Current and Emerging Pharmacological Targets for the Treatment of Alzheimer’s Disease

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Abstract. No cure or disease-modifying therapy for Alzheimer’s disease (AD) has yet been realized. However, a multitude of pharmacological targets have been identified for possible engagement to enable drug discovery efforts for AD. Herein, we review these targets comprised around three main therapeutic strategies. First is an approach that targets the main pathological hallmarks of AD: amyloid-β (Aβ) oligomers and hyperphosphorylated tau tangles which primarily focuses on reducing formation and aggregation, and/or inducing their clearance. Second is a strategy that modulates neurotransmitter signaling. Comprising this strategy are the cholinesterase inhibitors and N-methyl-D-aspartate receptor blockade treatments that are clinically approved for the symptomatic treatment of AD. Additional targets that aim to stabilize neuron signaling through modulation of neurotransmitters and their receptors are also discussed. Finally, the third approach comprises a collection of ‘sensitive targets’ that indirectly influence Aβ or tau accumulation. These targets are proteins that upon Aβ accumulation in the brain or direct Aβ-target interaction, a modification in the target’s function is induced. The process occurs early in disease progression, ultimately causing neuronal dysfunction. This strategy aims to restore normal target function to alleviate Aβ-induced toxicity in neurons. Overall, we generally limit our analysis to targets that have emerged in the last decade and targets that have been validated using small molecules in in vitro and/or in vivo models. This review is not an exhaustive list of all possible targets for AD but serves to highlight the most promising and critical targets suitable for small molecule drug intervention.

Keywords: Alzheimer’s disease, amyloid, drug discovery, therapeutics, toxicity

INTRODUCTION

Statistical projections estimate that 14 million Americans will be living with Alzheimer’s disease (AD) by 2050 [1]. This level of affliction will raise the economic costs to care for AD patients to a projected $1.1 trillion [1]. These numbers, representing both patient and family suffering and economic impact, greatly highlight the importance of AD research for the identification of disease-modifying therapy. It has been more than a century since the disease was identified in 1906 by Alois Alzheimer, a clinical psychiatrist and neuroanatomist who reported: “A peculiar severe disease process of the cerebral cortex” [2]. His report noted protein...
aggregation intracellularly and extracellularly. These proteins were later identified to be neurofibrillary tangles which accumulated inside of neuron cells and amyloid-β (Aβ) plaques which aggregate between the neurons [3, 4]. Drug discovery research in AD has proven to be highly challenging, and no cure has been identified. However, expanding research knowledge has translated to the clinic, allowing for changes to the criteria of diagnosing patients of AD into three main stages. An early, preclinical stage with no symptoms, a middle stage of mild cognitive impairment (MCI) and a final stage marked by symptoms of dementia [5]. These stages go beyond the possibility or probability criteria historically used. These criteria were determined by neuropsychological testing or a diagnosis of exclusion [6], yet failed to detect the early stages of AD, the pre-symptomatic phase. This latter phase is believed to be the stage where AD progresses and initiates the neurodegenerative process of inflammation and brain atrophy that is identified in the later stages of MCI and dementia [7]. The new criteria to diagnose AD employs biomarkers that quantify the accumulation of the pathogenic proteins, Aβ or tau, to understand their role in disease progression. These same biomarkers are also used in clinical trials to evaluate the effect of pharmacological interventions. [8, 9]. Examples of currently available biomarkers include measuring levels of Aβ in cerebrospinal fluid (CSF), amyloid imaging-positron emission tomography (PET), fluorodeoxyglucose-PET scanning, or measuring levels of tau protein in CSF [10]. Further research is warranted to identify more sensitive and accurate biomarkers for AD and for additional information regarding this area of research the reader is directed to a number of excellent reviews summarizing the subject [11, 12].

