹.

The atomic structure of γ -secretase in a substrate free condition was disclosed in 2015 using single particle cryoelectron microscopy (cryo-EM) with a resolution of 3.4 Å. The total molecular weight of these proteins is about 170 kDa (kDa), with an extra 30–70 kDa of glycosylation present in the nicastrin extracellular domain. The catalytic component, presenilin, has nine TMs. The TM6 and TM7 regions of presenilin are autocatalytically cleaved into the NTF and the CTF upon association with PEN2. Nicastrin and APH-1 combine to form a stable subcomplex that interacts with presenilin's CTF. A sizable extracellular domain found in nicastrin is assumed to be in charge of substrate recruitment. More than 150 missense mutations found in AD patients demonstrate the critical function of presenilin in the γ -secretase complex⁷⁷.

The catalytic subunit of the complex present in PS1 and PS2 in the aspartyl protease presenilin⁸⁰. PS1 is proteolyzed and converted into the building of two: an NTF and a CTF. The complex involves PEN2 for maturation, whereas APH1 is significant for its stability. Lately, NTC was discovered to show a vital role in APP binding (Fig. 7)⁷⁴. The distinctive patterns YD on TM6 and GxGD protease on TM7 distinguish Presenilin from other TMs.

Presenilin initiates an autocatalytic breakage during γ -secretase assembly, yielding an amino NT fragment containing TMs 1–6 and a CTF containing TMs 7–9. More than two-thirds of the 300 mutations taken from patients with FAD are localized to PS1, and roughly a third each to PS2 and APP. Nicastrin has a single TM and a huge extracellular domain that is highly glycosylated and is supposed to target the NT of substrate proteins⁷⁷.

The hydrophobic nature of the substrate binding sites on the membrane-embedded protease complex led to the detection of extremely powerful inhibitors that can pass the BBB. The inhibitor was discovered to bind with amino acids I143, M146, W165, L166, ser169, M233, F283, G384, and F388, which form a requisite motif that also contains the catalytic dyad of D257/D385 in the crystal structure of γ -secretase assembly⁷⁴.

The γ -secretase modulators (GSMs) prevent the enzyme's production of A β_{42} without reducing total A β levels. This potential impact was initially detected in a subclass of NSAIDs, including naproxen, ibuprofen, and sulindac sulfide (Fig. 8). Despite their low efficacy in reducing A β_{42} production, these compounds provided vital proof that such selective reduction could be accomplished, resulting in low nanomolar compounds with promising pharmacokinetic properties⁸¹.

Currently, several γ -secretase inhibitors (GSIs) are being investigated, and among them, some are in clinical trials, such as semagacestat (LY450139) nonselective GSIs. Phase III trial results of semagacestat on patients involving mild to moderate AD were abruptly terminated in two of these trials⁸¹. It was also found that the use of GSIs was associated with adverse liver, spleen, and skin reactions. γ -Secretase activating protein, a protein that is a part of the amyloidogenic pathway, has been studied in patients who were suffering from Down's Syndrome (DS) and has been found to produce β -amyloid without displaying acute toxicity, so its inhibition might be a target for development⁸². Following the disaster of GSIs in the clinical trials, it was evident that GSMs would be a more secure solution. Because of its multiple substrates, inhibiting

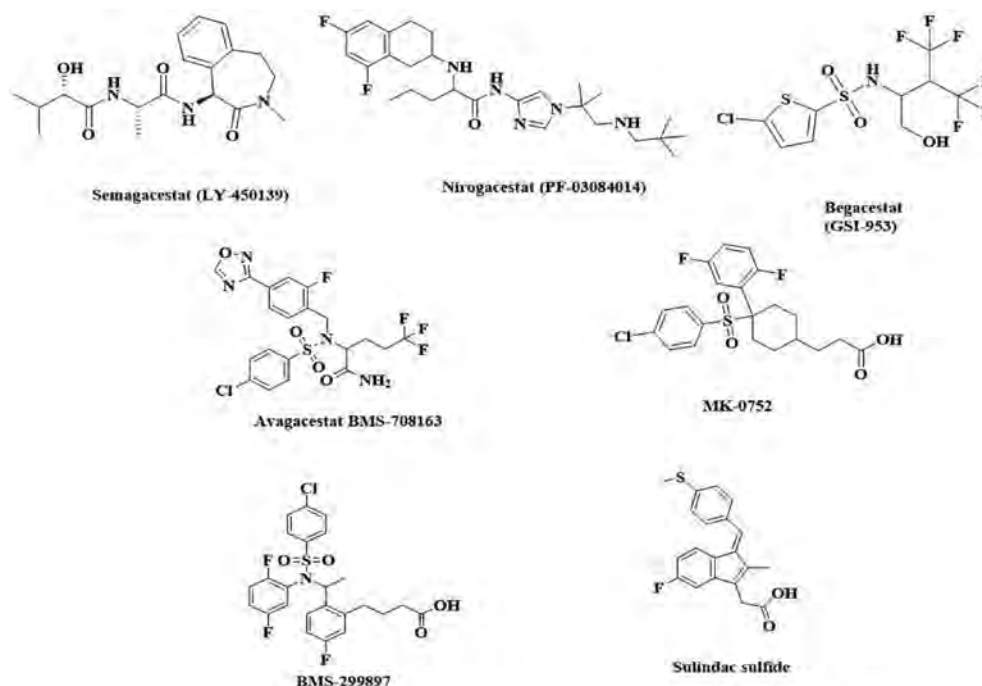


Figure 8 The structures of γ -secretase inhibitors which have extended clinical trials.

GS may not be tolerated in a continuous dosage paradigm; however, boosting its process ability with GSMs may fix the essential molecular shortfall in FAD. Clinical trials of BMS-299897 were the first of their kind. Bristol Myers Squibb developed the inhibitor; however, no clinical data was released. Many secretase inhibitors like ELND-006 (Fig. 4), GSI-953, PF-3084014, LY-450139, MK-0752, and BMS-708163 (Fig. 8) are in clinical studies, among there the data of LY-450139 are opened for public reference. The inability of GSIs to distinguish between APP and Notch causes critical difficulties of Notch related toxicity, as depicted in Table 2^{40,47,83-88}.

3.3.2. γ -Secretase modulators (GSMs)

Inhibiting $A\beta_{1-42}$ aggregation in AD patients without increasing CTF- β deposition, meddling with Notch signaling and APP intracellular domain (AICD) release, appears to be a logical way for containing AD causatively. GSMs with increased potency for reducing $A\beta_{1-42}$ production have been designed and developed in recent years. In $A\beta_{1-42}$ inhibitory potency has to be augmented and selectivity for other γ -secretase substrates be preserved. Some NSAIDs have been demonstrated to inhibit Notch-1's S3 cleavage site. The concentration-dependent dissociation of inhibitory effects on multiple cleavage sites appears to leave a "window of modulation". The reduction of microglia mediated cytokine release is another mechanism that may be implicated in the favourable behavioural and neuropathological effects of many NSAIDs (the best recognized being ibuprofen), reduction of microglia peroxidase and inhibition of neuronal cell

death. γ -Secretase modulators like NSAIDs face challenges in brain penetration because of the high binding to plasma protein; the unbound fraction is only 3–5 percent of the total concentration, limiting the quantity of medication that may enter the CNS. Most NSAID GSMs are massive lipophilic molecules with a high prevalence of non-selective and off-target pharmacology. Finally, improved comprehension of the target(s) with which these γ -secretase modulators bind will aid future drug development for AD⁴¹. Tarenflurbil (Fig. 9) modulates the γ -secretase activity by reducing $A\beta_{42}$ synthesis and generating less toxic $A\beta$ fragments while sparing other γ -secretase substrates, including Notch. Tarenflurbil is the *R*-enantiomer of Flurbiprofen that unexpectedly portrayed righteous safety and clinical results in a phase II trial⁸⁹. The clinical advancement of γ -secretase modulators for AD treatment is shown in Table 3^{41,81,90-95}.

3.3.3. β -Secretase (BACE1) inhibitors

The β -secretase, as an enzyme responsible for the cleavage of β -site APP (BACE1; also known as Asp2, memapsin 2)⁹⁶. BACE1 is a complex structure protein resembling other aspartyl proteases, a family of enzymes found in the human body, including pepsin BACE2, cathepsin D (CatD), renin, and cathepsin E (CatE). BACE1 hydrolyses the APP in the lumina and step that restricts speed in the production of $A\beta$. Inhibition of BACE1 has several advantages; it prevents $A\beta$ formation early in the APP processing. Furthermore, knockout homozygote BACE1 mice showed a complete loss of $A\beta$ formation with no considerable side effects. Initially, APP is broken by α - or β -secretase, followed by which

Table 2 Clinical development of γ -secretase (Chemical structures shown in Fig. 8).

Sr. No.	Name of compound	Pros	Cons	Clinical trial identifier	Status	Ref.
1	Semagacestat (LY-450139)	Reduces the amount of newly synthesised $A\beta$ in healthy people's CSF. (first GSI to enter phase 3 clinical trials.)	In AD patients, it worsens cognitive function in a dose-dependent manner. In AD patients, the risk of skin cancer and infection increases.	NCT01035138 NCT00762411 NCT0059456	Discontinued	84
2	Avagacestat BMS-708163	Notch sparing. Lowers $A\beta$ conc. In CSF of normal person	Increases in reversible renal tubule effects and nonmelanoma skin cancer. Rises in brain atrophy rates. Patients with AD have poor tolerability.	NCT00890890	Phase II	85
3	Begacestat (GSI-953)	Potent $A\beta$ reduction and <i>in vitro</i> notch processing selectivity. <i>In vivo</i> plasma and CSF $A\beta$ reduction.	Inadequate information on cerebral plaque aggregation in Tg mice. Produces rebounds $A\beta$ -dependent cognitive deficits in Tg mice	WAY 210953	Phase II	86
4	Nirogacestat PF-3084014	Notch sparing. Well CNS permeation. Long term activity on $A\beta$ conc. No rebound effect on plasma $A\beta$ in animals	Inadequate information on cerebral plaque deposition in Tg mice. Inadequate knowledge regarding the behavioral impact of AD in animal models. Undesirable patient's PK/PD profile	NIR-DT-301	Discontinued	40,87
5	MK-0752	Reductions $A\beta_{40}$ conc. In CSF of normal person	Prevents notch cleavage. Major gastro-intestinal toxicity in patients	NCT00645333	Discontinued for AD	40,88
6	Scyllo-inositol (ELND005)	Notch sparing. Well brain permeation. Reduce brain $A\beta$ load in transgenic mice	No cognitive improvement; Toxicity in the greater doses (infections and death)	NCT00568776	Phase II	47

CNS, central nervous system; CSF, cerebrospinal fluid; GSI, gamma secretase inhibitor; PK, pharmacokinetic; PD, pharmacodynamic; Tg, transgenic.

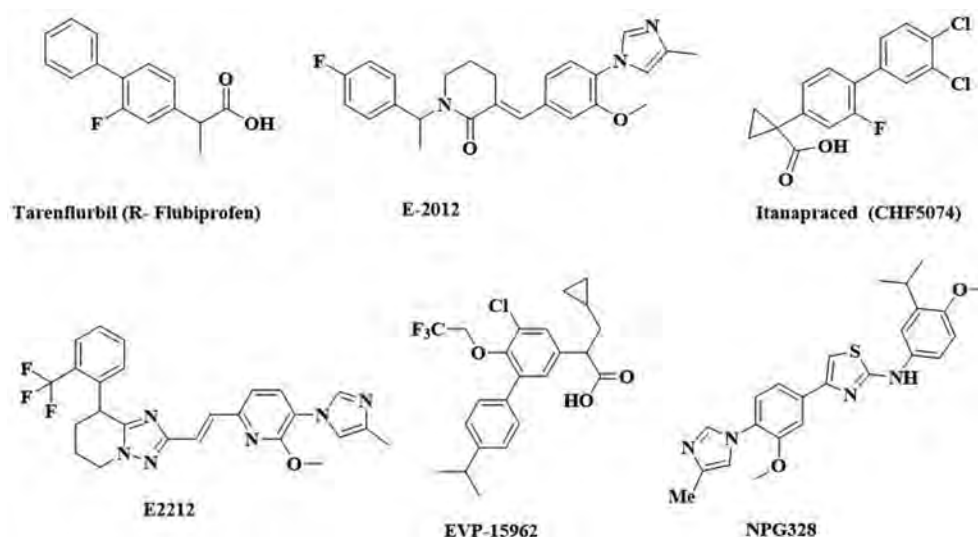


Figure 9 The structures of γ -secretase modulators, which has extended clinical trials.

Table 3 γ -Secretase modulators in clinical advancement for AD treatment (Chemical structures shown in Fig. 9).

Sr. No.	Name of Compound	Pros	Cons	Clinical Trial identifier	Status of compound	Ref.
1	Tarenfluril	High safety profile for patients with AD	Less <i>in vitro</i> effectiveness; Less BBB permeation; No effect in phase 3 trials	NCT00105547	Discontinued	90
2	E2012	Notch sparing without deposition of $A\beta$; No rebound actions on human plasma; Decreases plasma levels of $A\beta_{42}$ and $A\beta_{40}$ differently in healthy humans	Rats have lenticular opacity; there is a scarcity of information on brain plaque aggregation in transgenic mice; Data on behavioral effects are scarce	NCT01221259	Discontinued	91,92
3	CHF5074	Notch sparing; No deposition of CTF β ; In Tg mice, it reduces brain pathology; Increases cognitive deficit in tg mice; <i>In vivo</i> neuroprotective action	Little micromolar inhibitory potency on $A\beta_{42}$; Incomplete brain penetration	NCT01203384	Phase II	93
4	E2212	Well safety profile in animals; High inhibitory potency on $A\beta_{1-42}$	Inadequate information on brain plaque deposition in transgenic mice; Data on behavioural effects are scarce	NCT01221259	Phase I	Clinical trials. Gov Identifier NCT0122125G
5	EVP-0015962	Notch sparing; Well tolerated and reduces brain $A\beta$ aggregates in Tg2576 mice; Enhances cognitive deficiencies in tg mice	Cognitive effects in Tg mice found at comparatively high dose	—	Preclinical	94,95
6	NPG-328	Nanomolar strength; Notch-sparing; In tg mice, it reduces brain pathology	Lack of information on behavioural activity	—	Preclinical	41,81

γ -secretase processes the membrane-attached fragments⁹⁷. BACE1 hydrolyses APP at the Asp+1 amino acid of the $A\beta$ -sequence, resulting in the NT of the peptide. The hydrolysis

affords two fragments: the ectodomain (APPs β) and the second CT part, which is membrane bound. C99 is then acted by γ -secretase, thus forming the CT of the $A\beta$ protein and the AICD.

The synthesis of $A\beta$ in the brain is attributed to the successive proteolytic breaking of APP by β - and γ -secretase^{96,98}.

3.3.3.1. Crystal structure of BACE1. According to electron microscopy, regular and dystrophic presynaptic terminals are where BACE1 primarily produces subcellular⁹⁹. BACE1 crystal structures with and without inhibitor bound in the active site have been resolved repeatedly. An NT protease domain, a linking strand, a transmembrane domain, and a cytosolic domain are all present in BACE1¹⁰⁰. It has an aspartic protease structure; however, its active site is much more open than pepsins. The substrate binding site (the “cleft”) is situated in the middle of the NT and CT lobes, with the catalyzed dyad of asp32/asp228 at the center of the cleft¹⁰¹. A flap or hairpin loop (aa, residues 67–75) is situated at the NT lobe and controls substrate access through conformational variations. The flap of BACE1 was found to implement an open conformation in apo structures (Fig. 10)¹⁰². However, due to the considerable pharmaceutical attention paid to developing BACE1 inhibitors, some structures of complexes of BACE1 crystallized with nonpeptide inhibitors have been identified in recent years, and a diversity of flap conformations has been reported. Numerous side conformations of the conserved flap amino acid residue Y71 and BACE1 crystal structures, particularly self-inhibitory ones, have also been identified^{101,103}. Numerous pharmaceutical industries have developed inhibitors of BACE1 to combat this terrible disease, many of which have proceeded to clinical studies, and inhibition of BACE1 is thus considered a key AD treatment strategy⁹⁸.

Numerous hurdles must be overcome to build BACE1 inhibitors with low unintended consequences. Selectivity is critical in BACE1 inhibition without harming other proteases to avoid off-target side effects¹⁰³. The size of BACE1's active site, which includes catalytic aspartic acid residues, a flap, and a 10S loop, has also been identified as a challenge⁹⁷. Another concern is the potential of these chemicals to cross the BBB. Additionally, several of the discovered BACE1 inhibitors were susceptible to P-glycoprotein (Pgp) efflux, a limiting factor that hampers drug

entrance into the brain even when BBB penetration is achieved¹⁰³.

Despite the challenges above, numerous laboratories have succeeded in generating potent, selective, and orally bioavailable BACE1 inhibitors. In clinical trials, a number of them have displayed hopeful results, but one has reached the FDA approval stage. Clinical trials are currently conducted with several β -secretase inhibitors, including PF-05297909, AZD3293, LY2886721, and MK-8931. In its phase I clinical trial, CTS-21166 proved a dose dependent decline in plasma $A\beta$ levels (Fig. 11)¹⁰⁴. AZD3293 is presently being studied in phase II/III clinical trials to test its disease modifying properties¹⁰⁵. A few of these new classes of BACE1 inhibitors have advanced to clinical development in recent years, including AZD3839 and LY2811376, which have also advanced to phase II a/b clinical development. CoMentis conducted clinical development of CTS21166, which was shown to reduce human plasma $A\beta$. Further structure based design efforts aim to meet varied challenges posed by therapeutic inhibition of the BACE1 target¹⁰⁴. The clinical advancement of γ -secretase modulators for AD treatment is shown in Table 4¹⁰⁵⁻¹¹⁰.

3.3.4. α -Secretase activators

The metalloprotease ADAM10, also known as α -secretase, cleaves APP primarily in the transmembrane domain, inhibiting the formation of $A\beta$ ¹¹¹. Several proteases, including the ADAM family ($A\beta$ disintegrin and metalloprotease) ADAM9, ADAM10, ADAM17, and tumour necrosis factor- α convertase (TACE), meet few of the α -secretase criteria¹¹². Despite amyloidogenic processing, APP is converted by α - and γ -secretase in the non-amyloidogenic pathway. α -Secretase proteolyzes APP at the domain, particularly between K16 and L17. Interestingly, along with the formation of $A\beta$, it elevates the release of NT fragment, sAPP α , a neuroprotective and neurotrophic that enhances long-term potential released by α -secretase cleavage. In AD patients, decreased concentrations of sAPP were reported in CSF^{113,114}. Following the breakage of APP by α - and γ -secretase that cleaves the 83 residues membrane-anchored C-terminal fragment C83, retaining p3 (isoforms $A\beta_{17-40}$ and $A\beta_{17-42}$) and AICD is released¹¹⁵. *In vitro*, p3 isoform $A\beta_{17-42}$ induces neuronal apoptosis, but less potently than $A\beta_{1-42}$ ¹¹⁶. AICD is a second α -secretase cleaved product that may be neuroprotective. Increasing AICD levels improved memory and synaptic plasticity in transgenic mice. AICD is produced by both APP processing pathways (Fig. 12) but mostly by the non-amyloidogenic APP processing pathway. α -Secretase proteolysis of APP stops pathogenic $A\beta$ peptide formation and releases neuroprotective APP cleavage products. This suggests that this enzyme activity may protect healthy people against AD and prevent the generation of $A\beta$ aggregates and plaques^{117,118}. α -Secretase appears to be specific for various peptidic bonds, not only the scissile bond. The enzyme's proximity to the membrane appears to be important, as 12 and 13 residues of the N-terminal to the membrane are cleaved by α -secretase¹⁰⁴.