AMYLOID AND TAU FORMATION

The two pathological hallmarks of AD are the accumulation of insoluble oligomers of Aβ protein, known as Aβ plaques and the intracellular accumulation of the microtubule-associated protein tau, known as neurofibrillary tangles. The formation of Aβ begins through a transmembrane protein, amyloid-β protein precursor (AβPP), which is sequentially cleaved by the aspartate proteases β- and γ-secretase, that leads to the formation of Aβ peptide (1–42) and a degenerated C-terminus (Fig. 1) [13–15]. Amyloid then aggregates to form dimers, tetramers, and oligomers [16, 17]. The deposition of Aβ plaques extracellularly hinders neurotransmitter signaling between neurons causing synaptic dysfunction [18]. AβPP can also be cleaved by α-secretase which leads to the formation of soluble AβPPα (sAβPPα), a neuroprotective fragment [19]. Further cleavage of sAβPPα by γ-secretase in the plasma membrane compartments before its release to the extracellular space leads to the formation to Aβ peptide (1–40) [20, 21], which has a pathological function in modulating synaptic function and repairing leaks in the blood-brain barrier (BBB) [22, 23]. However, a definitive role of this pathological protein is not fully understood. It is now accepted that the oligomer form of Aβ42 is the most toxic species and correlates well with severity of the disease [16]. In addition to Aβ, the hyperphosphorylated tau protein which forms the neurofibrillary tangles is neurotoxic [24]. Tau protein acts as a physiological stabilizer to neuronal microtubules and contributes to axon stability and overall neuronal function [25]. In AD, increased levels or dysfunction of candidate kinases such as cyclin-dependent kinase 5 (CDK5) and glycogen synthase kinase-3 beta (GSK3β) activity leads to the hyperphosphorylation of tau which causes its dissociation from microtubules and its accumulation to tangles (Fig. 2) [26, 27]. In the early stages of AD, cognitive decline occurs at the same time that tau tangles are seen to accumulate in patients [28, 29].

The focus of this review is to highlight current and emerging therapeutic targets for AD. Strategies towards identifying a drug that can halt the progression of AD can be split into three main categories:

1. Strategies that act on the pathological proteins, Aβ and tau, with the aim to inhibit their...
formation, aggregation or induce their clearance.

2. Modulation of the function of neurotransmitters or their receptors to stabilize neuron health and downstream cascades.

3. Identification of protein targets that are sensitive to Aβ-induced toxicity and by restoring, or protecting, the function of the target, this strategy aims to alleviate AD pathology.

In reality, the drug that can achieve all three strategies would be optimal, but until (or if) then, a combination therapy may be the desired approach in the search for a treatment [30].

STRATEGIES THAT ACT ON PATHOGENIC PROTEINS

Novel therapeutic candidates aimed toward inhibition of Aβ aggregation or tau accumulation have been known for decades. These approaches, represented by a large number of novel chemical entities, are currently in the preclinical and clinical trial stages. However, of those that have completed early stage clinical trials, most fail to translate to therapeutic value. Pharmacological targets that are currently being investigated include targets that inhibit Aβ protein aggregation, immunotherapeutic targets that induce inflammation response, antibodies that degrade specific aggregate formation, a decrease of Aβ formation through modulation of an enzyme’s activity, regulating Aβ transport, enhancing Aβ-degrading enzymes, immunotherapy against tau aggregation and blockage of tau hyperphosphorylation.

Inhibition of Aβ protein aggregation

Neurons are continuously producing Aβ in small quantities in various forms, the increase in Aβ₄₂ production via an increase in β- and γ-secretase activity or by a decrease in degradation by Aβ-degrading enzymes such as prolyl endopeptidase (PreP) and insulin-degrading enzyme (IDE) induces the tendency to form oligomeric aggregates [31–33]. This oligomeric form causes synaptic dysfunction and leads to the neurodegeneration seen in AD. Due to an incomplete understanding of the mechanism of oligomer formation, very few small molecules that can inhibit aggregation have moved into clinical studies [34]. Lead compounds such as 2-amino-4-chlorophenol, 4-aminophenol, 4-aminoanisole, 3,4-dihydroxybenzoic acid, and 2-hydroxy-3-ethoxy-benzaldehyde inhibit aggregate formation (Fig. 3) [35]. Others, that act by binding to Aβ monomers and maintaining them in a non-fibrillar form, as an example, the small molecule...
Fig. 3. Structures of small molecules that act to inhibit Aβ aggregation.

Tramiprosate (Fig. 3) progressed into two large Phase III trials but did not demonstrate efficacy in improving cognitive function [36, 37]. Further analysis of the data from one of the latter trials revealed the beneficial effect of Tramiprosate in stabilizing cognitive performance for 78 weeks of testing in patients with mild cognitive dysfunction and carrying a homozygous form of Apolipoprotein E (ApoE4) mutation, a risk factor gene for late-onset AD [38]. The drug, however, showed unwanted side effects leading to the development of an L-Valyl ester prodrug, ALZ-801 (Fig. 3) [39]. The prodrug improved the pharmacokinetic properties of Tramiprosate, and it proved more tolerable in a phase I safety study [40]. A phase III trial of ALZ-801 is due to start recruiting shortly with a dose of 265 mg twice daily [40]. Naturally occurring polyphenolic compounds prevent Aβ aggregation and attenuate cognitive deterioration; as an example, Luteolin and Transilin were identified as potent inhibitors of Aβ fibril formation (Fig. 3) [41]. Further, small molecules such as resveratrol, coumarin, and D737 reverse the structure of the soluble oligomer of Aβ and inhibit its aggregation (Fig. 3) [42–44].

The oligomeric form of Aβ42 has been widely studied as being the toxic form [45]. However, whether inhibiting aggregate formation is a possible therapeutic target or not, is still debated. For instance, Elnd005, an endogenous inositol stereoisomer (Fig. 3) [46], acts by binding and inhibiting the aggregation of Aβ42 peptide. The small molecule showed in vitro studies its activity to inhibit Aβ42 aggregation and formation of oligomers [47, 48]. Moreover, in transgenic mice, Elnd005 preserved synaptic density and improved learning deficits, making the molecule a good candidate for a clinical trial [49]. However, the molecule failed to reach desired endpoints in trials, not showing an improvement in behavioral or cognitive functions compared to placebo (NCT01735630) [50]. In summary, one can speculate that generating a potent small molecule that targets aggregation of the oligomeric form may not be feasible. Alternatively, it may be the case that the mechanism of action of protein oligomerization needs further validation to aid in design of such a potent small molecule.

Immunotherapy against Aβ using active immunization

Active immunization that can stimulate the host immune response toward clearing Aβ deposits in the brain has recently gained traction. In the past two decades, studies have shown that live vaccines do not
just prevent the formation of new Aβ plaques but also contribute to the elimination of existing plaques [51]. By definition, an active immunization acts by activating the host immune response toward releasing antibodies against a given antigen [52]. This adaptive immune response is accomplished in the brain by microglial activation [53]. One can argue that the function of an aging immune system in elderly patients is not stable and that instead of producing the appropriate antibodies, it can generate autoimmune side effects [54]. Moreover, microglia function is not entirely understood in the human brain [55]. This speculation makes the approach of active immunization highly challenging. Despite these issues, numerous studies have been established in the search for active immunization therapy. One of which is CAD106, a novel immunotherapy designed to stimulate the generation of antibodies against a small Aβ peptide fragment (Aβ1-6) and block subsequent Aβ accumulation. The peptide is then coupled to a carrier that contains 180 copies of the coat protein of bacteriophage Qβ, to induce the immune response. In AβPP transgenic mice, CAD106 showed clearance of Aβ plaques [56]. Further, CAD106 is currently in clinical trials to evaluate its efficacy in AD subjects at risk for onset of clinical symptoms in an attempt to halt the disease before progression (NCT02565511) [57]. Other active vaccines are in development, such as MER5101, which has been successful in preclinical studies [58] and Lu AF20513 which is currently in clinical trials (NCT02388152) [59].

**Immunotherapy using passive immunization**

Among AD phase III clinical trial studies, passive immunization is the most widely investigated approach. It was first considered when Bapineuzumab, a humanized anti-Aβ monoclonal antibody directed against the Aβ N-terminus was developed [60]. Although phase II trials were questionable as ApoE4 carriers did not show treatment differences from the placebo group, non-carriers showed significant improvement in cognitive and functional endpoints. The antibody proceeded to phase III, however, no success was achieved [61]. Nevertheless, several passive immunization methods are currently under investigation. Intravenous administration of immunoglobulin preparations (IVIg) containing high levels of human anti-Aβ42 antibodies which can bind to soluble Aβ and improve cognitive performance are currently in clinical trial (NCT01300728) [62].