α -Secretases can cleave APP depending on APP's position on the plasma membrane, the site of processing (endosomes or membrane), and the pH of the environment, resulting in non-amyloidogenic compounds¹¹⁹. Protein kinase C (PKC) can be stimulated to increase α -secretase, which has been shown to prevent the formation of $A\beta_{42}$ in test animals. An agonist of the M1 receptor has been shown to elevate APPs' non-amyloidogenic proteolysis and to reduce $A\beta_{42}$ levels. Activation of the α -secretase, specifically ADAM-10, may be more advantageous than other targets for treating AD because it inhibits $A\beta$ peptide formation¹¹².

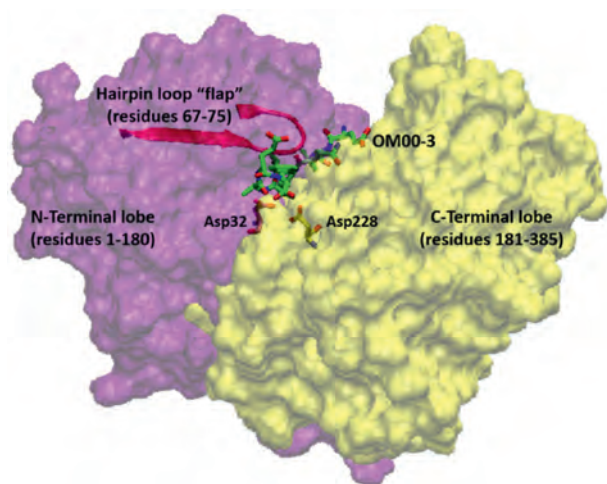


Figure 10 Human BACE1's three-dimensional structure (PDB ID: 1FKN). The NT lobe is magenta in colour, whereas the CT lobe is green. The active site is shared by the co-crystallized inhibitor OM00-3 and is located in the centre of the two lobes⁷⁴. Reprinted with the permission from Ref. 74. Copyright © 2020 The authors.

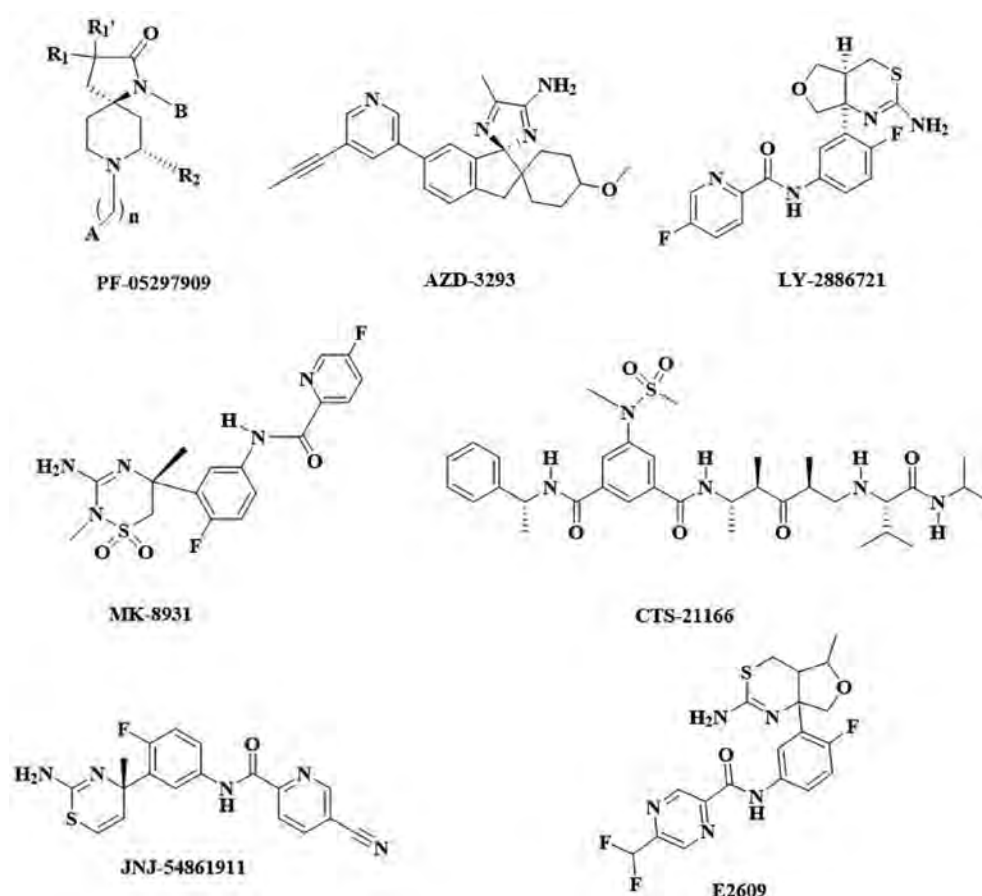


Figure 11 The structures of β -secretase inhibitors.

3.4. Tau targeting therapy

Neuropathologically, AD is described as the occurrence of neurofibrillary lesions in the intraneuronal space constituted of tau proteins¹²¹. Tau, a microtubule associated protein, polymerizes tubulin into microtubules and assists in maintaining complex neuronal cell microarchitectures, such as microtubule formation and stabilization, mainly in the axon. The MAPT gene transcripts

express six main tau splicing isoforms from exons 2, 3, and 10¹²². In the NT projection domain of tau, exons 2 and 3 express twenty-nine residue acidic inserts, while exon 10 encodes a 31 residue microtubule attaching repeat in the CT domain. Microtubule binding repeats of 352–441 amino acids are found in whole tau isoforms. Tau is a phosphoprotein whose activity is controlled by phosphorylation. Its isoforms are susceptible to disorder in aqueous solutions because of their high polar, glycine, proline, and

Table 4 β -Secretase inhibitors in clinical advancement for AD treatment (chemical structures shown in Fig. 11).

Sr. No.	Name of compound	Pros	Cons	Status of compound	Ref.
1	PF-05297909	Well tolerated. Reduction in plasma A β (A β 40 and A β 42)	No functional or cognitive progress; Well tolerated and more side effects	Discontinued in 2018	106,107
2	AZD 3293	Reduction of CSF A β	No functional or cognitive progress	Discontinued status for AD	105
3	LY2886721	Potent small molecule, active site inhibitor of BACE1	Rat toxicology; Pigment epithelium of the eye	Terminated in 2013	108
4	MK-8931	Not any significant outcome	No functional or cognitive progress; More side effects	Discontinued	109
5	CTS21166	Reduction in plasma A β and plaque load	Reduces central penetration/exposure	Terminated in 2014	107
6	JNJ-54861911	Reduction of CSF A β concentrations by 80%	Worse cognition; More adverse effects (hepatotoxicity)	NCT02406027 (II) NCT02569398(II/III)	110
7	E2609	Dose dependent reduction in A β 40 and A β 42 Guinea pigs in levels in brain and CSF of SD rats	Abnormal liver biochemical tests.	Terminated phase II clinical trial	107

BACE1, β -site APP cleavage; CSF, cerebrospinal fluid; SD, Sprague–Dawley.

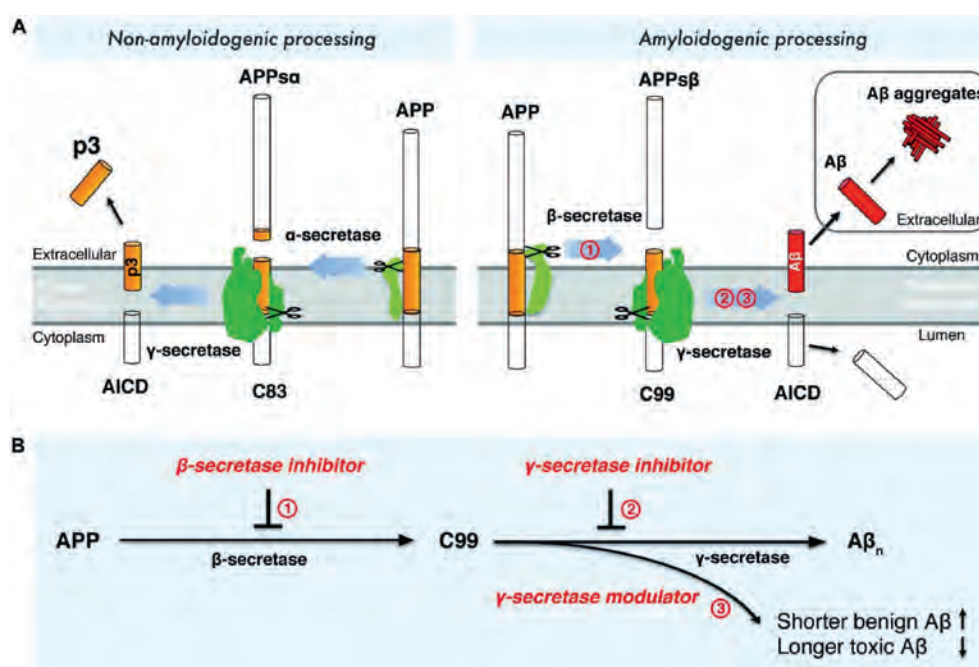


Figure 12 APP is cleaved by either α - or β -secretase in the non-amyloidogenic and amyloidogenic pathways. The non-amyloidogenic pathway generates sAPP- α and C83 (left). The amyloidogenic pathway produces sAPP- β and C99 (right). Gamma-secretase cleaves C99 to produce AICD and A β (right)¹²⁰. Reproduced integrally with permission from Ref. 120. Copyright © 2011 The authors.

low hydrophobic content. Some of them have cysteine residues, which can react with oxygen and produce adsorbed molecules. Solvent exposed hydrophobic orders create the vital areas of filamentous clumps in tauopathies¹²³. When the control of kinases and phosphatases are imbalanced, that disconnects the aggregated tau from microtubules¹²⁴. It can also impact the plasma membrane and microtubules in neurons. Moreover, tau increases neuronal development and synaptic function in synapses and dendrites¹²⁵. Alterations in synaptic supply and disturbance of synaptic protein interactions can impair neuronal activity and cause AD. If the abnormal inclusions occur in neuronal cell bodies, they are known as NFTs and threads if they are produced in dendrites or axons. This research suggested that AD could be caused by tau mis-sorting⁴³.

The aberrant tau hyperphosphorylation is distinct from typical and temporary tau hyperphosphorylation in development, anaesthesia, and hypothermia. ADP-tau is sedimentable/oligomeric and likely promotes neurodegeneration by sequestering normal microtubule associated proteins and disturbing the microtubule network. In frontotemporal dementia, tau abnormalities may induce neurodegeneration by increasing aberrant tau hyperphosphorylation. Paired helical or straight filament (PHF/SF) creates neurofibrillary tangles from AD P-tau. Tau truncation in the AD brain enhances PHF/SF self-assembly and does not sequester MAPs or damage microtubules like AD P-tau. Thus, preventing aberrant tau hyperphosphorylation may be a promising treatment for AD and tauopathies (Fig. 13)^{126,127}.

3.4.1. Development of tau directed therapies

Several target pathways could be imagined based on tau biology and its role in neurodegeneration. Post translational tau changes could be addressed by blocking kinases like glycogen synthase kinase-3 β (GSK3 β) and protein phosphatase (PP2A) or altering phosphorylation and tau aggregation is another objective. Another

target might be total tau or pathologically mutated tau species. Few drugs that target tau or its principal target, microtubules, have made it to clinical trials. TRx0237 (modified methylene blue, Rember) is a tau aggregation inhibitor, BMS-241027 (epothilone D) is a microtubule stabilizer, and medications to lower tau protein or phospho-tau are available (davunetide, sGC-1061, immunization approaches).

Molecules that reduce the load of p-Tau protein are also in development, which is supposed to be the direct cause of symptoms in AD. In animal studies, many tau vaccinations have demonstrated efficacy and safety. The drug that was tested on mice showed an excellent safety profile and even motivated a positive immune reaction in a human patient¹²⁷. Microtubule associated tau has undergone many post translational modifications in several neurodegenerative complications, including AD. These changes are strongly linked to tau aggregation in AD. Tau hyperphosphorylation, truncation, glycosylation, glycation, nitration, and ubiquitination are among the post-translational modifications identified in tau¹²⁸.

Several therapies and drugs targeted other diseases that affect tau, such as propylthiouracil (PTU) (Fig. 14), an antithyroid molecule that causes tau phosphorylation in AD patients by boosting pro-inflammatory cytokines. Radiotherapy was used to treat numerous types of brain cancers, can expose the brain to ionizing radiation. In AD patients, radiation exposure to a 0.5 or 2 Gy beam causes amplification of tau phosphorylation. Simultaneously, oxidative stress, a factor from ionizing radiation, entices the same progression¹²². Clinical trials for AADvac1 (liposomal based vaccination) have commenced, while trials for ACI-35 (liposomal-based vaccine) have also begun. This treatment aims to prevent clusters of paired, helically twisted strands of hyperphosphorylated tau from forming in neurofibrillary tangles. There has been research into tau protein phosphorylation inhibitors, such as tideglusib (Fig. 14), an irreversible GSK

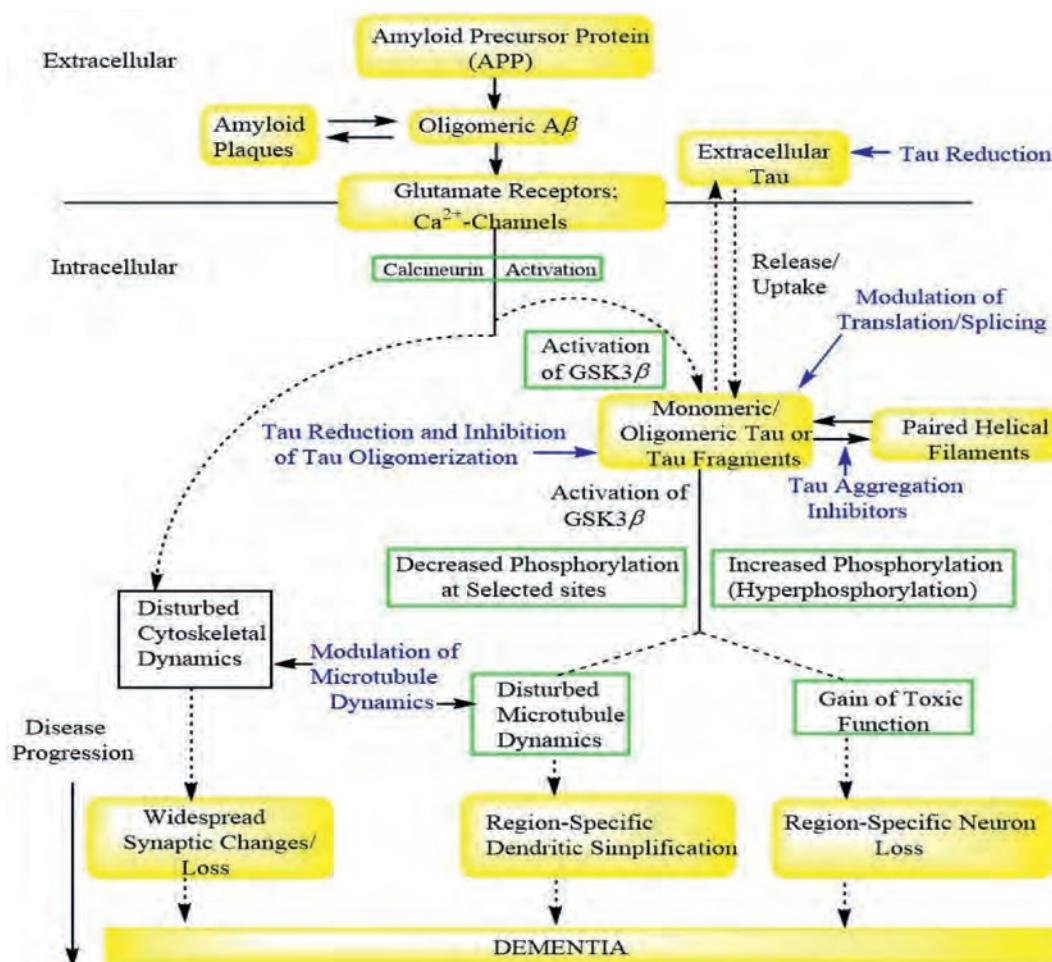


Figure 13 The neurodegenerative triad and the altered amyloid cascade hypothesis in AD. Oligomeric A β causes the neurodegenerative triad of synaptic alterations, dendritic simplicity, and neuron death through tau-dependent and tau-independent pathways. The locations of potential tau disease-targeted therapies are highlighted in blue. For more information, see the text. GSK3 β stands for glycogen synthase kinase 3 β ¹²⁷. Reprinted with permission from Ref. 127. Copyright © 2016 The authors.

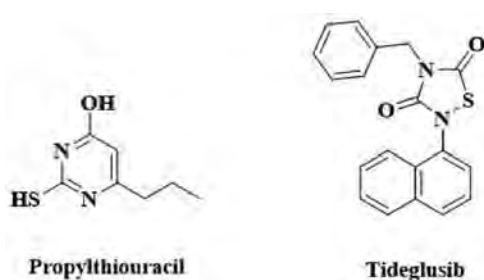


Figure 14 Structures of anti-thyroid drug and an irreversible GSK inhibitor respectively.

inhibitor, but no statistically meaningful benefits have been discovered yet. Other promising inhibitors include *N*-phenylamines, anthraquinones, phenyl thiazolyl-hydrazides, rhodamine, phenothiazines, and benzothiazoles¹²⁹.

3.4.1.1. Kinase inhibitors. The hyperphosphorylation of tau by protein kinase is required for its toxicity; however, kinases play a vital part in regulating cell activity and maintaining a healthy

physiological state. A tau targeted treatment is difficult to create since kinase interactions are redundant, and it is unclear which enzyme catalyzes the phosphorylation¹³⁰.

The first family of tau inhibitors lowers associated kinase activity since an imbalance between GSK3 β and PP2A promotes Tau hyperphosphorylation and NFT formation. This is supported by GSK3 β 's influence on cellular signalling and gene transcription¹³¹. GSK3 is a ubiquitously expressed, constitutively active serine/threonine kinase involved in various physiological activities, including gene transcription and glycogen metabolism. GSK-3 interacts with several parts of the amyloid system that form plaques and take part in the phosphorylation of Tau. This microtubule binding protein helps generate neurofibrillary tangles and affects presenilin and other AD associated proteins. Mammals have two closely related GSK3 isoforms, GSK-3 α and β , with similar biochemical properties and 98% homology in their catalytic domains. The catalytic domains of the isoforms are similar, but their N-terminal sections differ dramatically¹³².

According to studies, GSK3 α , but not GSK3 β , has been shown to restrict APP cleavage, leading to an increase in the production of A β . Neuronal exposure to A β increases GSK3 β activity by inhibiting PI3 kinase signalling. Blocking either GSK3 β expression or activity

prevents A β induced neurodegeneration. Increased GSK3 activity would serve to enhance A β production and in turn, tau hyperphosphorylation and neuronal degeneration in both FAD and sporadic instances, following the amyloid cascade hypothesis of AD, even though it is not the major cause of illness in this scenario¹³³.

A polymorphism in the GSK3 promoter has recently been linked to a risk factor for late onset AD, which may explain changes in GSK3 expression in illness. However, it is acknowledged that there is currently little direct evidence for this and that some research shows no alteration in GSK3 activity or reduced GSK3 activity. These findings collectively suggest that GSK3 activity might be increased in AD through changes in its phosphorylation state in addition to expression levels¹³³. GSK3 β is newly discovered and is responsible for 31 and 16 pathogenic tau phosphorylation sites and colocalized with NFTs in the post-mortem brain¹³⁴. Toxic A β enhances GSK3 β activity, implying that GSK3 β is a possible therapeutic target. The search for GSK-3 inhibitors is a particularly active area in both academic institutions and pharmaceutical firms¹³⁵.

In preclinical studies, a non-ATP competitive GSK3 β inhibitor NP-031112 (NP-12) reduces tau phosphorylation and the amyloid burden, thus preventing cell death and improving spatial memory¹³⁶. Several GSK3 inhibitors, including cations like lithium or small compounds have been studied as a promising strategy for treating AD. Lithium lowered tau phosphorylation and restored tauopathy in animal models but not in Alzheimer's patients¹³⁷.

Organic GSK-3 inhibitors of both natural and synthetic origin are highly varied in structure and span a variety of chemical regions. Most of the effects seen are from *in vitro* and cellular research. SB216763 corrects responses such as GSK3 activity and is connected with elevations in amounts of p-Tau, caspase-3, neuronal DNA fragmentation, the tau kinase phospho-c-jun N-terminal kinase (pJNK), and gliosis. In contrast, a single administration was linked with the induction of neurodegenerative markers and behavioural deficits. A negative result ended the phase 2b trial, and some GSK3 inhibitors from the paullone, indirubin, and maleimide families are in the process but are hampered by concerns about cytotoxicity¹³⁸. The maleimide compounds SB415286 and SB216763 (Fig. 15) are neuroprotective but might not specifically target cyclic dependent kinases (CDKs); they decreased GSK3 activity by battling for the ATP binding site. The thiadiazolidinones are ATP non-

competitive, albeit these findings need to be confirmed. Most recently, AstraZeneca revealed a powerful and selective inhibitor of GSK3 (AR-A014418) that is effective against cdk2, cdk5, and additional kinases tested¹³⁹.

CDK5 is another kinase linked to tau disease. Pathological tau phosphorylation is caused by the CDK5 regulatory protein present in the AD brain. Preclinical CDK5 selective inhibitors have been shown to permeate BBB and thus lower the increased A β levels by controlling CDK5. Insufficient efficacy or significant side effects led to the discontinuation of trials for other medicines targeting different protein kinases¹⁴⁰.

3.4.1.2. Tau aggregation inhibitors. Prevention of tau aggregation or promoting tau assembly disassociation are two alternative options. Preclinical evidence showed that Rember (methylene blue) can reverse learning deficits, and a phase II trial showed reduced AD progression with high bioavailability. Also known as leucomethylthionium (TRx0237), it has increased absorption, bioavailability, and tolerance. TRx0237 has been extensively studied since 2008 with evidence that it increases neuroprotection, A β clearance in transgenic mice, and spatial learning in rats. Few literatures indicated the anti-aggregation characteristics, and phase III investigations are still ongoing⁶. Phase II/III RCT enrolling persons with dementia are in the evaluation on six month regimen of 4 mg TRx0237 (Fig. 16) twice daily to a placebo¹³⁰.

Most tau stabilizers (*e.g.*, paclitaxel and epothilone D) (Fig. 16) had hazardous adverse effects. TPI 287 has shown promising results in mild to moderate AD patients, chronic supranuclear palsy, and corticobasal syndrome, with positive effects on cognitive function and/or nerve cell activity. In a mouse model, nicotinamide lowers phosphorylated tau and protects microtubule stability¹⁴¹. Phase II clinical trial is in progress in people with mild-to-moderate AD.

3.5. Neurotransmitter related putative therapies

Depletion of acetylcholine and synaptic impairment are two hallmarks of AD. Consequently, two hypotheses were proved: cholinergic and glutamatergic. FDA approved therapies, AChE inhibitors, and NMDA receptor antagonists were created to alleviate AD symptoms. Even though medications that regulate transmitter

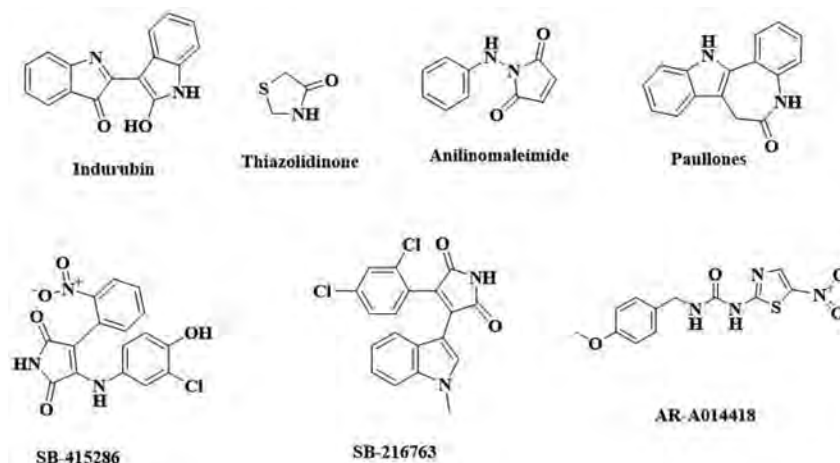


Figure 15 The structures of GSK3 inhibitors which has extended clinical trials.

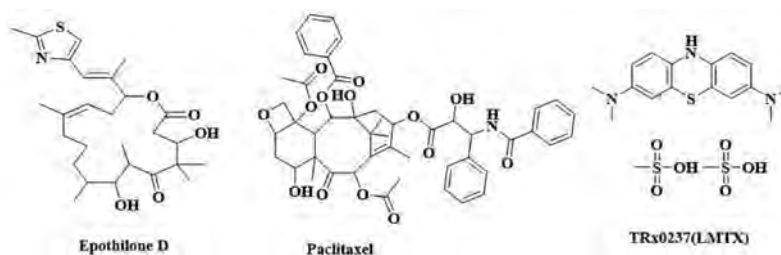


Figure 16 Structures of tau aggregation inhibitors (TRx0237) and tau stabilizers (Paclitaxel and epothilone D).

generation, release, and recycling cannot halt AD progression, the quest for new receptor agonists and antagonists continues¹⁰.

The cholinergic impairment changes cognitive and neuropsychiatric disorders in AD patients. Acetylcholine, a key neurotransmitter, is degraded by acetylcholinesterase and butyrylcholinesterase (BuChE). The main therapeutic focus of cholinesterase inhibitors (ChEI) therapy for AD has been AChE inhibition. AChE positive neurons modulate cortical processing and reactions to novel inputs. These neurons may be important in attention, executive function, emotional memory, and behaviour. Inhibiting BuChE may thus provide further benefits. The substrate selectivity, expression, enzyme kinetics, activity in multiple brain regions, and gene regulation complex of the two enzymes are significantly different. According to a new study, AChE and BuChE play roles other than co-regulatory esterase actions at the end of ACh-mediated neural transmission¹⁴².

3.5.1. Cholinesterase inhibitors

For more than a quarter century, AD pathogenesis has been connected to a lack of the brain cholinergic neurotransmitter ACh,

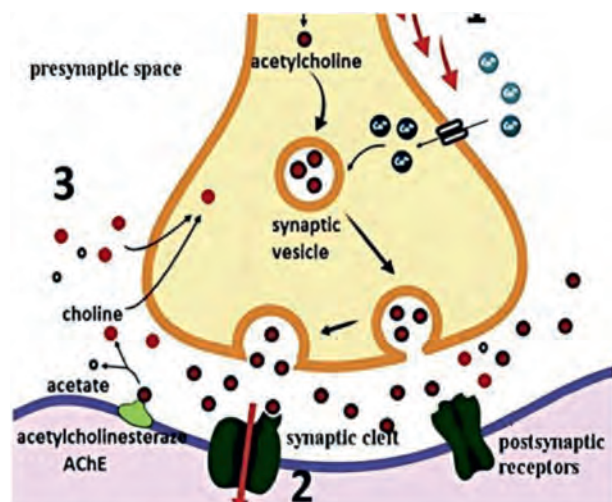


Figure 17 The acetylcholine release cycle and cholinergic hypothesis of AD are depicted schematically. AChE catalyses the breakdown of acetylcholine, and choline molecules are taken up by the presynaptic neuron. (1) Action potential causes Ca²⁺ influx and subsequent membrane docking of synaptic vesicles. (2) Acetylcholine binds to receptors and initiates a graded depolarization in the postsynaptic cell. (3) Acetyl-CoA is acetyl coenzyme A; ACh is acetylcholine; and AChE is acetylcholinesterase¹⁴⁶. Reproduced integrally with permission from Ref.146. Copyright © 2019 The authors.

based on research that linked cholinergic system problems to cognitive decline¹⁴³. The formation and degradation of ACh is schematically portrayed in Fig. 17. As a result, one potential therapeutic approach is to reduce acetylcholinesterase's biological function to increase cholinergic levels in the brain. AChEIs are used to limit ACh breakdown, which can improve brain cell function by boosting AChE concentration¹⁴⁴.

AChE hydrolyses ACh, releasing choline and acetate ions. It possesses a big hydrophobic cavity along with an esteratic subsite and an anionic substrate binding site. Also, other cationic substrates and inhibitors can be bound by the active site. Ser200, Glu327, and His440 comprise the ES catalytic triad. Approximately 20 from the enzyme surface, at the end of a gorge that deepens towards the bottom. Ser200 is involved in proton transfer hydrolysis of choline esters. A cation interaction occurs between quaternary ammonium of ACh and aromatic amino acid. The AS of Torpedo California AChE (TcAChE), a prototype ACh binding protein, contains many aromatic residues (14 amino acids). When Trp84 is substituted for alanine, the reactivity of the AChE is reduced by 3000-fold. AChE also has an acyl pocket that specifies substrates and an oxyanion hole that interacts with negative oxygen ions throughout catalysis, increasing the efficiency of AChE^{145,146}.

AChE hydrolyses 90% of ACh in the normal human brain and abandons its function further; BuChE plays a minor role. AChE levels drop in AD patients, whereas BuChE levels rise in some brain areas¹⁴². Recent research suggests that selective or nonselective inhibition of BuChE may have neuroprotective and disease modifying effects. These include indolinone, coumarin-3-carboxamides with *N*-benzyl piperidine moiety, coumarin-3-carboxamides with tryptamine moiety, 7-hydroxycoumarin derivatives, tacrine analogues and indole based hydrazide-hydrazone derivatives¹⁴⁷.

The three drugs include inhibitors of AChE exemplified by donepezil, rivastigmine, and galantamine are in clinical use (Fig. 1). However, these medications have limited efficacy and have exhibited varied dose related adverse effects, especially at greater doses¹⁴⁸. Galantamine and donepezil suppress AChE, while rivastigmine inhibits AChE and BuChE. The damage of cholinergic neurons in the CNS and the loss of nerve transmission are the primary reasons for cognitive function impairment in AD patients¹⁴⁹. Another important element in AD pathogenesis is the increased synthesis and accumulation of amyloid¹⁵⁰. As a result, dual inhibitors based on tacrine hybrids¹⁵¹ and donepezil is being designed to decrease both AChE activity and A β accumulation. Numerous AChE and A β cleaving enzyme 1 dual inhibitors have been created using computational techniques¹⁵².

Early dementia is accompanied by cholinergic neuron loss, so both preclinical and clinical investigations revealed that

cholinesterase inhibitors improved memory and learning¹⁵³. Later, in animal models, a strong link between weak cholinergic neuronal activity and memory loss was established¹⁵⁴. Thus, improving the cholinergic system, including binding acetylcholine nicotinic receptors, excites the postsynaptic neuron, which is crucial for long term potentiation (LTP) and memory development. After completing the phase I/II trials that demonstrated safety and well tolerance, EVP-6124 was recently studied in the phase III trials to assess cognitive aids. Also, some trials with nicotinic agonists are in various stages of clinical trials (ladostigil hemitartrate, phase II), including RO5313534 or Pozanicline (ABT-089) (Fig. 18)³⁵. Serotonin (5-HT) is a neurotransmitter affecting cell death and memory loss. Memory and learning deficits could be improved by increasing cholinergic transmission by inhibiting the 5-HT6 receptor. 5-HT6 antagonists have been shown to reverse anticholinergic drug-induced amnesia^{10,155}. Two HT antagonists, PRX-03140 (5-HT4 antagonist) and Intepirdine (SB-742457; 5-HT6 antagonist) have been evaluated in clinical phase II trials. Idalopirdine (Lu AE58054; 5-HT6) has newly entered phase III evaluation consisting of 930 mild to moderate Alzheimer's patients¹⁰.

3.6. N-methyl-D-aspartate receptor (NMDAR) antagonist

NMDAR antagonists pose a greater therapeutic potential in a variety of CNS illnesses. When administered at levels within their presumed therapeutic range, several NMDA receptor antagonists generated highly potential effects. As a result, it has been concluded that NMDAR antagonism is a viable treatment strategy¹⁵⁶. The excitatory glutamatergic nerve transmission is regulated by the NMDAR which is required for synapse formation and cell survival. However, elevated NMDAR activity results in excitotoxicity and cell death thus implicating a probable nerve degenerative process in AD. The huge number of excitatory neurotransmissions in the human CNS are mediated by glutamate and its receptors, especially ligand gated ionotropic glutamate receptors (iGluRs). iGluRs perform critical roles in synaptic plasticity, the chemical mechanism governing learning and memory. Disruption of normal signaling via iGluR has been connected to a variety of neuropathological illnesses and diseases. These conditions include epilepsy and brain injury, AD, PD, Huntington's disease, and multiple sclerosis¹⁵⁷.

3.6.1. NMDAR subtypes linkage to neurological disorders

It is now more evident that NMDAR hyperactivity or hypo-function can have negative repercussions. Changes in NMDAR

presence/function can add value to CNS disease in several ways: their over activation can induce neuronal death, as in stroke and possibly in Huntington's disease, or their inhibition can alter the balance of inhibition and excitation in neural circuitry, influencing CNS functions, as is likely in schizophrenia. For example, improved NMDAR action on excitatory neurons may result in improved synaptic plasticity of excitatory neurons, whereas improving NMDAR function on inhibitory neurons is likely to increase inhibition (reducing excitation as a result) and decrease in excitatory neurons' synaptic plasticity as a result. Accordingly, alterations in NMDAR expression or activity, depending on their locus, can influence the ratio of excitation to inhibition, impairing circuit and brain function¹⁵⁸.

The membrane topology of all ionotropic glutamate receptor subunits, such as the seven GluNs, is defined by three transmembrane parts (M1, M3, and M4) and a re-entrant pore loop (M2). The long N-terminus is extracellular, while the C-terminus is intracellular and engages with a variety of cytosolic proteins. Glutamate binds to GluN2 subunits in an NMDAR binding pocket formed by two regions in the proximal N-terminal domain and the lengthy extracellular loop among both M3 and M4 (S1 and S2, respectively). The re-entrant M2 loop is a component of the channel pore and contains a crucial asparagine residue that defines the channel's calcium permeability and facilitates the magnesium blockade¹⁵⁹. There are seven NMDAR subunits namely, NR1, four NR2 (A, B, C, and D), and NR3A and NR3B. The NR1 subunit has eight splice variants, as do the NR2 and NR3 subunits (excluding NR2A). Generally, NMDARs are hetero tetramers with two NR1 and two NR2 subunits each and each receptor of NR1–NR2 dimer is considered the basic useful structure. NR3A can form a receptor complex with NR1/NR2, also some NMDARs are heterotrimers containing two NR1 and two NR2 subunits¹⁶⁰. NR3A protein expression drops from neonatal week 7 to week 21, while NR3B protein expression increases and NR3A was found to be low while NR3B was reported high in adults. In humans, NR1 expression begins to rise during pregnancy and then plateaus until puberty. NR3A levels are low during the embryonic day, quickly surge post-birth and then slowly decline (Fig. 19)^{161,162}.

The majority of NMDARs have receptors for GluN1, GluN2B, GluN2A, or a combination of the two. NMDAR subunit expression varies throughout the brain and changes dramatically during its development. In the temporal area of the cerebral cortex and the hippocampus of the embryonic brain, these genes are expressed mildly, but they are widely expressed in the neonatal brain. Following that, it gradually declines to adult levels, reaching adult levels by the third postnatal week. Glutamate overstimulation of NMDA receptors has the potential to cause neuronal depolarization and Ca^{+2} influx. Several Ca^{+2} dependent enzymes are induced by intracellular Ca^{+2} over accumulation and these enzymes have the potential to harm and kill neurons (excitotoxicity).

As a successful method to slow the detrimental effects of excitotoxicity in the evolution of several disorders, including AD, ischemic stroke, neuropathic pain, PD, and depression, selective antagonism of GluN2B subunit-containing NMDA receptors has developed¹⁶³. In the fatal brain, inside the hippocampus and temporal portion of the cerebral cortex, the NR2B subunit is barely expressed¹⁵⁹. In adult granule cells that integrate into circuits with strong and linked synaptic activity, GluN2B-containing NMDARs encourage synapse activation. Thus, depending on the environment, GluN2B-containing NMDARs can have a bidirectional effect on synapse formation¹⁶⁴. Ifenprodil (Fig. 20) is a brand-new NMDA receptor antagonist that specifically inhibits NR2B

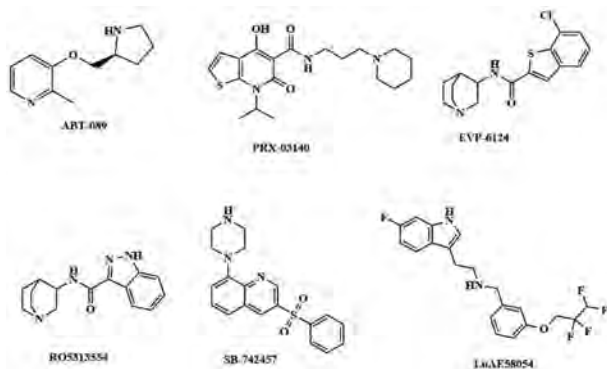


Figure 18 The structures of cholinesterase inhibitors which has extended clinical trials.