Extensive efforts are underway to identify monoclonal antibodies that can bind to Aβ protein or its plaques. The outcome of the five most recent antibodies in phase III trials is summarized in Table 1. A total of 23 phase III clinical trials are registered to study antibody effect to improve clinical function or slow disease progression. Of the 23 trials, 16 completed trials failed to define a clinical benefit and seven others are still ongoing. The antibodies worked as intended and reduced Aβ accumulation in the brain. However, no cognitive benefit to patients was observed. With

<table>
<thead>
<tr>
<th>Drug Name (dose)</th>
<th>Stage of Disease</th>
<th>Outcome (Ongoing Trial identifier)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bapineuzumab (0.5 or 1 mg/kg every 13 weeks)</td>
<td>Mild-to-Moderate (ApoE4 carriers and non-carriers)</td>
<td>No cognitive or functional improvement after treatment in seven phase III trials. Antibody engaged its target but with no benefit.</td>
<td>[61, 63]</td>
</tr>
<tr>
<td>Solanezumab (400 mg once a month)</td>
<td>At risk, Prodromal and Mild-to-Moderate Stages</td>
<td>No treatment related benefit from five phase III trials. The antibody is being tested in two ongoing trials to evaluate its effect on patients asymptomatic and at risk of AD (NCT02008357, NCT01760005).</td>
<td>[64–67]</td>
</tr>
<tr>
<td>Gantenerumab (105, 225 or 1200 mg once a month)</td>
<td>At Risk, Prodromal and Mild Stages</td>
<td>Clinical outcome remains under investigation with five ongoing phase III trials (NCT03443973, NCT03444870, NCT02051608, NCT01224106, NCT01760005)</td>
<td>[68, 69]</td>
</tr>
<tr>
<td>Aducanumab (low-dose titration to 3 or 6 mg/kg based on ApoE4 carrier status, high-dose titration to 10 mg/kg)</td>
<td>Prodromal-to-Mild Stage</td>
<td>No clinical outcome from two phase III trials.</td>
<td>[70–72]</td>
</tr>
<tr>
<td>Crenezumab (60 mg/kg once a month)</td>
<td>Prodromal-to-Mild Stage</td>
<td>No clinical outcome from two phase III trials.</td>
<td>[73, 74]</td>
</tr>
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the overall disappointing results from immunotherapies in clinical trials (Table 1), perhaps, it is time to reconsider the use of passive immunization for AD.

Secretase modulators

α-secretase

The α-secretase protein emerged as a therapeutic target due to the observation that enhancing the activity of the protein facilitates proteolysis of AβPP in non-amyloidogenic pathways and result in prevention of Aβ₄₂ formation [75]. A Disintegrin and metalloprotease (ADAM) family protein regulates the activity of α-secretase [76]. The complex is membrane-bound and raising the expression of proteins such as ADAM10, ADAM17, and ADAM9 has been shown to reduce Aβ levels in the AβPP/PS-I transgenic mouse model [77]. In phase II clinical trials, Acitretin, a vitamin A analog increased the mature ADAM10 stimulation process resulting in enhanced activity of α-secretase in neuroblastoma cells [78, 79]. Improving α-secretase activity will not only reduce the amyloidogenic pathway of AβPP but will also increase the assembly of sAβPP, a potential neuroprotective protein (Fig. 1) [19, 75].

The α-secretase enhancer, Etazolate (Fig. 4), is currently in preclinical development [80–82]. Another α-secretase enhancer, the macrolide lactone Bryostatin (Fig. 4), has completed a phase II clinical trial wherein seven doses of 20 μg administered over a 12-week treatment period in moderately severe to severe AD patients, showed improvement in the severe impairment battery test versus placebo (Computer analysis set, \( p < 0.07 \)) [83]. These findings collectively demonstrate that enhancing α-secretase activity holds promise for AD drug discovery.