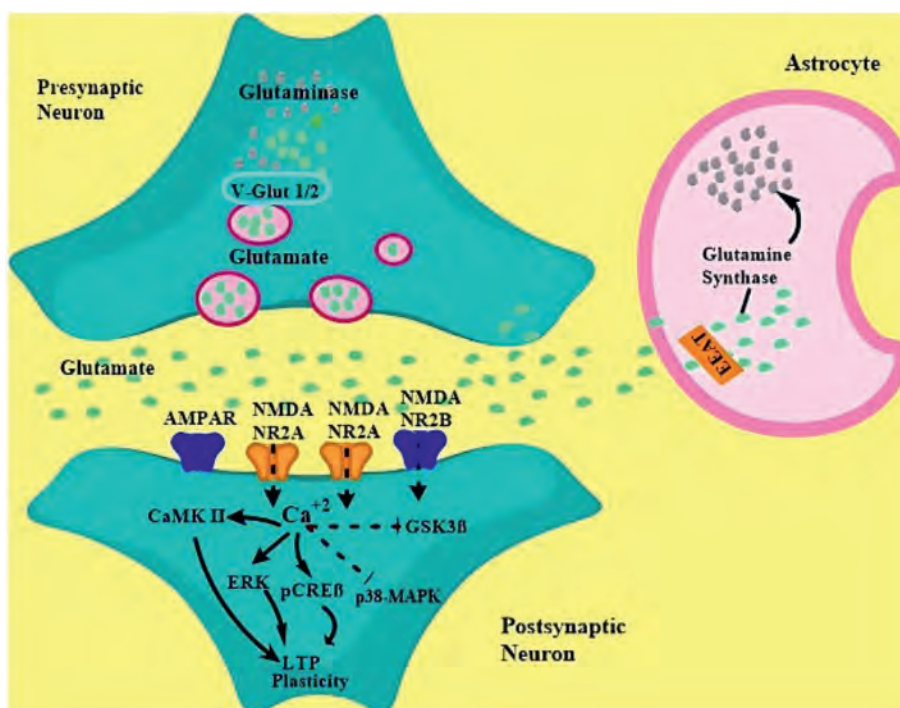


Figure 19 Transmission of glutamates in a healthy brain. By activating the postsynaptic terminal glutamate receptors, glutamate released from presynaptic terminals has an effect. Multiple metabolic pathways, specifically CaMK, ERK, and CREB, which are in charge of anabolic activation and long-term potentiation (LTP) process activation, are promoted by the interaction among glutamate and the NMDA receptor. Excess glutamate is carried into astrocytes by the excitatory amino acid transporter (EAAT) and converted to glutamine there by the glutamine synthase. Glutaminase then transforms glutamine into glutamate, which is then packaged into vesicles using certain transporters (VGlut). Vesicular glutamate transporter (VGlut); excitatory amino acid transporter (EAAT); *N*-methyl-D-aspartate NR2A and NR2B subunits (NMDANR2A and NMDANR2B subunits); extracellular signal-related kinase (ERK); calcium calmodulin-dependent kinase II (CaMKII); phosphorylated cyclic AMP response element binding protein (pCREB); p38-mitogen activated protein kinase (p38-MAPK)¹⁶². Reprinted with the permission from Ref. 162. Copyright © 2014 copyright holder.

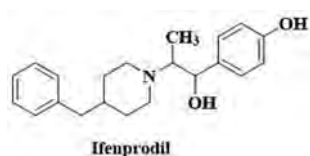


Figure 20 Structure of a brand-new NMDA receptor antagonist.

subunit containing receptors. As a result, it has found widespread usage as a tool for molecular investigations of the characteristics and control of NMDA receptors, as well as a method for studying NMDA receptor subtypes both *in vitro* and *in vivo*. Ifenprodil's mode of action may entail a rise in proton inhibition of NMDA receptors and exhibit an uncommon type of activity dependence. Analogues or derivatives of ifenprodil, some of which may be lead substances for therapeutically effective NMDA antagonists, possess several characteristics. Such antagonists can be used as analgesics, neuroprotectants, anticonvulsants, and for treating Parkinson's disease and other nervous system illnesses.

Some of the mutations have been reported in NMDARs; it is tacit that the mutation of the postsynaptic NR2B subunit is linked to alterations in synaptic architecture. Numerous coding variations on the risk haplotype encoding *rs1806201* may have a part in the susceptibility to AD. An increased frequency of the Ht2-AG haplotype

in AD patients revealed that the GRIN3A mutation may be a risk factor for AD^{165,166}. People with insCGTT, an NMDAR NR3B subunit mutation that adds four bases to the coding area, are predisposed to schizotypal personality features¹⁶⁷. The p. N615K mutation in GRIN2A deletion mutation in a little girl with epileptic encephalopathy reduced Ca^{2+} permeability in NR1–NR2A receptor currents. The NR2 subunit of NMDARs' electrical equilibrium is disrupted, resulting in various neuropathologies¹⁶⁵.

A study found that regulating NMDA receptors reduces glutamate-induced excitotoxicity and improves AD symptoms. This novel neurochemical technique differs from the cholinomimetic process employed in all currently approved AD treatments¹⁶⁷. NMDAR antagonists approved for the treatment of moderate to severe AD in the United States and Europe may have the potential to alleviate other neurological conditions such as vascular dementia and Parkinson's disease. Memantine has been shown in animal models to be a neuroprotective agent that improves both vascular and neurodegenerative mechanisms. While high glutamate levels cause neurotoxicity due to the overactivation of NMDARs, memantine, as a partial NMDAR antagonist, inhibits the NMDA glutamate receptors to normalize the glutamatergic system and enhance cognitive and memory deficits¹⁶⁸. A therapeutic approach with high affinity antagonists of NMDA receptor like MK-801 and phencyclidine is impractical because of unwanted effects; however, memantine, a low, moderate affinity,

non-competitive, and strongly voltage dependent NMDA receptor antagonist, is well tolerated and was finally approved by the US Food and therapies for the cure of moderation¹⁶⁹.

3.7. Triggering receptor expressed on myeloid cells 2

TREM2 is an immunoglobulin superfamily transmembrane receptor that binds lipids, lipoproteins (apoE, apoJ, apoAI, apoAII), and ligands linked to damage or pathogen-related molecular patterns (*e.g.*, lipopolysaccharides, bacteria)¹⁷⁰. It has an extracellular domain, transmembrane part, and cytoplasmic tail and is encoded by a gene on chromosome 6p21.1. *TREM2* gene mutations and polymorphisms have been related to an elevated risk of AD^{171,172}. Unusual *TREM2* gene mutations have been shown to raise an individual's risk of AD by up to thrice¹⁷³. Polycystic lipo membranous osteodysplasia with sclerosing leukoencephalopathy (PLOS; also known as Nasu-Hakola disease NHD) and frontotemporal dementia are caused by an unusual biallelic gene mutation¹⁷⁴. *TREM2*'s basic roles in the brain must be better understood before new therapeutic targets may be discovered.

3.7.1. *TREM2* variants and risk for AD

TREM2 has 46 genetic variants that have been linked to AD, out of these genetic variants, p. Arg47His (rs75932628), has been demonstrated to double or triple the risk of AD in numerous European and North American populations¹⁷⁵. A proxy of rs75932628 was discovered to be associated with AD in black patients¹⁷³ and pArg47His was not observed in late onset AD patients or healthy controls in Chinese cohorts^{176,177}. No such link

was discovered in the Iranian¹⁷⁸ or Japanese population¹⁷⁹, indicating that *TREM2* is demographic specific. Recent studies have related AD to polymorphisms in microglia expressed genes such as *TREM2*³⁷, *CD33*¹⁸⁰ and *CR*¹⁸¹. The R47H missense mutation is encoded by the rs75932628T polymorphism in *TREM2*, which carries the highest risk. *TREM2* deficits in neurological illness models like cuprizone-induced demyelination¹⁸².

3.7.2. *TREM* locus and *TREM2* expression

TREM2 is situated within a gene cluster on chromosome 6p21.1, in proximity to *TREM1*, triggering receptors expressed on myeloid cells like *TREML1*, *TREML2*, *TREML3P*, *TREML4*, and natural cytotoxicity triggering receptor 2 (*NCR2*) genes. The genes in this cluster share a lot of similarities and are primarily immunological¹⁷³. *TREM2* is an immunoglobulin superfamily transmembrane receptor expressed in myeloid cells such as microglia and osteoclasts that modulates the immune system¹⁸³. *TREM2* transcripts have the greatest signal in the basal ganglia, spinal cord, medulla oblongata, and corpus callosum. *TREM2* interacts with anionic ligands such as bacterial lipopolysaccharides, DNA, and phospholipids¹⁸⁴.

Ligand binding to *TREM2* initiates a signalling cascade that phosphorylates the *TREM2*-associated intracellular adaptor TYROBP. Phosphorylated TYROBP binds to a tyrosine kinase found in the spleen (SYK), which further activates phosphatidylinositol 3 kinases (PI3K), AKT serine/threonine-kinase (AKT), mitogen activated protein kinase (MAPK), Ca^{2+} mobilization and other downstream substrates¹⁸³. The proliferation and differentiation of cells are regulated by these mechanisms (Fig. 21)^{174,183}.

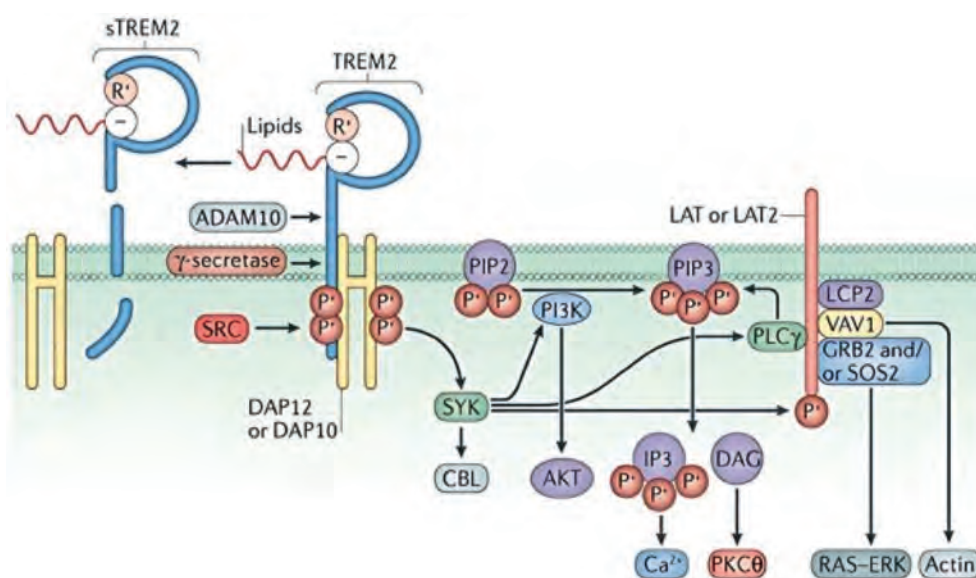


Figure 21 *TREM2* signalling pathways. *TREM2* binds positively charged arginine residues in its extracellular domain (R+) to negatively charged lipid ligands. The protein kinase SRC tyrosine phosphorylates the *TREM2*-associated adaptor DAP12 upon ligand binding, recruiting the tyrosine protein kinase SYK. Signalling proteins include phospholipase C (PLC), lymphocyte cytosolic protein 2 (LCP2; also called as SLP76), protooncogene vav (VAV1), growth factor receptor-bound protein 2 (GRB2), and others. All of these pathways result in the stimulation of protein kinase C (PKC), activation of the RAS-ERK pathway, and actin remodelling. SYK also stimulates the PI3K–AKT signalling pathway and CBL, an E3 ubiquitin-protein ligase that inhibits the *TREM2* signalling pathway. *TREM2* is also linked to DAP10, a PI3K attractant and activator. *TREM2* is released from the cell surface by secretase, disintegrin, and metalloproteinase domain-containing protein 10 (ADAM10) (s*TREM2*)¹⁸³. Reprinted with the permission from Ref. 183. Copyright © 2016 Macmillan Publishers Limited.

TREM2 signalling has been found to reduce toll like receptor responsiveness in dendritic cells¹⁸⁵.

3.7.3. *TREM2 cleavage and sTREM2*

TREM2 can be sequentially proteolyzed, after the p. H157Y residue. α -Secretases and metalloproteinase (ADAM10 and ADAM17) cleave the protein. Thus releasing the ectodomain and soluble TREM2 followed by α -secretase mediated cleavage of the C-terminus^{186,187}.

3.7.3.1. *TREM2 and AD.* TREM2 inactivating mutations were originally discovered in Nasu–Hakola disease (NHD) patients, this condition causes PLOSL in the brain and bones^{188,189}. Given that microglia and osteoclasts express TREM2 signifying that these cells need TREM2 to function effectively in the CNS and bones. A low frequency TREM2 variation was recently revealed as a genetic problem for nonfamilial AD in genome wide association studies (GWAS). In two huge patient cohorts, the mutation has generated an extracellular Ig domain arginine-47-histidine (p.R47H) substitution which increased the prevalence of AD¹⁷¹.

The discovery of a TREM2 variation as an AD risk element confirmed the long-held idea that changed microglial activity contributes to the disorder's etiology¹⁸². GWAS have found uncommon variations of immune receptor genes produced by microglia as risk elements for AD. Some examples are the inhibitory receptor myeloid cell surface antigen (CD33)¹⁸⁹ and another system method identified immune-related gene networks as AD regulators. The function of TREM2, its adaptor DAP12, and the downstream signalling pathway was emphasized¹⁹⁰.

Discovery of the R47H variant linked to AD has inspired comprehensive studies of TREM2 polymorphisms in the human population that disclosed the less common variants including the D87N substitution (rs142232675) in human population¹⁹¹. Additional TREM family receptor variants, including TREM and TREM like transcript protein 2 (TREML2) have been linked to AD susceptibility or safety the independent of a TREM2 genetic linkage. Some mutations were detected in the extracellular TREM receptor exons, while others were discovered in intronic areas that affect gene expression and/or splicing. An example of a TREM1 risk allele lowered TREM1:TREM2 expression¹⁸³.

3.7.4. *TREM2 mediated neuroprotection in microglia*

TREM2 is expressed by myeloid cells such as microglia, granulocytes, dendritic cells, bone marrow, and monocyte derived macrophages as well as tissue macrophages (e.g., Osteoclasts)¹⁷⁴. TREM2 delivers intracellular signals via DAP12 after ligand binding. TREM2–DAP12 complex then recruits Src followed by phosphorylation of various downstream cascades (e.g., PLC, PI3K, and ERK). TREM2 signalling dysfunction causes aberrant phagocytosis, cytokine secretion, defective cell proliferation, and survival. In other words, a reduction in TREM2 brain expression may contribute to a neurodegenerative microglia phenotype^{171,192}. TREM2's role in AD is complicated by its participation in A β reactive microgliosis. This procedure causes microglia to congregate around A β plaques thus allowing A β elimination. Recent evidence suggests that TREM2 deficiency reduces the amount of microglia cells around plaques. TREM2 deletion lowers phagocytosis, although haplo-deficiency lessens plaque compaction and axonal dystrophy without affecting amyloid phagocytosis. A β lipidation phase in TREM2 mediates microglial polarisation, processing, and plaque encapsulation. TREM2 is also

activated by lipidic components of a lipoprotein complex, allowing it to perceive the milieu and phagocytize dead neurons, myelin, and amyloid plaques^{193,194}. These findings support the concept that TREM2 is responsible for brain structure modeling and the pathophysiology of AD.

3.7.5. *TREM2 directed therapeutics*

TREM2 has been linked to a variety of NDDs, implying that it could be a target for a variety of disorders. TREM2 directed treatments may be a novel target for neurodegenerative disorders, in addition to its biomarker potential. Several factors influence the design of TREM2 therapies, TREM2 variants increase the risk of AD as much as one ApoE4 allele, and the minor allele occurrence of TREM2 variants is much lesser than ApoE4¹⁷³¹⁹⁵. Some have proposed that rectifying TREM2 mutations may be a useful therapeutic method, but this is unlikely to be a widely applicable way. Rather, investigating TREM2 polymorphisms associated with neurodegenerative disorders will elucidate immune response components critical to disease control and will pave the way for creating immune-directed treatments. Even in the case of AD, just activating or suppressing TREM2 does not appear to be beneficial. sTREM2 levels in CSF have been reported to differ between male and female subjects in certain investigations, but not all^{196,197}. Similarly, in women, a TREM2 variation was associated with indicators of systemic inflammation. Raising or lowering TREM2 is unlikely to be a universal treatment for neurodegenerative disorders due to the absence of strong biomarkers and the variation in clinical development across patients¹⁹⁸.

3.7.6. *Future perspectives of TREM2*

Numerous mutations have been identified and one such mutation is p. R47H, it is unclear if the other TREM2 variations reported in AD patients are also dysfunctional. Protein expression and folding may be affected by the glutamine-33-stop (Q33X) and T66M mutants allied to NHD and frontotemporal dementia^{184,191}. It will also offer structural support for the participation of specific amino acid residues in lipid binding. TREM2 has the ability to bind non-lipidic ligands like heat shock protein 60 (HSP 60) or act as a co-receptor for the transmembrane semaphorin Sema6D^{190,199}. Also, soluble fusion proteins with the TREM2 extracellular domain can bind to numerous cells, especially near amyloid plaques²⁰⁰.

3.8. *Bridging integrator (BIN1)*

BIN1 was first discovered as a cancer suppressor containing a BAR (Bin1/Amphiphysin/RVS167) domain, a CT SH3 domain, and a MYC interacting domain. It is now the second utmost prevalent genetic susceptibility locus in LOAD next to APOE and it is expected to have an impact on AD risk primarily through the Tau pathway. BIN1 is engaged in membrane trafficking and clathrin-mediated endocytosis. As this gene family is involved in membrane transport and actin dynamics and BIN1 can influence A β processing, production, and clearance²⁰¹. However, a study found that the strongly related SNP at BIN1 had the highest odds ratio and population attributable fraction among non-APOE risk loci. BIN1 expression is altered in transgenic aging mouse models of AD²⁰¹, AD brains and elevated levels of BIN1 expression have been linked to a later age of onset in AD patients²⁰².

3.8.1. *Biochemical features of BIN1*

Biochemical features of BIN1 were initially identified with 19 exons, but a missed exon 6a was discovered between exons 6 and

⁷²⁰³. BIN1 transcripts undergo substantial differential splicing and a wide group of BIN1 splice variants with variable tissue spread is formed²⁰⁴. The primary versions differ principally in the presence of 4 exons namely 6a, 10, 12 (which included a sequence of alternate brain specific exons, 12A–D), and 13^{205,206}. Exon 12 encodes a central insert domain that interacted with clathrin and AP2/a-adaptin, while some brain splice variants have a potential coiled coil part in the BAR domain, in which exon 6a encodes a 31 residue attachment (NT insert domain) incorporated. A 15 residue region expressed by exon 10 in the muscle-specific isoform comprises a putative nuclear localization sequence and lipid binding sequence. Exon 13, which encodes a portion of the MYC binding domain and is tissue independently spliced. According to a study, two of the BIN1 cDNAs seem to encode splice variants that lack a Carboxy terminal SH3 domain²⁰¹.

Numerous BIN1 splice variants have aberrant electrophoretic motilities, as seen on polyacrylamide gel electrophoresis, implying that post translational alteration of this protein might show a role. Even though the longest BIN1 transcript in humans, rats, and mice have a projected mass of just 65 kDa, the brain BIN1 isoforms is 85 kDa in humans and 92 kDa in rats. Likewise, the muscle isoform transfers at a position corresponding to 60–70 kDa in polyacrylamide gels despite its expected size of 50 kDa²⁰¹.