β-secretase

The β-secretase enzyme, known as beta-site amyloid precursor protein cleaving enzyme (BACE), is predominantly located in the intracellular acidic compartments and is a type I transmembrane aspartyl protease containing two aspartic acid residues in its active site, namely Asp32 and Asp228, present in a large hydrophobic cleft [84]. The enzyme regulates the first step in the proteolytic processing of AβPP toward forming Aβ₄₂, making it an ideal target to intervene and halt the production of toxic Aβ₄₂. The overexpression and knockdown of BACE, respectively, increased and reduced levels of Aβ₄₂. Moreover, BACE activity is increased in sporadic forms of AD [85]. Numerous compounds have been developed to target BACE activity, but only a few proceeded to clinical trials. One of which is Verubecestat, a highly active and structurally distinctive BACE inhibitor (Fig. 5). The compound has an IC₅₀ of 2.1 nM toward BACE activity in HEK293 cells expressing human AβPP [86]. The selective inhibitor proved to be safe in phase I trials, cleared Aβ deposits in phase II and was demonstrated to be well tolerated and ideal for once-a-day dosing due to a 20 h half-life [87]. However, in a randomized, placebo-controlled, parallel group and double-blind phase III clinical trial for the assessment of safety and efficacy in patients suffering from prodromal AD, the BACE inhibitor showed discouraging results. Although the compound cleared Aβ plaques from the brain, no improvement in cognitive function was observed. Moreover, when Merck announced the end of phase III trials, the results showed worsening of cognitive function in prodromal AD patients (40 mg/day for 104 weeks versus placebo, \( p = 0.01 \)) [88], and showed no prospective benefit in mild-to-moderate AD patients [89]. Thus, the continued failure of BACE inhibitors calls into question if this therapeutic target will ever yield a clinical AD drug.
γ-secretase

The γ-secretase enzyme is a protease complex protein that contains four subunits present in equal stoichiometry: presenilin 1 (PSEN1) or presenilin 2 (PSEN2), nicastrin, anterior pharynx defective 1 (APH1) and presenilin enhancer 2 [90]. The enzyme is involved in catalyzing the second step of Aβ42 formation (Fig. 1), making it an ideal target for inhibiting the formation of the toxic peptide. One crucial role for the enzyme is the formation of Notch protein that regulates cell proliferation, development, differentiation, growth, cell communication, and cell survival status [91]. This feature of the enzyme directed efforts toward the modulation of enzyme activity, rather than its inhibition. This conclusion also came to light after the failure of γ-secretase inhibitors in clinical trials. The γ-secretase modulators (GSMs) have a Notch sparing effect, and through their binding to an allosteric site, they do not interfere in the normal processivity of the enzyme. A new generation of GSMs is under development after the failure of the first generation which was attributed to their poor BBB permeability [92, 93], a necessity for any AD therapeutic [94], and undesired neuro-pharmacokinetic properties [95]. This new generation of GSMs included novel natural products identified from the extract of Actaea racemosa, the well-known botanical black cohosh and other natural products such as luteolin, curcumin and its derivatives (Fig. 3) [96–98]. Additional classes of compounds under evaluation as enzyme modulators include non-steroidal anti-inflammatory drug (NSAID)-derived carboxylic acids and non-NSAID-derived heterocyclic chemotypes [93]. An example of a GSM that is in clinical trials is NGP 555 (Fig. 6), where preclinical studies demonstrated the reduction of Aβ42 formation with improvement in cognitive function in rodents [99]. The clinical study for safety was completed, but the results are yet to be published (NCT02534480).

In summary, the activity of the family of secretases are essential in the pathophysiology of AD. However, a critical perspective here is that these enzymes play a central role in neuroplasticity through their actions on AβPP, activating or inhibiting these enzymes may not have a beneficial effect clinically. Inhibition of BACE or γ-secretase will decrease the normal non-α-catabolism of AβPP and likely produce serious problems without alleviating AD pathology. The reader is directed to the referenced review and included references therein for more in-depth analysis of secretase modulators in clinical trials [100].