PICALM, a gene connected to AD, is also linked to endocytosis. The average expression of BIN1 and PICALM has newly been revealed to be more significant in the white matter of the CNS¹⁸⁹. The N-terminal N-BAR domain, which adheres to lipid membranes and starts membrane curvature in T-tubules in muscular cells, endocytic pits in neuronal and non-neuronal cells, and probably cytoplasmic endosomes, also present in all known alternatively spliced BIN1 forms^{207,208}. BIN1 seems to connect the microtubule cytoskeleton to the cellular membrane through tubular membrane structures²⁰⁸, which may have an impact on the production of neurofibrillary tangles. Lastly, BIN1 is required to activate cell senescence and apoptotic^{209,210}. Similarly, BIN1 has been found to play a vital role in oncogene-induced senescence in primary cells, preventing cancer in its early stages²¹¹.

3.8.2. A possible role for BIN1 in AD

The concept of the amyloid cascade may be important further than the monogenic forms of AD, as LOAD is linked to a minor but pervasive dysfunction in the capacity to remove peripheral A β peptides²¹². Additional discoveries, however, suggested that drug discovery goals must concentrate more on emerging disease pathways such as endocytosis, synaptic damage, immune system, and lipid metabolism^{61,213}. Unlike APOE, clusterin (CLU), and CR1, which have vast information from the perspective of AD, the in-depth role of BIN1 on neurodegeneration is still a mirage. Thus, more investigation is required into the function of BIN1 in the pathogenesis of AD and putative pathways that affect AD risk. Even though the mechanisms behind BIN1's harmful character in AD are unknown, various plausible pathways have been discovered. Furthermore, BIN1's activity in multiple circumstances may aid us in grasping a potential function in AD²¹⁴.

3.8.3. BIN1 as a potential AD therapeutic target

Overall, the potential functions of BIN1 in the development of neurodegeneration create various new avenues for research into AD. The relations with Tau pathology must be prioritized, among them since it has the potential to alter neurofibrillary tangles. Further prominently, it has been shown that knocking down BIN1

reduces Tau mediated neurotoxicity²¹⁵, proposing that targeting BIN1 could be a novel approach to nerve protection and AD treatment. As previously indicated, blocking IDO1, a BIN1 immunoregulatory target, has proven therapeutic advantage for AD²¹⁶. Provisionally, BIN1 may influence Ca(v)1.2 trafficking and calcium channel blocker selectivity, which could reduce Tau load and increase autophagy role²¹⁷, a trait that could be crucial for effective treatment. Indeed, autophagy has been a critical reason for numerous neurodegenerative disorders, including frontotemporal dementia, AD, Parkinson's disease, and Huntington's disease. In AD, the pathogenesis has also been attributed to the endosomal lysosomal system precisely A β amyloidogenesis. Functional abnormalities in lysosomal pathways and over-expression of hydrolases lead to the formation of elevated levels of A β ²¹⁸.

3.9. Targeting infectious agents

Several medications in the current AD therapeutic development pipeline target infections and inflammation. COR388 inhibited the generation of gingipain by *P. gingivalis* and blocked A β _{1–42} production by reducing neuroinflammation and thus rescuing neurons in the hippocampus of mice²¹⁹. One study found that the Herpesviridae family, Epstein Barr virus (EBV), Herpes simplex virus-1 (HSV-1), Human herpesvirus 6 (HHV-6), and Chronic progressive nephropathy (Cpn) infection were related to a greater risk of AD in a meta-analysis of case control studies²²⁰.

HSV-1 antibodies were found in people with schizophrenia in the 1960s and 1970s. Some studies analyzed nucleic acid sequences of HSV-1 in the brains of manic and psychiatric patients and detected the HSV-1 genome in brain samples from elderly dementia patients. Various researchers looked for a link between viral infection and late onset sporadic AD however, some of such studies were futile. Hepatitis B virus (HBV) and influenza A and B viruses were among the viruses studied. Serum antibody titres to CMV, adenovirus, HSV, influenza A/B/measles virus, *Coxiella burnettii*, chlamydia group B, influenza A/B/measles virus and Mycoplasma pneumonia were not associated with AD^{221,222}. In certain cases, negative results can be attributed to the approaches that were not sensitive enough to detect viral genomes. Utilizing the polymerase chain reaction, other researchers found HSV genomes in the serum or brains of AD patients. Previously, spirochetes were suspected of causing AD. Recently, two additional bacteria, Chlamydia pneumonia, and Helicobacter pylori were linked to AD and developed a vascular hypothesis²²².

3.10. Carboxylesterase Notum

Notum, a secreted palmitoyl protein carboxylesterase, has newly been discovered to be a Wnt signalling negative modulator. Notum works by taking away a crucial palmitoyl moiety from Wnt proteins thus converting them to inactive form. It may be a more tractable therapeutic target for regulating Wnt signalling since it has a known high resolution crystal structure²²³. Notum appears to play a crucial role in human disease and study results suggest that targeting Notum may change a new therapeutic approach for the treatment of osteoporosis, cancer, and neurodegenerative disorders²²⁴.

Planarians through humans are all metazoans that have notum orthologues and they all have the recognizable S–H–D sequence catalytic triad of α/β -hydrolases. Early theories suggested that

Notum may hydrolyze the glycosaminoglycan chains of glypicans thereby changing their capacity to interact with Wnts and somehow modify signaling activity. This idea was sparked by Notum's sequence resemblance to plant pectin acetyl esterases. Later, it was shown that Notum causes cultured cells to release glypican, possibly *via* cleaving the GPI anchor. It is currently believed that Notum is a phospholipase that is peculiar to glypicans. However, glypican based interactions also affect Hedgehog, fibroblast growth factor, Dpp (*Drosophila* TGF), and Wingless signalling. Therefore, it stands to reason that these bio-pathways would be susceptible to Notum-induced glypican release. Wnt signalling activates notum expression in *Drosophila*, planarian worms, zebrafish, and hepatocarcinoma; on the other hand, Notum appears to preferentially inhibit Wnt signalling²²⁵.

Wnt proteins are a type of lipoprotein that binds to a wide range of cell surface receptors and co-receptors to trigger multiple intracellular signalling pathways. Three crucial Wnt signalling pathways that impact cytoskeleton remodelling and/or changes

in gene expression are planar cell polarity (PCP), canonical Wnt/ β -catenin, and Wnt/ Ca^{2+} . The well-studied Wnt pathway regulates the expression of Wnt target genes by stabilizing cytoplasmic β -catenin and promoting cellular cytoskeleton remodelling. GSK3 and CK1 phosphorylate β -catenin in the absence of Wnt ligands, forcing it to disintegrate²²⁶. Wnts are morphogens that are produced and are required for embryonic homeostasis. Wnt signalling dysregulation is linked to a variety of developmental defects and illnesses, including cancer, fibrosis, and osteoporosis. When Wnts interact with cell surface receptors, both β -catenin dependent and independent pathways are activated. Frizzled, LRP 5/6 (low density lipoprotein receptor associated proteins), and G protein coupled receptors. Because the palmitoleate group fits into the frizzled cysteine rich domain's hydrophobic groove for better engagement with receptors frizzled, a conjugation between the mono-unsaturated palmitoleic acid and a serine residue is vital. Wnt secretion requires palmitoylation, which is mediated by the ER enzyme Porcupine. Wnt secretion is inhibited by either

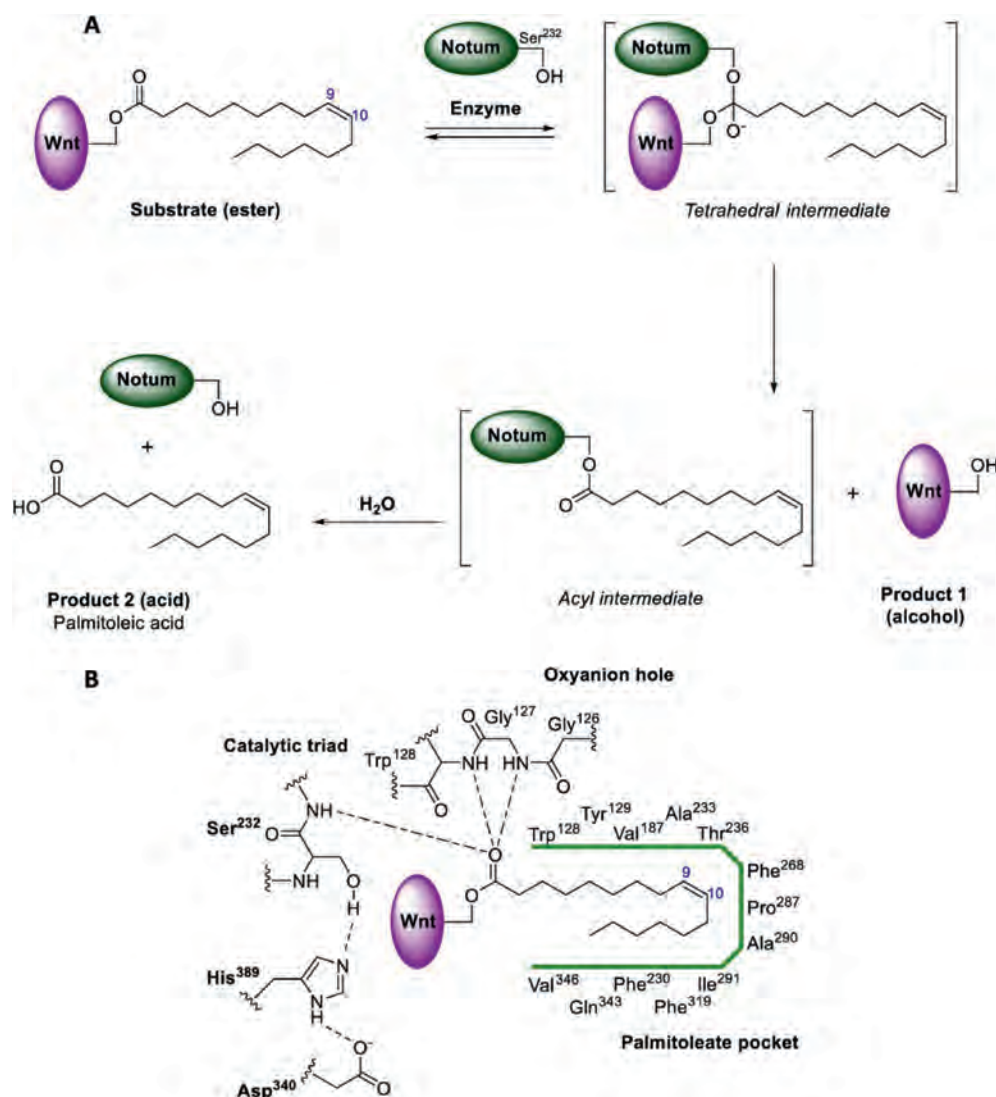


Figure 22 (A) Chemical reaction involving Notum's carboxylesterase activity operating on Wnt to release the palmitoleate group. (B) A 2D representation of the binding interactions. In addition to the traditional Ser232–Ala233 and Gly126–Gly127 amides, the Gly127–Trp128 amide plays role in the generation of the oxyanion hole. The catalytic triad (Ser232, His389, Asp340) is shown in bold²³⁰ © 2019. This work is openly licensed *via* CC BY 4.0. <https://creativecommons.org/licenses/by/4.0/>.

changing the conserved serine residue in Wnt or reducing PORCN enzymatic activity, which stops Wnts from binding with their carrier protein Wntless (WLS)²²⁷.

3.10.1. Wnt pathway and Notum function determination

Two groups studying the regulation of *Wingless* signalling in *Drosophila* independently discovered Notum. An enhancer trap screen for genes activated by *Wingless* signalling led to the discovery of a mutation that caused the expansion of presumptive wing tissue in Gerlitz and Basler's group²²⁸. At the same time, Giraldez et al.²²⁹ discovered that overexpression of the same gene resulted in the opposite phenotype, wing tissue loss, and an enlargement of the notum, an anatomical structure at the back of the fly. The latter phenotype prompted the authors to name the gene *notum*, which somehow surpassed *wingful*. Both studies found Notum to be a target of *Wingless* signalling and its protein product to be a powerful inhibitor of *Wingless* signaling²³⁰.

The fact that a signal peptide was present early on suggested that Notum functions in the extracellular environment. Given that Notum and plant pectin acetyl esterase have proteins with similar amino acid sequences, it is possible that Notum could change the glycosaminoglycans of glypicans, which are known to bind to Wnts and other growth factors *via* glycosylphosphatidylinositol anchored proteoglycans²³¹. Further biochemical studies, however, challenged this theory and proposed that Notum might be a phospholipase that breaks down proteins the GPI anchor of glypicans, releasing the glypicans from the cell surface along with any bound Wnt²³². Since many extracellular proteins other than Wnts bind to glypicans, one would anticipate additional pleiotropic effects if the molecular target of Notum was a glypican (*e.g.*, Hedgehog, FGF, BMP). The phospholipase model was ruled

out by structural analysis and enzymatic assays, which revealed that Notum is a carboxylesterase (Fig. 22)²³⁰.

3.10.2. Crystal structure of Notum

The Notum structure is based on the “canonical” α/β -hydrolase superfamily protein shape, with an eight-stranded β -sheet core hidden by α -helices (α B, α C and α F) and loops. The helices α A, α D, and α E and loops make up a movable lid domain, which can be ‘opened’ or ‘closed’ by shifting the helices far from or closer to the catalytic pocket, which is a unique property of lipases (Fig. 23A). An open state enables substrate entry, whereas a closed state is believed to allow catalytic substrate processing. The palmitoleated substrate bound Notum (S232A) structure demonstrates this by acquiring a closed conformation. However, few small molecule inhibitors are attached to Notum in an open conformation. The crystal structures of Notum exhibit a distinct, large (about 380 Å) active site with a hydrophobic pocket next to the catalytic triad created by S232, H389, D340 that houses the Wnt palmitoleate group (PDB id: 4UZQ) (Fig. 23B). Extended fatty acid chains need a turn in their structure to fit into the hydrophobic binding pocket, which can take lengthy carbon chains up to C8–10. The conventional S232–A233 and G126–127 peptides and the G127 to W128 amide produce the oxyanion hole, which offers additional stability to the tetrahedral transition state throughout ester hydrolysis. Entry into the pocket is relatively narrow, yet it has excellent flexibility. Because of these structural characteristics, this Notum pocket has been classified as highly druggable²²³.

Notum's crystal structure also exhibited a hydrophobic pocket that might hold *cis*-palmitoleate, concluding that Notum might hydrolyze the O-linkage of palmitoleate to Wnts. MALDI analysis of palmitoylated peptides treated with recombinant Notum confirmed this *in vitro*. Surprisingly, the structural study revealed multiple heparin binding sites on Notum's surface. These sites

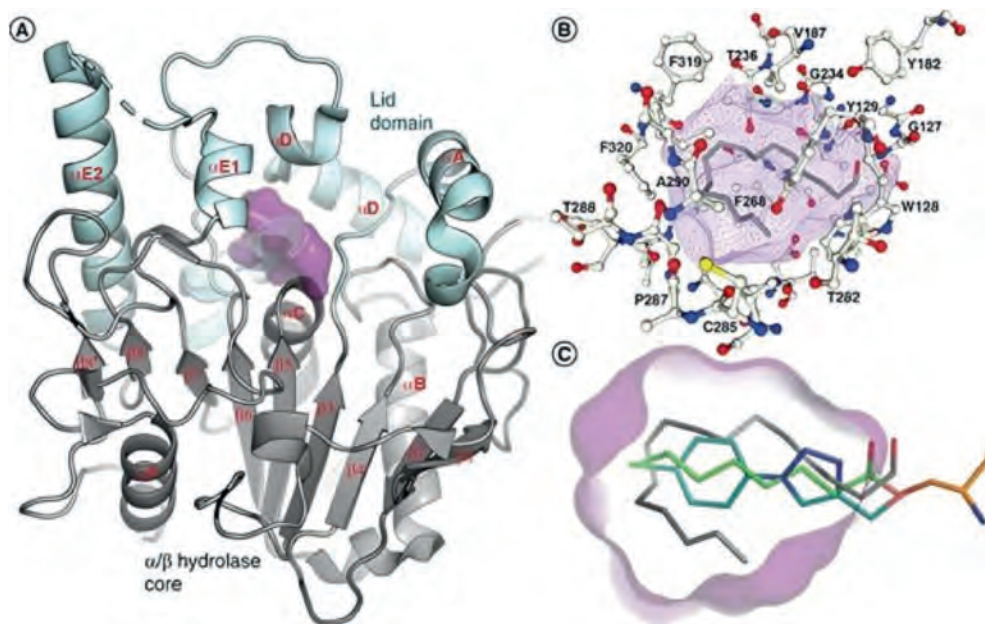


Figure 23 Notum structure as illustrated in a cartoon. (A) A grey cartoon of the enzyme core is shown, with the lid domain in pale cyan. A purple surface delineates the lipophilic pocket. (B) Notum pocket-forming residues (white ball and sticks) and palmitoleic acid substrate (grey sticks) within the pocket (purple mesh). (C) A close-up view of a pocket (purple) showing the alignment of Wnt palmitoleate (grey) and ghrelin octanoyl lipid (green/orange) substrates, as well as a representative inhibitor (teal)²²³. Reprinted with the permission from Ref. 223. Copyright © 2021 The authors.

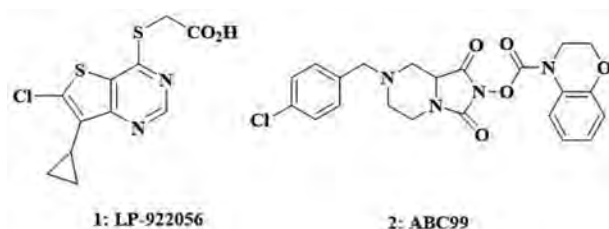


Figure 24 The structures of cholinesterase inhibitors.

explain Notum's potential to bind glypicans and give a molecular foundation for the genetic interactions among Notum and glypicans that have been recorded²²⁹. This pocket is where Notum's substrates and most inhibitors bind (Fig. 23C)²²³.

3.10.3. Notum inhibitors

Inhibitors of notum can bring back Wnt signalling, which could help treat diseases like osteoporosis and AD. Notum inhibitors have been shown to revitalize aged colon stem cells, enhance cortical bone strength and width, and raise elderly neuronal progenitors. They are being studied as a possible therapy for neurodegenerative diseases like AD, in which Wnt signalling is frequently downregulated. The discovery of 4-(indolin-1-yl)-4-oxo butanoate esters, a new class of covalent Notum inhibitors, has been reported. According to high resolution crystal structures, the nucleophile S232 and hydrolyzed butyric esters create a common covalent adduct in the Notum inhibitor complexes²³².

Only a few NOTUM inhibitors, which are heteroaryl fused thiophenes, have been described so far. These compounds are considered reversible type inhibitors, but their specificity inside and outside the serine hydrolase family is unclear. Ureas and activated carbamates have previously been shown to be dynamic serine hydrolases that are irreversible inhibitors, particularly once combined with activity based protein profiling to optimize effectivity and selectivity. Suci et al.²³³ isolated *N*-hydroxy hydantoin (NHH) carbamates from a structurally varied set of activated carbamates and urease to serve as specific and compelling inhibitors of NOTUM. In animals treated with ABC99, inhibition of Notum generated by Paneth cells led to the regeneration of aging intestinal epithelium²³⁴. In the brain, the Notum controls Wnt signaling, which regulates neurogenesis in the subventricular zone (SVZ), and inhibition of the Notum with ABC99 (Fig. 24) activates Wnt signaling and increases more in the SVZ²³⁵.