Aβ transport

Clearing Aβ is another strategy that has gained attention lately after a gene mutation in chromosome 19 was first identified in 1993 [101]. The gene carried the code for ApoE, a significant carrier of apolipoprotein and cholesterol in the brain and contributes to the clearance of Aβ from the brain. There are three major human isoforms: ApoE2, ApoE3, and ApoE4. The possession of an ApoE4 allele can considerably bring forward the age of disease onset and is recognized as the major genetic risk factor for AD [102]. The difference between ApoE3 and ApoE4 is a single amino acid; ApoE4 contains an arginine residue at position 112, whereas ApoE3 has a cysteine at this position [103]. This difference between the two isoforms has raised a debate in the field as to whether E4 is a risk factor because it has gained toxic properties relative to E3, or because it has lost useful E3 function. A theoretical hypothesis for ApoE4 mutation is identifying small molecules that bind to ApoE4 and block the intramolecular domain interaction that is characteristic of this isoform, thus converting it into an ApoE3-like structure [104, 105]. This hypothesis is opposed by the finding that ApoE4 knock-in mice when cross-bred with AβPP transgenic mice, showed that ApoE4 caused less Aβ deposition than the knockout mice [106]. This reduction in Aβ level suggests that the increase in ApoE4 expression, or its remodeling, may provide an avenue for therapeutic intervention in AD [107–109]. It is likely that ApoE increases passage of Aβ from blood to brain mediated by the low-density lipoprotein receptor-related protein (LRP) and with age, the LRP expression tends to decrease, leading to the increase of Aβ deposits in the brain [110]. This hypothesis is supported by the findings of antibodies that block LRP resulting in the decrease of Aβ efflux from the brain, and that administration of soluble LRP increased the efflux [111, 112]. For further information regarding the subject of ApoE4 and its relevance in AD pathology the reader is directed to the referenced reviews [113, 114].
Another protein that is involved in Aβ transport is the receptor for advanced glycation end products (RAGE). The receptor is located in the BBB and binds to Aβ with high affinity, facilitating its entry to the central nervous system (CNS); this contributes to inflammation and neuronal death caused by Aβ accumulation [115]. Moreover, nuclear factor-κB (NF-κB) signaling pathways are activated following Aβ-RAGE interactions, which may promote apoptosis and inflammatory responses [116]. Thus, preventing Aβ from binding to RAGE has gained traction as a pharmacological target [117]. On the other hand, in clinical trials a small molecule inhibitor of RAGE, Azeliragon (Fig. 7) failed to reach the desired effect of improved cognitive function and the trial was terminated before projected completion in June 2019 (NCT02916056). The trial concluded that targeting RAGE showed no benefit to AD patients as their disease progression was not affected by the inhibitor.

Aβ degrading enzymes

Proteases that can degrade Aβ are an emerging target for AD. One such protease is neprilysin (NEP). Transgenic mice with overexpression of NEP demonstrated a decrease in plaque formation but with no cognitive improvement since it did not target the oligomeric form [118]. Moreover, the overexpression of NEP can lead to degradation of other substrates of NEP that may lead to off-target effects [119]. Another Aβ degrading enzyme, IDE, specifically targets the β-structured proteins [120]. The expression level of the protease is reduced in the early stages of AD [121]. Moreover, increasing expression of IDE has been shown to decrease plasma levels of Aβ and lower the progression rate to late-onset AD [122]. In vivo studies showed the upstream modulation of AβPP to IDE, AβPP−/− mice express high levels of mRNA, protein, and activity of IDE compared with wild-type controls [123]. Enhancing IDE activity through the use of resveratrol (Fig. 3) has been shown to increase enzyme activity towards degrad-

ing Aβ42 fragments. These data suggest the allosteric modulation of the enzyme is a valid therapeutic target for AD [124]. Decreased proteolytic activity of various proteases in in vitro studies and in AD transgenic mice has led to the identification of tens of other proteases that are involved in degrading Aβ, and for further information, the reader is directed to excellent reviews regarding the subject [125–127].

In summary, targeting Aβ as a pathogenic protein has faced challenges, and none of the approaches that aim to inhibit synthesis or increase clearance from the brain has reached the desired endpoint in clinical trials to date. However, studying Aβ and its pathological activity has moved the field closer to understanding its role in the complex pathophysiology of AD. An optimal level of Aβ is needed for the survival of the neurons and synaptic plasticity, while excessive accumulation of extracellular Aβ results in synaptic dysfunction and the hyperphosphorylation of tau [88, 128].

Targeting tau protein

Tau hyperphosphorylation and aggregation are strongly correlated with AD pathology (Fig. 2). The presence of tau marks the onset of AD symptoms which correlates with disease progression and represents the second hallmark of AD. Tau itself appears to be a major contributor to many other forms of brain disease, including Pick disease, progressive supranuclear palsy, Huntington’s disease, frontotemporal dementia with parkinsonism-17, corticobasal degeneration, and argyrophilic grain disease. Targeting tau toxicity is ultimately focused on inhibiting its dissociation from microtubules along with inhibiting its hyperphosphorylation to stabilize microtubule function [129].

Immunotherapy against tau

Similar to Aβ immunotherapy, a targeted antibody that can cross the BBB and accumulate in neurons would subsequently bind to tau aggregates, leading to a specific antibody response against phosphorylated tau protein [130]. The concept of tau immunotherapy was raised in the last decade and in vivo studies show that injection of tau oligomer monoclonal antibody in mice expressing mutant human tau, reversed tau pathology. However, no effect was observed in hyperphosphorylated tau or neurofibrillary tangles [131]. In tau transgenic mice, anti-tau oligomer passive immunization ameliorated tau toxicity and cognitive impairment [132]. These findings suggest that target-
ing the tau oligomer may be an emerging target in the treatment of AD. Active immunotherapy against tau pathology aims to stimulate the production of antibodies that target tau tangles, prevent tau aggregation and promote its clearance. In clinical trials, AADvac1 live vaccine demonstrated safety and tolerability in a phase I trial and triggered antibody production [133]. Further phase II clinical trials are currently on-going in patients with mild AD (NCT03174886). For more information regarding tau immunotherapy, the reader is directed to a review that summarizes the progress of this approach [134].

Targeting tau phosphorylation

Elevated levels of kinases lead to the hyperphosphorylation of tau and microtubule instability. Hence, inhibiting such kinases has evolved as a pharmacological strategy in the treatment of AD. The kinase GSK3\(^\beta\) is one of the most studied kinases that is involved in tau phosphorylation. It is well established now that GSK3\(^\beta\) phosphorylates Ser/Thr moieties within tau, leading to its hyperphosphorylation and aggregation which ultimately leads to tau tangle formation [135]. A phase III clinical trial performed with the GSK3\(^\beta\) inhibitor, Tideglusib, however, showed no clinical benefit in AD patients [136]. Different classes of GSK3\(^\beta\) inhibitors are being studied to elucidate their effect in AD, and for more information regarding the subject, the reader is directed to referenced review [135]. Another kinase that is well known to be involved in tau phosphorylation is CDK5; P25 and P35 neuron-specific activators enhance its activity. The calcium-dependent calpain protease leads to the progression of P25 and P35. This signaling leads to the phosphorylation of tau by CDK5. Tamoxifen (Fig. 8) has been identified to regulate the kinase function by inhibiting the activation of the kinase by p25 signaling [137]. For information regarding CDK5 and its role in AD, the reader is directed to referenced review [138]. Other molecules have also been studied to assess their ability to inhibit tau aggregation. One such compound is the methylene blue derivative, LMTM. However, a phase III trial to evaluate its efficacy in the treatment of mild to moderate AD patients showed no improvement in cognitive function [139]. A selection of emerging kinases that represent possible targets for AD is summarized in Table 2. These kinases show a direct link to tau phosphorylation and aggregation and further correlation with the cognitive dysfunction in AD. Despite the clear understanding of the function of candidate kinases such as GSK3\(^\beta\), CDK5, and others not discussed in this review, in relation to their phosphorylation of tau, their relevance as targets toward inhibiting tau hyperphosphorylation is still unclear. The large number of phosphorylated moieties in tau suggests that it is not a single kinase that regulates the hyperphosphorylation mechanism but multiple. Moreover, selectively targeting an individual kinase using small molecules relies on adenosine triphosphate (ATP) recognition motifs [140]. Thus, targeting a single kinase is a significant challenge. Another perspective is that the sequence of tau phosphorylation and aggregation in AD is not well established. It has been hypothesized that tau phosphorylation is the driver for tau aggregation; however, it is yet to be proven. An alternative hypothesis is that tau aggregates before phosphorylation, which hinders the action of phosphatases on hyper-phosphorylated aggregates. Nevertheless, essential kinases can alter \(\alpha\)PP processing and \(\alpha\)\(\beta\) deposition in addition to aiding the development of neurofibrillary tangles, which makes inhibiting kinase activity of significance to neuroprotection in AD. However, to investigate such approaches further, detailed understanding of tau phosphorylation and its relevance to AD is needed.