Notum melatonin complex crystal structure could assist in developing more highly effective brain accessible drugs that could help cure neurodegenerative disease. Melatonin has previously shown neuroprotective action in aging and AD animal models by reducing the deposition of A β and hyperphosphorylated Tau.



Figure 25 Notum inhibitors associated with the hormone melatonin.

Stimulation of Wnt signalling can prevent GSK3, a component of the "destruction complex", thus minimizing tau hyperphosphorylation. Zhao et al.²³⁶, investigated the XChem platform output to find Notum inhibitors, and the fragment hit *N*-[2-(5-fluoro-1*H*-indol-3-yl) ethyl]-acetamide (IC₅₀ 37.2 μ mol/L; PDB 6TR7) was emphasized because of its structural resemblance to melatonin. By immersing (PDB 6TR5) and *N*-acetyl serotonin (PDB 6TR6) in Notum crystals (Fig. 25), various structural studies were done, and high-resolution structures of related groups were produced. Among these structures, two compounds interact with Notum: one at the catalytic region of the enzyme and the other at the pocket's edge against the substrate entrance²³⁶.

Caffeine and, to a lesser extent, theophylline can inhibit notum activity (Fig. 26). Both biochemical and biophysical methods were used to characterize the caffeine Notum interaction thoroughly. High resolution structures of (PDB 6TV4) and (PDB 6TUZ) show that both compounds bind in the palmitoleate pocket but in distinct manners. This structural information could aid in the discovery of more potent Notum inhibitors²²⁹.

In the development of 1-phenyl-1,2,3-triazoles and 5-phenyl-1,3,4-oxadiazol-2(3*H*)-ones, (1-(4-chlorophenyl)-1*H*-1,2,3-triazol-4-yl) methanol (IC₅₀ 11.5 μ mol/L) was a notable hit from this fragment set and was chosen as the beginning point for a hit-to-lead programme (Fig. 27). According to the X-ray structure

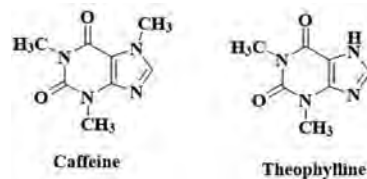


Figure 26 Caffeine and theophylline are Notum inhibitors.

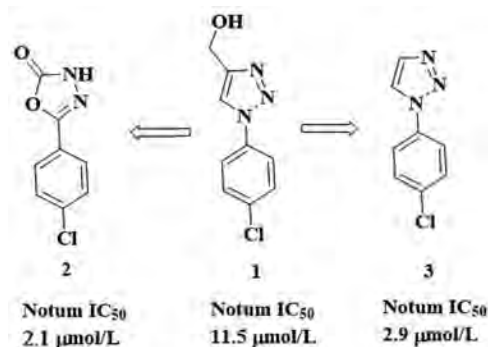


Figure 27 Oxadiazole 2 and triazole 3 were two complimentary leads produced by a fragment.

of 1 (PDB 6ZUV), the 4-chlorophenyl ring occupied the palm-toleate pocket and formed a stacking contact with the residue Phe268. Trp128 and the triazole head group may interact *via*—stacking, and the triazole's N2 and Trp128's peptidic backbone may form a hydrogen bond. Moreover, residue Trp128 forms a hydrogen bond with the methyl alcohol group's oxygen. Modification of 1 by alteration of the heterocyclic head group found two complimentary leads: oxadiazole 2 and triazole 3²²⁹.

4. Conclusions

Alzheimer's disease is one of the dreadful diseases that has blown a full whistle for the cure of patients. Numerous pathophysiological changes have been recognized involving multiple signaling systems for the gradual increase in the deteriorating condition of AD. According to the existing data, one must instead consider a causal polymicrobial community that impacts immune/inflammatory reactions in the brain and the periphery and interacts with many factors such as genetics, environment, and age. Thus, AD should be viewed as a complicated illness involving the dysfunction of the brain's immune system. Despite technical advancement and artificial intelligence, early AD detection is needed for an hour; once detected, the treatment depends on the use of the drug for symptomatic treatment. Future treatments (and prevention) of AD will not be a single simple molecule but a multimodal complicated approach. Putative molecular mechanisms in the progression of AD have indicated the involvement of amyloid proteins and complicated neuroinflammation, TREM2, notum, tau accumulation, and degeneration, etc., with numerous supporting scientific proofs. A few significant numbers of new minor molecule hits have been reported against the modulation of the aforementioned molecular targets, and some have advanced to higher stages of clinical trials. The successful molecule as a clinical drug for treating AD is eagerly anticipated in the present and future.

Acknowledgments

All the authors express heartfelt gratitude to BVDU for providing ample facilities. Ashwini Patil expresses sincere gratitude to Chhatrapati Shahu Maharaj Research, Training and Human Development Institute (SARTHI) (An Autonomous Institute of Govt. of Maharashtra) for providing scholarships under CSMNRF-2021 (India).

Author contributions

All of the authors approved the final version of the manuscript.

Conflicts of interest

All the authors express no conflict of interest with any agencies.

References

1. Kwan P, Konno H, Chan KY, Baum L. Rationale for the development of an Alzheimer's disease vaccine. *Hum Vaccin Immunother* 2020;**16**: 645–53.
2. Chen XQ, Mobley WC. Alzheimer disease pathogenesis: insights from molecular and cellular biology studies of oligomeric A β and tau species. *Front Neurosci* 2019;**13**:659.
3. Coman H, Nemeş B. New therapeutic Targets in Alzheimer's disease. *Int J Gerontol* 2017;**11**:2–6.
4. Cenini G, Voos W. Mitochondria as potential targets in Alzheimer disease therapy: an update. *Front Pharmacol* 2019;**10**:902.
5. Minati L, Edginton T, Grazia Bruzzone M, Giaccone G. Reviews: current concepts in Alzheimer's disease: a multidisciplinary review. *J Alzheimers Dis Other Dement* 2009;**24**:95–121.
6. Vaz M, Silvestre S. Alzheimer's disease: recent treatment strategies. *Eur J Pharmacol* 2020;**887**:173554.
7. Breijyeh Z, Karaman R. Comprehensive review on Alzheimer's disease. *Molecules* 2020;**25**:5789.
8. Chi S, Yu JT, Tan MS, Tan L. Depression in Alzheimer's disease: epidemiology, mechanisms, and management. *J Alzheimers Dis* 2014;**42**:739–55.
9. Lipton S. The molecular basis of memantine action in Alzheimer's disease and other neurologic disorders: low-affinity, uncompetitive antagonism. *Curr Alzheimer Res* 2005;**2**:155–65.
10. Jia Q, Deng Y, Qing H. Potential therapeutic strategies for Alzheimer's disease targeting or beyond β -Amyloid: insights from clinical trials. *BioMed Res Int* 2014;**2014**:837157.
11. Yan Q, Zhang J, Liu H, Babu-khan S, Vassar R, Biere AL, et al. Anti-inflammatory drug therapy alters β -amyloid processing and deposition in an animal model of Alzheimer's disease. *J Neurosci* 2003;**23**: 7504–9.
12. Ravindra GK, Achaiiah G, Sastry GN. Molecular modeling studies of phenoxyrimidiny l imidazoles as p38 kinase inhibitors using QSAR and docking. *Eur J Med Chem* 2008;**43**:830–8.
13. Jiayi Zhou BL. Alzheimer's disease and Prion protein. *Intractable Rare Dis Res* 2013;**2**:35–44.
14. Vaz M, Silvestre S. Alzheimer's disease: recent treatment strategies. *Eur J Pharmacol* 2020;**887**:173554.
15. Chung E, Ji Y, Sun Y, Kascak RJ, Kascak RB, Mehta PD, et al. Anti-PrP^C monoclonal antibody infusion as a novel treatment for cognitive deficits in an Alzheimer's disease model mouse. *BMC Neurosci* 2010;**11**:130.
16. Moreira PI, Cardoso SM, Santos MS, Oliveira CR. The key role of mitochondria in Alzheimer's disease. *J Alzheimers Dis* 2006;**9**:101–10.
17. Cole SL, Vassar R. The Alzheimer's disease β -secretase enzyme, BACE1. *Mol Neurodegener* 2007;**2**:22.
18. Çokuğraş AN. Butyrylcholinesterase: structure and physiological importance. *Turk J Biochem* 2003;**28**:54–61.
19. Hur JY, Gertsik N, Johnson DS, Li YM. γ -Secretase inhibitors: from chemical probes to drug development. In: Wolfe MS, editor. *Developing therapeutics for Alzheimer's disease: progress and challenges*. Amsterdam: Elsevier Inc.; 2016. p. 63–76.
20. Singh Y, Gupta G, Shrivastava B, Dahiya R, Tiwari J, Ashwathanarayana M, et al. Calcitonin gene-related peptide (CGRP): a novel target for Alzheimer's disease. *CNS Neurosci Ther* 2017;**23**: 457–61.
21. García-Osta A, Cuadrado-Tejedor M, García-Barroso C, Oyarzábal J, Franco R. Phosphodiesterases as therapeutic targets for Alzheimer's disease. *ACS Chem Neurosci* 2012;**3**:832–44.
22. Abrams P, Andersson KE, Buccafusco JJ, Chapple C, De Groat WC, Fryer AD, et al. Muscarinic receptors: their distribution and function in body systems, and the implications for treating overactive bladder. *Br J Pharmacol* 2006;**148**:565–78.
23. Ford CP. The role of D2-autoreceptors in regulating dopamine neuron activity and transmission. *Neuroscience* 2014;**282**:13–22.
24. Mandal PK, Kansara K, Dabas A. The GABA—working memory relationship in Alzheimer's disease. *J Alzheimers Dis Rep* 2017;**1**: 43–5.
25. Ramsey CP, Glass CA, Montgomery MB, Lindl KA, Ritson GP, Chia LA, et al. Expression of Nrf2 in neurodegenerative diseases. *J Neuropathol Exp Neurol* 2007;**66**:75–85.
26. Srivastava A, Das B, Yao AY, Yan R. Metabotropic glutamate receptors in Alzheimer's disease synaptic dysfunction: therapeutic opportunities and hope for the future. *J Alzheimers Dis* 2020;**78**: 1345–61.

27. Repici M, Giorgini F. DJ-1 in Parkinson's disease: clinical insights and therapeutic perspectives. *J Clin Med* 2019;**8**:1377.
28. Tao L, Zhu Y, Wang R, Han J, Ma Y, Guo H, et al. N-myc downstream-regulated gene 2 deficiency aggravates memory impairment in Alzheimer's disease. *Behav Brain Res* 2020;**379**:112384.
29. Vieira MNN, Lyra e Silva NM, Ferreira ST, De Felice FG. Protein tyrosine phosphatase 1B (PTP1B): a potential target for Alzheimer's therapy?. *Front Aging Neurosci* 2017;**9**:7.
30. Behl T, Kaur D, Sehgal A, Singh S, Sharma N, Zengin G, et al. Role of monoamine oxidase activity in Alzheimer's disease: an insight into the therapeutic potential of inhibitors. *Molecules* 2021;**26**:3724.
31. SantaCruz KS, Yazlovitskaya E, Collins J, Johnson J, DeCarli C. Regional NAD(P)H: quinone oxidoreductase activity in Alzheimer's disease. *Neurobiol Aging* 2004;**25**:63–9.
32. O'Brien RJ, Wong PC. Amyloid precursor protein processing and Alzheimer's disease. *Annu Rev Neurosci* 2011;**34**:185–204.
33. Jiang Q, Heneka M, Landreth GE. The role of peroxisome proliferator-activated receptor- γ (PPAR γ) in Alzheimer's disease: therapeutic implications. *CNS Drugs* 2008;**22**:1–14.
34. Necula D, Riviere-Cazaux C, Shen Y, Zhou M. Insight into the roles of CCR5 in learning and memory in normal and disordered states. *Brain Behav Immun* 2021;**92**:1–9.
35. Hoskin JL, Al-Hasan Y, Sabbagh MN. Nicotinic acetylcholine receptor agonists for the treatment of Alzheimer's dementia: an update. *Nicotine Tob Res* 2019;**21**:370–6.
36. Gebre AK, Altaye BM, Atey TM, Tuem KB, Berhe DF. Targeting renin-angiotensin system against Alzheimer's disease. *Front Pharmacol* 2018;**9**:440.
37. Deming Y, Li Z, Benitez BA, Cruchaga C. Triggering receptor expressed on myeloid cells 2 (TREM2): a potential therapeutic target for Alzheimer's disease?. *Expert Opin Ther Targets* 2018;**22**:587–98.
38. Giuffrida ML, Copani A, Rizzarelli E. A promising connection between BDNF and Alzheimer's disease. *Aging (Albany NY)* 2018;**10**:1791.
39. Lauretti E, Dincer O, Praticò D. Glycogen synthase kinase-3 signaling in Alzheimer's disease. *Biochim Biophys Acta Mol Cell Res* 2020;**1867**:118664.
40. Suh YH, Checler F. Amyloid precursor protein, presenilin, and α -synuclein: molecular pathogenesis and pharmacological applications in Alzheimer's disease. *Pharmacol Rev* 2002;**54**:469–525.
41. Imbimbo BP, Giardina GAM. γ -Secretase inhibitors and modulators for the treatment of Alzheimer's disease: disappointments and hopes. *Curr Top Med Chem* 2011;**11**:1555–70.
42. Hernández-Zimbrón LF, Rivas-Arancibia S. Deciphering an interplay of proteins associated with amyloid β_{1-42} peptide and molecular mechanisms of Alzheimer's disease. *Rev Neurosci* 2014;**25**:773–83.
43. Kametani F, Hasegawa M. Reconsideration of amyloid hypothesis and tau hypothesis in Alzheimer's disease. *Front Neurosci* 2018;**12**:328460.
44. Hardy J. The amyloid hypothesis for Alzheimer's disease: a critical reappraisal. *J Neurochem* 2009;**110**:1129–34.
45. Zhang Y, He M lin. Deferoxamine enhances alternative activation of microglia and inhibits amyloid beta deposits in APP/PS1 mice. *Brain Res* 2017;**1677**:86–92.
46. Penke B, Szucs M, Bogár F. Oligomerization and conformational change turn monomeric β -amyloid and tau proteins toxic: their role in Alzheimer's pathogenesis. *Molecules* 2020;**25**:1659.
47. Salloway S, Sperling R, Keren R, Porsteinsson AP, Van Dyck CH, Tariot PN, et al. A phase 2 randomized trial of ELND005, Scyllo-inositol, in mild to moderate Alzheimer disease. *Neurology* 2011;**77**:1253–62.
48. Hey JA, Yu JY, Versavel M, Abushakra S, Kocis P, Power A, et al. Clinical pharmacokinetics and safety of ALZ-801, a novel prodrug of tramiprosate in development for the treatment of Alzheimer's disease. *Clin Pharmacokinet* 2018;**57**:315–33.
49. Amijee H, Scopes DIC. The Quest for small molecules as amyloid inhibiting therapies for Alzheimer's disease. *J Alzheimers Dis* 2009;**17**:33–47.
50. Mandel SA, Amit T, Kalfon L, Reznichenko L, Weinreb O, Youdim MBH. Cell signaling pathways and iron chelation in the neurorestorative activity of green tea polyphenols: special reference to epigallocatechin gallate (EGCG). *J Alzheimers Dis* 2008;**15**:211–22.
51. Exley C, Price NC, Kelly SM, Birchall JD. An interaction of β -amyloid with aluminium *in vitro*. *FEBS Lett* 1993;**324**:293–5.
52. Murayama M, Hono K. Pre-precipitate clusters and precipitation processes in Al-Mg-Si alloys. *Acta Mater* 1999;**47**:1537–48.
53. McLachlan D, Kruck T, Kalow W, Andrews D, Dalton A, Bell M, et al. Intramuscular desferrioxamine in patients with Alzheimer's disease. *Lancet* 1991;**337**:1304–8.
54. Cherny RA, Legg JT, McLean CA, Fairlie DP, Huang X, Atwood CS, et al. Aqueous dissolution of Alzheimer's disease A β amyloid deposits by biometal depletion. *J Biol Chem* 1999;**274**:23223–8.
55. Cherny RA, Atwood CS, Xilinas ME, Gray DN, Jones WD, McLean CA, et al. Treatment with a copper-zinc chelator markedly and rapidly inhibits β -amyloid accumulation in Alzheimer's disease transgenic mice. *Neuron* 2001;**30**:665–76.
56. Fredericksen CJ, Bush AI. *Synaptically released zinc: physiological functions and pathological effects*, vol. 14. Netherlands: Kluwer Academic Publishers; 2001.
57. Mclean CA, Cherny RA, Fraser FW, Hons B, Fuller SJ, Smith MJ, et al. Soluble pool of A β amyloid as a determinant of severity of neurodegeneration in Alzheimer's disease. *Ann Neurol* 1999;**46**:860–6.
58. Wilson CA, Doms RW, Lee VM. Intracellular APP processing and A β production in Alzheimer's disease. *J Neuropathol Exp Neurol* 1999;**58**:787–94.
59. Solomon B, Koppel R, Frankel D, Hanan-Aharon E. Disaggregation of Alzheimer β -amyloid by site-directed mAb. *Proc Natl Acad Sci USA* 1997;**94**:4109–12.
60. Wilcock DM, Colton CA. Anti-amyloid- β immunotherapy in Alzheimer's disease: relevance of transgenic mouse studies to clinical trials. *J Alzheimers Dis* 2008;**15**:555–69.
61. Menéndez-gonzález M, Pérez-piñera P, Martínez-rivera M, Muñoz AL, Vega JA. Immunotherapy for Alzheimer's disease: rational basis in ongoing clinical trials. *Immunother Alzheimer's Dis* 2011;**17**:508–20.
62. Steinitz M. Immunotherapy for Alzheimer's disease. *Immunotherapy* 2009;**1**:461–9.
63. Wilcock DM, Colton CA. Anti-Amyloid- β immunotherapy in Alzheimer's disease: relevance of transgenic mouse studies to clinical trials. *J Alzheimers Dis* 2008;**15**:555–9.
64. Morgan D. Immunotherapy for Alzheimer's disease. *J Alzheimers Dis* 2006;**9**:425–32.
65. Mikulca JA, Nguyen V, Gajdosik DA, Teklu SG, Giunta EA, Lessa EA, et al. Potential novel targets for Alzheimer's pharmacotherapy: II. Update on secretase inhibitors and related approaches. *J Clin Pharm Ther* 2014;**39**:25–37.
66. Delrieu J, Ousset PJ, Vellas B. Gantenerumab for the treatment of Alzheimer's disease. *Expert Opin Biol Ther* 2012;**12**:1077–86.
67. Doody RS, Thomas RG, Farlow M, Iwatsubo T, Vellas B, Joffe S, et al. Phase 3 trials of solanezumab for mild-to-moderate Alzheimer's disease. *N Engl J Med* 2014;**370**:311–21.
68. Richard E, Den Brok MG, Van Gool WA. Bayes analysis supports the null hypothesis of anti-amyloid beta therapy in Alzheimer's disease. *Alzheimers Dement* 2021;**17**:1051–5.
69. Dyck CH. Anti-Amyloid- β monoclonal antibodies for Alzheimer's disease: pitfalls and promise. *Biol Psychiatry* 2018;**83**:311–9.
70. Dovey HF, John V, Anderson JP, Chen LZ, De Saint Andrieu P, Fang LY, et al. Functional gamma-secretase inhibitors reduce beta-amyloid peptide levels in the brain. *J Neurochem* 2001;**76**:173–81.
71. Wolfe MS, Kopan R. Intramembrane proteolysis: theme and variations. *Science* 2004;**305**:1119–23.
72. Struhl G, Adachi A. Requirements for presenilin-dependent cleavage of notch and other transmembrane proteins. *Mol Cell* 2000;**6**:625–36.

73. Bai X, Rajendra E, Yang G, Shi Y, Scheres SH. Sampling the conformational space of the catalytic subunit of human γ -secretase. *J Biophys Struct* 2015;**4**:e11182.
74. Mouchlis VD, Melagraki G, Zacharia LC, Afantitis A. Computer-aided drug design of β -secretase, γ -secretase and anti-tau inhibitors for the discovery of novel Alzheimer's therapeutics. *Int J Mol Sci* 2020;**21**:703.
75. Wolfe MS. Structure and function of the γ -secretase complex. *Biochemistry* 2019;**58**:2953–66.
76. Zhang X, Li Y, Xu H, Zhang YW. The γ -secretase complex: from structure to function. *Front Cell Neurosci* 2014;**8**:427.
77. Bai X, Yan C, Yang G, Lu P, Sun L, Zhou R, et al. An atomic structure of human γ -secretase. *Nature* 2015;**525**:212–7.
78. Yang G, Zhou R, Zhou Q, Guo X, Yan C, Ke M, et al. Structural basis of notch recognition by human γ -secretase. *Nature* 2019;**565**:192–7.
79. Hur JY. γ -Secretase in Alzheimer's disease. *Exp Mol Med* 2022;**54**:433–46.
80. Wolfe MS, Xia W, Ostaszewski BL. Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and γ -secretase activity. *Nature* 1999;**117**:513–7.
81. Panza F, Frisardi V, Solfrizzi V, Imbimbo B P, Logroscino G, Santamato A, et al. Interacting with γ -secretase for treating Alzheimer's disease: from inhibition to modulation. *Curr Med Chem* 2011;**18**:5430–47.
82. Chu J, Wisniewski T, Pratic D. GATA1-mediated transcriptional regulation of the c-secretase activating protein increases A β formation in Down syndrome. *Am Neurol Asso* 2015;**79**:138–43.
83. Kumar D, Ganeshpurkar A, Modi G, Gupta SK, Singh SK. Secretase inhibitors for the treatment of Alzheimer's disease: long road ahead. *Eur J Med Chem* 2018;**148**:436–52.
84. Doody RS, Raman R, Farlow M, Iwatsubo T, Vellas B, Joffe S, et al. A Phase 3 trial of semagacestat for treatment of Alzheimer's disease. *N Eng J Med* 2013;**369**:341–50.
85. Coric V, Salloway S, Van Dyck CH, Dubois B, Andreasen N, Brody M, et al. Targeting prodromal Alzheimer disease with avagacestat: a randomized clinical trial. *JAMA Neurol* 2015;**72**:1324–33.
86. Martone RL, Zhou H, Atchison K, Comery T, Xu JZ, Huang X, et al. Begacestat (GSI-953): a novel, selective thiophene sulfonamide inhibitor of amyloid precursor protein γ -secretase for the treatment of Alzheimer's disease. *J Pharmacol Exp Ther* 2009;**331**:598–608.
87. Qui R, Willavize S, Fullerton T, Gastonguay MR. Modelling and simulation of plasma A β in humans after multiple oral doses of PF-3084014, a potent gamma-secretase inhibitor. *Pap Knowled Tow A Med Histo Doc* 2009;**5**:253.
88. Stone JA, Rosen LB, Plump JA, Yuan J, Harrison T, Flynn M, et al. The gamma secretase inhibitor Mk-0752 acutely and significantly reduces CSF A β 40 concentrations in humans. *Alzheimers Dement* 2006;**2**:S79.
89. Wilcock GK, Black SE, Hendrix SB, Zavitz KH, Swabb EA, Laughlin MA. Efficacy and safety of tarenflurbil in mild to moderate Alzheimer's disease: a randomised phase II trial. *Lancet Neurol* 2008;**7**:483–93.
90. Green RC, Schneider LS, Amato DA, Beelen AP, Wilcock G, Swabb EA, et al. Effect of tarenflurbil on cognitive decline and activities of daily living in patients with mild Alzheimer disease: a randomized controlled trial. *JAMA* 2009;**302**:2557–64.
91. Nagy C, Schuck E, Ishibashi A, Nakatani Y, Rege B, Logovinsky V. P3-415: E2012, a novel gamma-secretase modulator, decreases plasma amyloid-beta (A β) levels in humans. *Alzheimers Dement* 2010;**6**:S74.
92. Business Review. In: *Eisai Co., Ltd. PharmaDeals review*. vol. 2004; 2004. Available from: <https://doi.org/10.3833/pdr.v2004i46.819>.
93. Imbimbo BP, Del Giudice E, Colavito D, D'Arrigo A, Dalle Carbonare M, Villetti G, et al. 1-(3',4'-Dichloro-2-fluoro [1,1'-biphenyl]-4-yl)-cyclopropanecarboxylic acid (CHF5074), a novel γ -secretase modulator, reduces brain β -amyloid pathology in a transgenic mouse model of Alzheimer's disease without causing peripheral toxicity. *J Pharmacol Exp Ther* 2007;**323**:822–30.
94. Rogers K, Felsenstein KM, Hrdlicka L, Tu Z, Albayya F, Lee W, et al. Modulation of γ -secretase by EVP-0015962 reduces amyloid deposition and behavioral deficits in Tg2576 mice. *Mol Neurodegener* 2012;**7**:61.
95. Rogers K, Leventhal L, Hopp S, Miller MJ, Yang Z, Hrdlicka L, et al. P4-022: the gamma secretase modulator EVP-0015962 improves cognitive deficits in Tg2576 mice concomitant with decreases in A β ₄₂. *Alzheimers Dement* 2010;**6**:e26–7.
96. Haass C. Take five—BACE and the γ -secretase quartet conduct Alzheimer's amyloid β -peptide generation. *EMBO J* 2004;**23**:483–8.
97. Moussa-Pacha NM, Abidin SM, Omar HA, Alniss H, Al-Tel TH. BACE1 inhibitors: current status and future directions in treating Alzheimer's disease. *Med Res Rev* 2020;**40**:339–84.
98. Vassar R, Kuhn PH, Haass C, Kennedy ME, Rajendran L, Wong PC, et al. Function, therapeutic potential and cell biology of BACE proteases: current status and future prospects. *J Neurochem* 2014;**130**:4–28.
99. Kandalepas PC, Sadleir KR, Eimer WA, Zhao J, Nicholson DA, Vassar R. The Alzheimer's β -secretase BACE1 localizes to normal presynaptic terminals and to dystrophic presynaptic terminals surrounding amyloid plaques. *Acta Neuropathol* 2013;**126**:329–52.
100. Hong L, Koelsch G, Lin X, Wu S, Terzyan S, Ghosh AK, et al. Structure of the protease domain of memapsin 2 (β -secretase) complexed with an inhibitor. *Science* 2000;**290**:150–3.
101. Xu Y, Li MJ, Greenblatt H, Chen W, Paz A, Dym O, et al. Flexibility of the flap in the active site of BACE1 as revealed by crystal structures and molecular dynamics simulations. *Acta Crystallogr D Biol Crystallogr* 2012;**68**:13–25.
102. Shimizu H, Tosaki A, Kaneko K, Hisano T, Sakurai T, Nukina N. Crystal structure of an active form of BACE1, an enzyme responsible for amyloid β protein production. *Mol Cell Biol* 2008;**28**:3663–71.
103. Yuan J, Venkatraman S, Zheng Y, McKeever BM, Dillard LW, Singh SB. Structure-based design of β -site APP cleaving enzyme 1 (BACE1) inhibitors for the treatment of Alzheimer's disease. *J Med Chem* 2013;**56**:4156–80.
104. Ghosh AK, Osswald HL. BACE1 (β -secretase) inhibitors for the treatment of Alzheimer's disease. *Chem Soc Rev* 2014;**43**:6765–813.
105. Eketjäll S, Janson J, Kaspersson K, Bogstedt A, Jeppsson F, Fälting J, et al. AZD3293: a novel, orally active BACE1 inhibitor with high potency and permeability and markedly slow off-rate kinetics. *J Alzheimers Dis* 2016;**50**:1109–23.
106. Bell J, O'Neill B, Brodney M, Hajos-Korcsok E, Lu Y, Riddell D, et al. P1–351: a novel BACE inhibitor (PF-05297909): a two-part adaptive design to evaluate safety, pharmacokinetics, and pharmacodynamics for modifying beta-amyloid in a first-in-human study. *Alzheimers Dement* 2013;**9**:287.
107. Bazzari FH, Bazzari AH. BACE1 inhibitors for Alzheimer's disease: the past, present and any future?. *Molecules* 2022;**27**:8823.
108. Alzforum. Therapeutics - LY2886721. Available from: www.alzforum.org. 2019-23-5.
109. Egan MF, Kost J, Tariot PN, Aisen PS, Cummings JL, Vellas B, et al. Randomized trial of Verubecestat for mild-to-moderate Alzheimer's disease. *N Engl J Med* 2018;**378**:1691–703.
110. Novak G, Streffer JR, Timmers M, Henley D, Brashear HR, Bogert J, et al. Long-term safety and tolerability of atabecestat (JNJ-5486191). *Alzheimers Res Ther* 2020;**12**:58.
111. Draffin JE, Knafo S, Heneka MT. Alzheimer's disease and mechanism-based attempts to enhance cognition. In: *Cognitive enhancement*. Bonn: Elsevier Inc.; 2015. p. 193–231.
112. Kojro E, Fahrenholz F. The non-amyloidogenic pathway: structure and function of α -secretases. In: Harris JR, Fahrenholz F, editors. *Alzheimer's disease. Subcellular biochemistry*. Boston: Springer; 2005. p. 105–27.

113. Zhang YW, Thompson R, Zhang H, Xu H. APP processing in Alzheimer's disease. *Mol Brain* 2011;**4**:3.
114. Lannfelt L, Basun H, Wahlund LO, Rowe BA, Wagner SL. Decreased α -secretase-cleaved amyloid precursor protein as a diagnostic marker for Alzheimer's disease. *Nat Med* 1995;**1**:829–32.
115. Higgins LS, Murphy Jr GM, Forno LS, Catalano R, Cordell B. P3 beta-amyloid peptide has a unique and potentially pathogenic immunohistochemical profile in Alzheimer's disease brain. *Am J Pathol* 1996;**149**:585–96.
116. Wei W, Norton DD, Wang X, Kusiak JW. $A\beta_{17-42}$ in Alzheimer's disease activates JNK and caspase-8 leading to neuronal apoptosis. *Brain* 2002;**125**:2036–43.
117. Huifang M, Lesné S, Kotilinek L, Steidl-Nichols JV, Sherman M, Younkin L, et al. Involvement of β -site APP cleaving enzyme 1 (BACE1) in amyloid precursor protein-mediated enhancement of memory and activity-dependent synaptic plasticity. *Proc Natl Acad Sci U S A* 2007;**104**:8167–72.
118. Spies PE, Verbeek MM, Van Groen T, Claassen JA. Reviewing reasons for the decreased CSF A β 42 concentration in Alzheimer's disease. *Front Biosci* 2012;**17**:2024–34.
119. Stakos DA, Stamatiopoulos K, Bampatsias D, Sachse M, Zormpas E, Vlachogiannis NI, et al. The Alzheimer's disease amyloid-beta hypothesis in cardiovascular aging and disease: JACC focus seminar. *J Am Coll Cardiol* 2020;**75**:952–67.
120. Zhao J, Liu X, Xia W, Zhang Y, Wang C. Targeting amyloidogenic processing of APP in Alzheimer's disease. *Front Mol Neurosci* 2020;**13**:137.
121. Kolata G. Down Syndrome Alzheimer's linked: down syndrome, adults get Alzheimer-like changes in their brains and many become demented, leading researchers to ask about the connection. *Science* 2016;**230**:1152–3.
122. Gao Y, Tan L, Yu J, Tan L. Tau in Alzheimer's disease: mechanisms and therapeutic strategies. *Curr Alzheimer Res* 2018;**15**:283–300.
123. Nanavaty N, Lin L, Hincley SH, Kuret J. Detection and quantification methods for fibrillar products of *in vitro* tau aggregation assays. In: Smet-Nocca C, editor. *Tau protein. Methods in molecular biology*. New York, NY: Humana Press; 2017. p. 101–11.
124. Gandhi NS, Kukic P, Lippens G, Mancera RL. Molecular dynamics simulation of tau peptides for the investigation of conformational changes induced by specific phosphorylation patterns. *Methods Mol Biol* 2017;**1523**:33–59.
125. Ferreira A, Ghoshal N. Inhibition of neuronal maturation in primary hippocampal neurons from τ deficient mice. *J Cell Sci* 2001;**114**:1179–87.
126. Khalid I, Liu F, Gong CX, Grundke I. Tau in Alzheimer's disease and related tauopathies. *Curr Alzheimer Res* 2010;**7**:656–64.
127. Bakota L, Brandt R. Tau biology and tau-directed therapies for Alzheimer's disease. *Drugs* 2016;**76**:301–13.
128. Chong FP, Ng KY, Koh RY, Chye SM. Tau proteins and tauopathies in Alzheimer's disease. *Cell Mol Neurobiol* 2018;**38**:965–80.
129. Mendiola-Precoma J, Berumen LC, Padilla K, Garcia-Alcocer G. Therapies for prevention and treatment of Alzheimer's disease. *BioMed Res Int* 2016;**2016**:2589276.
130. Gago M, Machado A, Rocha S. Current clinical approaches in neurodegenerative diseases. In: *Handbook of innovations in central nervous system regenerative medicine*. Braga: Elsevier Inc.; 2020. p. 79–124.
131. Winblad B, Pei J, Sjo M. Neurofibrillary degeneration in Alzheimer's disease: from molecular mechanisms to identification of drug targets. *Geriatr Psychiatry* 2008;**21**:555–61.
132. Kramer T, Schmidt B, Lo Monte F. Small-molecule inhibitors of GSK-3: structural insights and their application to Alzheimer's disease models. *Int J Alzheimers Dis* 2012;**2012**:381029.
133. Hooper C, Killick R, Lovestone S. The GSK3 hypothesis of Alzheimer's disease. *J Neurochem* 2008;**104**:1433–9.
134. Martin L, Latypova X, Wilson CM, Magnaudeix A, Perrin M, Yardin C, et al. Tau protein kinases: involvement in Alzheimer's disease. *Ageing Res Rev* 2013;**12**:289–309.
135. Martinez A, Castro A, Dorronsoro I, Alonso M. Glycogen synthase kinase 3 (GSK-3) inhibitors as new promising drugs for diabetes, neurodegeneration, cancer, and inflammation. *Med Res Rev* 2002;**22**:373–84.
136. Serenó L, McKee A, Simic G. A novel GSK-3 β inhibitor reduces Alzheimer's pathology and rescues neuronal loss *in vivo* related papers. *Neurobiol Dis* 2009;**35**:359–67.
137. Hampel H, Ewers M, Bürger K, Annas P, Mörtberg A, Bogstedt A, et al. Lithium trial in Alzheimer's disease: a randomized, single-blind, placebo-controlled, multicenter 10-week study. *J Clin Psychiatry* 2009;**70**:922–31.
138. Gao C, Hlscher C, Liu Y, Li L. GSK3: a key target for the development of novel treatments for type 2 diabetes mellitus and Alzheimer's disease. *Rev Neurosci* 2012;**23**:1–11.
139. Bhat RV, Haeblerlein SLB. Glycogen synthase kinase 3: a drug target for CNS therapies. *J Neurochem* 2004;**89**:1313–7.
140. Wen Y, Planel E, Herman M, Figueroa HY, Wang L, Liu L, et al. Interplay between cyclin-dependent kinase 5 and glycogen synthase kinase 3; mediated by neuregulin signaling leads to differential effects on tau phosphorylation and amyloid precursor protein processing. *J Neurosci* 2008;**28**:2624–32.
141. Green KN, Steffan JS, Martinez-coria H, Sun X, Schreiber SS, Thompson LM, et al. Nicotinamide restores cognition in Alzheimer's disease transgenic mice *via* a mechanism involving sirtuin inhibition and selective reduction of Thr231-phosphatase. *J Neurosci* 2008;**28**:11500–10.
142. Perry EK, Perry RH, Blessed RH, Tomlinson BE. Changes in brain cholinesterase in senile dementia of Alzheimer type. *Neuropathol Appl Neurobiol* 1978;**4**:273–7.
143. Perry EK, Tomlinson BE, Blessed G, Bergmann K, Gibson PH, Perry RH. Correlation of cholinergic abnormalities with senile plaques and mental test scores in senile dementia. *Br Med J* 1978;**2**:1457–9.
144. Author T, Society BG, Permissions F, Address UK. Acetylcholinesterase inhibitors for Alzheimer's disease: anti-inflammatories in acetylcholine clothing. *Age Ageing* 2006;**35**:336–8.
145. Sharma K. Cholinesterase inhibitors as Alzheimer's therapeutics (Review) *Mol Med Rep* 2019;**20**:1479–87.
146. Stanciu GD, Luca A, Rusu RN, Bild V, Chiriac SIB, Solcan C, et al. Alzheimer's disease pharmacotherapy in relation to cholinergic system involvement. *Biomolecules* 2020;**10**:40.
147. Coşar ED, Dincel ED, Demiray S, Sucularlı E, Tüccaroğlu E, Özsoy N, et al. Anticholinesterase activities of novel indole-based hydrazide-hydrazone derivatives: design, synthesis, biological evaluation, molecular docking study and *in silico* ADME prediction. *J Mol Struct* 2022;**1247**:131398.
148. Weinstock M. Selectivity of cholinesterase inhibition: clinical implications for the treatment of Alzheimer's disease. *CNS Drugs* 1999;**12**:307–23.
149. Silman I, Sussman JL. Acetylcholinesterase: 'classical' and 'non-classical' functions and pharmacology. *Curr Opin Pharmacol* 2005;**5**:293–302.
150. Bartus RT, Dean RL, Lippa A. The cholinergic hypothesis of geriatric memory dysfunctions. *Science* 1982;**217**:408–17.
151. Luo W, Li YP, He Y, Huang SL, Tan JH, Ou TM, et al. Design, synthesis and evaluation of novel tacrine-multialkoxybenzene hybrids as dual inhibitors for cholinesterase and amyloid beta aggregation. *Bioorg Med Chem* 2011;**19**:763–70.
152. Akrami H, Mirjalili BF, Khoobi M, Nadri H, Moradi A, Sakhteman A, et al. Indolinone-based acetylcholinesterase inhibitors: synthesis, biological activity and molecular modelling. *Eur J Med Chem* 2014;**84**:375–81.
153. Gupta S, Pandey A, Tyagi A, Mohan GA. Computational analysis of Alzheimer's disease drug targets. *Curr Res Inf Pharm Sci* 2010;**11**:1–10.
154. Santucci AC, Kanof PD, Haroutunian V. Effect of physostigmine on memory consolidation and retrieval processes in intact and nucleus basalis-lesioned rats. *Psychopharmacol* 1989;**99**:70–4.

155. Hirst WD, Abrahamsen B, Blaney FE, Calver AR, Aloj L, Price GW, et al. Differences in the central nervous system distribution and pharmacology of the mouse 5-hydroxytryptamine-6 receptor compared with rat and human receptors investigated by radioligand binding, site-directed mutagenesis, and molecular modelling. *Mol Pharmacol* 2003;**64**:1295–308.
156. Parsons CG, Danysz W, Quack G. Memantine is clinically well tolerated. *Pharmacol Res* 1999;**38**:735–67.
157. Wang R, Reddy PH. Role of glutamate and NMDA receptors in Alzheimer's disease. *J Alzheimers Dis* 2017;**57**:1041–8.
158. Zhou Q, Sheng M. NMDA receptors in nervous system diseases. *Neuropharmacol* 2013;**74**:69–75.
159. Sanz-clemente A, Nicoll RA, Roche KW. Review diversity in NMDA receptor composition: many regulators, many consequences. *Neuroscientist* 2013;**19**:61–75.
160. Zhang Y, Li P, Feng J, Wu M. Dysfunction of NMDA receptors in Alzheimer's disease. *Neurol Sci* 2016;**37**:1039–47.
161. Henson MA, Roberts AC, Salimi K, Vadlamudi S, Hamer RM, Gilmore JH, et al. Developmental regulation of the NMDA receptor subunits, NR3A and NR1, in human prefrontal cortex. *Cereb Cortex* 2008;**18**:2560–73.
162. Campos-Pea V, Antonio M. Alzheimer disease: the role of A β in the glutamatergic system. *Neurochem* 2014;**1**:150.
163. Liu W, Jiang X, Zu Y, Yang Y, Liu Y, Sun X, et al. A comprehensive description of GluN2B-selective N-methyl-D-aspartate (NMDA) receptor antagonists. *Eur J Med Chem* 2020;**200**:112447.
164. Kelsch W, Li Z, Eliava M, Goengrich C, Monyer H. GluN2B-containing NMDA receptors promote wiring of adult-born neurons into olfactory bulb circuits. *J Neurosci* 2012;**32**:12603–11.
165. Zhang Y, Li P, Feng J, Wu M. Dysfunction of NMDA receptors in Alzheimer's disease. *Neurol Sci* 2016;**37**:1039–47.
166. Hansen KB, Ogden KK, Yuan H, Traynelis SF. Distinct functional and pharmacological properties of triheteromeric GluN1/GluN2A/GluN2B NMDA receptors. *Neuron* 2014;**81**:1084–96.
167. Matsuno H, Ohi K, Hashimoto R, Yamamori H, Yasuda Y, Fujimoto M, et al. A naturally occurring null variant of the NMDA type glutamate receptor NR3B subunit is a risk factor of schizophrenia. *PLoS One* 2015;**10**:e0116319.
168. Olivares DK, Deshpande V, Shi YK, Lahiri DH, Greig NT, Rogers J, et al. N-Methyl D-aspartate (NMDA) receptor antagonists and memantine treatment for Alzheimer's disease, vascular dementia, and Parkinson's disease. *Curr Alzheimer Res* 2013;**9**:746–58.
169. Farlow MR. NMDA receptor antagonists. A new therapeutic approach for Alzheimer's disease. *Geriatrics* 2004;**59**:22–7.
170. Gussago C, Casati M, Ferri E, Arosio B. The triggering receptor expressed on myeloid cells-2 (trem-2) as an expression of the relationship between microglia and Alzheimer's disease: a novel marker for a promising pathway to explore. *J Frailty Aging* 2019;**8**:54–6.
171. Mecca C, Giambanco I, Donato R, Arcuri C. Microglia and aging: the role of the TREM2–DAP12 and CX3CL1–CX3CR1 axes. *Int J Mol Sci* 2018;**19**:318.
172. Aggarwal A, Mehta S, Gupta D, Sheikh S, Pallagatti S, Singh R, et al. Clinical & immunological erythematous patients characteristics in systemic lupus Maryam. *J Dent Educ* 2012;**76**:1532–9.
173. Carmona S, Zahs K, Wu E, Dakin K, Bras J, Guerreiro R. The role of TREM2 in Alzheimer's disease and other neurodegenerative disorders. *Lancet Neurol* 2018;**17**:721–30.
174. Jay TR, Von Saucken VE, Landreth GE. TREM2 in neurodegenerative diseases. *Mol Neurodegener* 2017;**12**:56.
175. Cuyvers E, Sleegers K. Genetic variations underlying Alzheimer's disease: evidence from genome-wide association studies and beyond. *Lancet Neurol* 2016;**15**:857–68.
176. Yu J, Jiang T, Wang Y, Wang H, Zhang W, Hu N, et al. Triggering receptor expressed on myeloid cells 2 variant is rare in late-onset Alzheimer's disease in Han Chinese individuals. *Neurobiol Aging* 2014;**35**:2013–5.
177. Schlepckow K, Kleinberger G, Fukumori A, Feederle R, Lichtenthaler SF, Steiner H, et al. An Alzheimer-associated TREM2 variant occurs at the ADAM cleavage site and affects shedding and phagocytic function. *EMBO Mol Med* 2017;**9**:1356–65.
178. Mehrjoo Z, Najmabadi A, Abedini SS, Mohseni M, Kamali K, Najmabadi H, et al. Association study of the TREM2 gene and identification of a novel variant in exon 2 in Iranian patients with late-onset Alzheimer's disease. *Med Princ Pract* 2015;**24**:351–4.
179. Miyashita A, Wen Y, Kitamura N, Matsubara E, Kawarabayashi T, Shoji M, et al. Lack of genetic association between TREM2 and late-onset Alzheimer's disease in a Japanese population. *J Alzheimers Dis* 2014;**41**:1031–8.
180. Bradshaw EM, Chibnik LB, Keenan BT, Ottoboni L, Raj T, Tang A, et al. CD33 Alzheimer's disease locus: altered monocyte function and amyloid biology. *Nat Neurosci* 2013;**16**:848–50.
181. Lambert J, Heath S, Even G, Campion D, Sleegers K, Hiltunen M, et al. Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. *Nat Genet* 2009;**41**:1094–9.
182. Poliani PL, Gilfillan S, Colonna M, Poliani PL, Wang Y, Fontana E, et al. TREM2 sustains microglial expansion during aging and response to demyelination. *J Clin Invest* 2015;**125**:2161–70.
183. Colonna M, Wang Y. TREM2 variants: new keys to decipher Alzheimer disease pathogenesis. *Nat Rev Neurosci* 2016;**17**:201–7.
184. Kleinberger G, Yamanishi Y, Suárez-Calvet M, Czirr E, Lohmann E, Cuyvers E, et al. TREM2 mutations implicated in neurodegeneration impair cell surface transport and phagocytosis. *Sci Transl Med* 2014;**6**:243ra86–243ra86.
185. Ito H, Hamerman JA. TREM-2, a triggering receptor expressed on myeloid cell-2, negatively regulates TLR responses in dendritic cells. *Eur J Immunol* 2012;**42**:176–85.
186. Feuerbach D, Schindler P, Barske C, Joller S, Beng-Louka E, Worringer KA, et al. ADAM17 is the main sheddase for the generation of human triggering receptors expressed in myeloid cells (hTREM2) ectodomain and cleaves TREM2 after histidine. *Neurosci Lett* 2017;**660**:109–14.
187. Ridha BH, Wherrett J, Hemelsoet D, Bleecker JL De, Magy L, Keen RW, et al. The genetic causes of basal ganglia calcification, dementia, and bone cysts. *Neurology* 2005;**64**:1502–7.
188. Paloneva J, Autti T, Raininko R, Partanen J, Salonen O, Puranen M, et al. CNS manifestations of Nasu–Hakola disease frontal dementia with bone cysts. *Neurology* 2001;**56**:1552–8.
189. Holton P, Ryten M, Nalls M, Trabzuni D, Weale ME, Hernandez D, et al. Initial Assessment of the pathogenic mechanisms of the recently identified Alzheimer risk loci. *Ann Hum Genet* 2013;**77**:85–105.
190. Takegahara N, Takamatsu H, Toyofuku T, Tsujimura T, Okuno T, Yukawa K, et al. Plexin-A1 and its interaction with DAP12 in immune responses and bone homeostasis. *Nat Cell Biol* 2006;**8**:615–22.
191. Guerreiro R, Wojtas A, Bras J, Carrasquillo M, Rogaeva E, Majounie E, et al. TREM2 variants in Alzheimer's disease. *N Engl J Med* 2013;**368**:117–27.
192. Deczkowska A, Keren-Shaul H, Weiner A, Colonna M, Schwartz M, Amit I. Disease-associated microglia: a universal immune sensor of neurodegeneration. *Cell* 2018;**173**:1073–81.
193. Ozben T, Ozben S. Neuro-inflammation and anti-inflammatory treatment options for Alzheimer's disease. *Clin Biochem* 2019;**72**:87–9.
194. Schoepp NG, Khorosheva EM, Schlappi TS, Curtis MS, Humphries RM, Angeles L, et al. TREM2 haploinsufficiency in mice and humans impairs the microglia barrier function leading to decreased amyloid compaction and severe axonal dystrophy. *Neuron* 2017;**55**:9557–61.
195. Gonzalez JD, Schmutz C, Munger C, Perkes A, Gustin A, Peterson M, et al. Assessment of TREM2 rs75932628 association with Alzheimer's disease in a population-based sample: the Cache County Study. *Neurobiol Aging* 2013;**36**:2587–96.
196. Piccio L, Deming Y, Del JL, Laura Á, Holtzman DM, Fagan AM, et al. Cerebrospinal fluid soluble TREM2 is higher in Alzheimer's

- disease and associated with mutation status. *Acta Neuropathol* 2016; **131**:925–33.
197. Suárez-calvet M, Kleinberger G, Ángel M, Caballero A, Brendel M, Rominger A, et al. sTREM 2 cerebrospinal fluid levels are a potential biomarker for microglia activity in early-stage Alzheimer's disease and associate with neuronal injury markers. *EMBO Mol Med* 2016; **8**: 466–76.
 198. Reiner AP, Beleza S, Franceschini N, Auer PL, Robinson JG, Kooperberg C, et al. Genome-wide association and population genetic analysis of c-reactive protein in African American and Hispanic American women. *Am J Hum Genet* 2012; **91**:502–12.
 199. Stefano L, Racchetti G, Bianco F, Passini N, Gupta RS, Bordignon PP, et al. The surface-exposed chaperone, Hsp60, is an agonist of the microglial TREM2 receptor. *J Neurochem* 2009; **110**:284–94.
 200. Hsieh CL, Koike M, Spusta SC, Niemi EC, Yenari M, Nakamura MC, et al. A role for TREM2 ligands in the phagocytosis of apoptotic neuronal cells by microglia. *J Neurochem* 2009; **109**:1144–56.
 201. Tan MS, Yu JT, Tan L. Bridging integrator 1 (BIN1): form, function, and Alzheimer's disease. *Trends Mol Med* 2013; **19**:594–603.
 202. Goate AM, Karch CM, Jeng AT, Nowotny P, Candy J, Cruchaga C. Expression of novel Alzheimer's disease risk genes in control and Alzheimer's disease brains. *PLoS One* 2012; **7**:e50976.
 203. Wechsler-Reya R, Sakamuro D, Zhang J, Duhadaway J, Prendergast GC. Structural analysis of the human BIN1 gene: evidence for tissue-specific transcriptional regulation and alternate RNA splicing. *J Biol Chem* 1997; **272**:31453–8.
 204. Pineda-Lucena A, Ho CS, Mao DY, Sheng Y, Laister RC, Muhandiram R, et al. A structure-based model of the c-Myc/Bin1 protein interaction shows alternative splicing of Bin1 and c-Myc phosphorylation are key binding determinants. *J Mol Biol* 2005; **351**:182–94.
 205. Ren G, Vajjhala P, Lee JS, Winsor B, Munn AL. The BAR domain proteins: molding membranes in fission, fusion, and phagy. *Microbiol Mol Biol Rev* 2006; **70**:37–120.
 206. Sakamuro D, Wechsler-Reya R, Prendergast GC. BIN1 is a novel MYC-interacting protein with features of a tumor suppressor. *Nat Genet* 1996; **14**:69–77.
 207. Muller AJ, Baker JF, DuHadaway JB, Ge K, Farmer G, Donover PS, et al. Targeted disruption of the murine bin1/amphiphysin II gene does not disable endocytosis but results in embryonic cardiomyopathy with aberrant myofibril formation. *Mol Cell Biol* 2003; **23**:4295–306.
 208. Meunier B, Quaranta M, Daviet L, Hatzoglou A, Leprince C. The membrane-tubulating potential of amphiphysin 2/BIN1 is dependent on the microtubule-binding cytoplasmic linker protein 170 (CLIP-170). *Eur J Cell Biol* 2009; **88**:91–102.
 209. Galderisi U, Di Bernardo G, Cipollaro M, Jori FP, Piegari E, Cascino A, et al. Induction of apoptosis and differentiation in neuroblastoma and astrocytoma cells by the overexpression of Bin1, a novel Myc interacting protein. *J Cell Biochem* 1999; **74**:313–22.
 210. Elliott K, Ge K, Du W, Prendergast GC. The c-Myc-interacting adaptor protein Bin1 activates a caspase-independent cell death program. *Oncogene* 2000; **19**:4669–84.
 211. Wajapeyee N, Serra RW, Zhu X, Mahalingam M, Green MR. Oncogenic BRAF induces senescence and apoptosis through pathways mediated by the secreted protein IGFBP7. *Cell* 2008; **132**:363–74.
 212. Lambert JC, Amouyel P. Genetics of Alzheimer's disease: new evidences for an old hypothesis?. *Curr Opin Genet Dev* 2011; **21**: 295–301.
 213. Carter C. Alzheimer's disease: APP, gamma-secretase, APOE, CLU, CR1, PICALM, ABCA7, BIN1, CD2AP, CD33, EPHA1, and MS4A2, and their relationships with herpes simplex, *C. pneumoniae*, other suspect pathogens, and the immune system. *Int J Alzheimers Dis* 2011; **1**:501862.
 214. Schlepckow K, Kleinberger G, Fukumori A, Feederle R, Lichtenthaler SF, Steiner H, et al. TREM-2, triggering receptor expressed on myeloid cell-2, negatively regulates TLR responses in dendritic cells. *EMBO Mol Med* 2017; **9**:1356–65.
 215. Chapuis J, Hansmann F, Gistelink M, Mounier A, Van Cauwenbergh C, Kolen KV, et al. Increased expression of BIN1 mediates Alzheimer's genetic risk by modulating tau pathology. *Mol Psychiatry* 2013; **18**:1225–34.
 216. Yu CJ, Zheng MF, Kuang CX, Huang W Da, Yang Q. Oren-gedokuto and its constituents with therapeutic potential in Alzheimer's disease inhibit indoleamine 2,3-dioxygenase activity *in vitro*. *J Alzheimers Dis* 2010; **22**:257–66.
 217. Anekonda TS, Quinn JF. Calcium channel blocking as a therapeutic strategy for Alzheimer's disease: the case for isradipine. *Biochim Biophys Acta, Mol Basis Dis* 2011; **1812**:1584–90.
 218. Funderburk SF, Marcellino BK, Zhenyu YP. Cell “self-eating” (autophagy) mechanism in Alzheimer's disease. *J Transl Pers Med* 2010; **77**:59–68.
 219. Sabbagh MN, Decourt B. COR388 (atuzaginstat): an investigational ginkgipain inhibitor for the treatment of Alzheimer's disease. *Expert Opin Investig Drugs* 2022; **31**:987–93.
 220. Ou YN, Zhu JX, Hou XH, Shen XN, Xu W, Dong Q, et al. Associations of infectious agents with Alzheimer's disease: a systematic review and meta-analysis. *J Alzheimers Dis* 2020; **75**:299–309.
 221. Renvoize EB, Awad LO, Hambling MH. A sero-epidemiological study of conventional infectious agents in Alzheimer's disease. *Age Ageing* 1987; **16**:311–4.
 222. Honjo K, van Reekum R, Verhoeff NP. Alzheimer's disease and infection: do infectious agents contribute to the progression of Alzheimer's disease?. *Alzheimers Dement* 2009; **5**:348–60.
 223. Zhao Y, Jolly S, Benvegnu S, Yvonne Jones E, Fish PV. Small-molecule inhibitors of carboxylesterase Notum. *Future Med Chem* 2021; **13**:1001–15.
 224. Mahy W, Willis NJ, Zhao Y, Woodward HL, Svensson F, Siphthorp J, et al. 5-Phenyl-1,3,4-oxadiazol-2(3H)-ones are potent inhibitors of notum carboxylesterase activity identified by the optimization of a crystallographic fragment screening hit. *J Med Chem* 2020; **63**:12942–56.
 225. Kakugawa S, Langton PF, Zebisch M, Howell S, Liu Y, Feizi T, et al. Europe PMC funders group notum deacylates Wnts to suppress signalling activity Europe PMC funders author manuscripts. *PMC* 2015; **519**:187–92.
 226. Palomer E, Buechler J, Salinas PC. Wnt signaling deregulation in the aging and Alzheimer's brain. *Front Cell Neurosci* 2019; **13**:227.
 227. Madan B, Ke Z, Lei ZD, Oliver FA, Oshima M, Lee MA, et al. NOTUM is a potential pharmacodynamic biomarker of Wnt pathway inhibition. *Oncotarget* 2016; **7**:12386–92.
 228. Gerlitz O, Basler K. Wingful, an extracellular feedback inhibitor of Wntless. *Genes Dev* 2002; **16**:1055–9.
 229. Giraldez AJ, Copley RR, Cohen SM. HSPG modification by the secreted enzyme Notum shapes the Wntless morphogen gradient. *Dev Cell* 2002; **2**:667–76.
 230. Bayle ED, Svensson F, Atkinson BN, Steadman D, Willis NJ, Woodward HL, et al. Carboxylesterase notum is a druggable target to modulate wnt signaling. *J Med Chem* 2021; **64**:4289–311.
 231. Kreuger J, Perez L, Giraldez AJ, Cohen SM. Opposing activities of Dally-like glypican at high and low levels of wingless morphogen activity. *Dev Cell* 2004; **7**:503–12.
 232. Zhao Y, Svensson F, Steadman D, Frew S, Monaghan A, Bictash M, et al. Structural insights into Notum covalent inhibition. *J Med Chem* 2021; **64**:11354–63.
 233. Suciu RM, Cognetta AB, Potter ZE, Cravatt BF. Selective irreversible inhibitors of the Wnt-deacylating enzyme notum developed by activity-based protein profiling. *ACS Med Chem Lett* 2018; **9**: 563–8.
 234. Pentimikko N, Iqbal S, Mana M, Andersson S, Iii ABC, Suciu RM, et al. Notum produced by paneth cells attenuates regeneration of aged intestinal epithelium. *Nature* 2019; **571**:398–402.
 235. Mizrak D, Bayin NS, Yuan J, Liu Z, Suciu RM, Niphakis MJ, et al. Single-cell profiling and scope-seq reveal lineage dynamics of adult ventricular-subventricular zone neurogenesis and notum as a key regulator. *Cell Rep* 2020; **31**:107805.
 236. Zhao Y, Ren J, Hillier J, Jones M, Lu W, Jones EY. Structural characterization of melatonin as an inhibitor of the Wnt deacylase Notum. *J Pineal Res* 2020; **68**:e12630.