STRATEGIES THAT STABILIZE NEURON TRANSMISSION

The modulation of neurotransmitters in the brain remains the only approach that has successfully yielded an approved treatment for AD. These approved drugs, however, only provide symptomatic relief and do not slow the progression of the disease. This section will address the dysregulation of neurotransmitter signaling and postsynaptic receptor expression in AD. The various approaches to stabilize this dysfunction through modulating neurotransmitter release from presynaptic neurons, regulation of postsynaptic receptor expression and respective downstream signaling will be highlighted.
## Table 2
Summary of selective kinases, their mechanism and proof of concept studies

<table>
<thead>
<tr>
<th>Target</th>
<th>Mechanism</th>
<th>Proof of concept studies</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>O-GlcNAcase (OGA).</td>
<td>Activation leads to the de-OGlcnAcylation of tau, which makes tau more vulnerable for phosphorylation.</td>
<td>Inhibition of OGA prevents cognitive decline in AD transgenic mice. An inhibitor of OGA, ASN120290, is in an ongoing phase I clinical trial.</td>
<td>[141–143]</td>
</tr>
<tr>
<td>Protein phosphatase 2A.</td>
<td>Decreased activity increases tau phosphorylation.</td>
<td>An activator of phosphatase 2A, Sodium Selenate, reduces tau hyperphosphorylation and completely abrogates tangle formation. The compound improves contextual memory and motor performance and prevents neurodegeneration in two independent transgenic tau mice models. Sodium Selenate showed safety and tolerability in a phase Ia clinical trial in Australia (ACTRN12611001200976).</td>
<td>[144–146]</td>
</tr>
<tr>
<td>Dual-specificity tyrosine phosphorylation-regulated kinase-1A (Dyrk1a).</td>
<td>Elevated activity increases tau phosphorylation. The kinase induces the phosphorylating AβPP which enhance its processing through BACE and the γ-secretase complex, which leads to the increase of overall Aβ42 levels.</td>
<td>Two molecules, EHT 5372 (Fig. 8) and Dyrk-inh, (structure not shown), have been shown to decrease tau phosphorylation and decrease Aβ42 production in HEK293 cells over-expressing AβPP and transgenic mice models of AD. The kinase is upregulated in postmortem human AD brains.</td>
<td>[147–150]</td>
</tr>
<tr>
<td>Thousand-and-one amino acid kinase (TAOK) 1 and 2.</td>
<td>Activation increases tau phosphorylation.</td>
<td>A TOAK inhibitor showed decreased tau phosphorylation in differentiated primary cortical neurons. Kinase activity was co-localized with tangles in post-mortem frontotemporal lobar degeneration (FTLD) brain tissue. Decreased tau phosphorylation was observed upon treatment of FTLD patient-specific pluripotent stem cell-derived neurons with a TAOK inhibitor. These findings were also validated in cortical neurons from a transgenic mouse model of tauopathy (Tau35 mice).</td>
<td>[151, 152]</td>
</tr>
<tr>
<td>Adenosine monophosphate-activated protein kinase (AMPK).</td>
<td>The exposure of neurons to Aβ induces a rise of intracellular Ca2+, which activates Ca2+/calmodulin dependent kinase kinase β (CaMKKβ) leading to the activation of AMPK. Inhibition of AMPK decreases tau phosphorylation.</td>
<td>AMPK has been shown to elevate phosphorylated tau in primary mouse neurons as well as PS19 tau transgenic mice.</td>
<td>[153–155]</td>
</tr>
<tr>
<td>Microtubule affinity regulating kinase (MARK) / partitioning defective gene 1 (PAR-1) family.</td>
<td>The kinase initiates the tau hyperphosphorylation cascade through phosphorylating Ser262, stabilizing microtubule-unbound tau in an early phase of tau hyperphosphorylation. Phosphorylation of Ser262 results in impaired binding of tau to microtubules which leads to the vulnerability of tau to phosphorylation by other kinases.</td>
<td>The MARK phosphorylation sites on tau are confirmed to be elevated in the early stages of disease in a transgenic mouse model of tauopathy. In a Drosophila model of AD, Aβ-induced toxicity is dependent on MARK/PAR-1 expression. The elevation of MARKs family has been demonstrated in AD brain, more specifically MARK/PAR-1.</td>
<td>[156–162]</td>
</tr>
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</table>

### Cholinergic system

The loss of cholinergic neurons is the major disruptor of the neural circuitry that contributes to AD pathology [163]. Four out of the five AD drugs approved by the U.S. Food and Drug Administration (FDA) are acetylcholinesterase inhibitors (tacrine [164], donepezil [165], rivastigmine [166], and galantamine [167]) (Fig. 9). Therefore, the cholinergic system continues to be targeted for treatment. The
function of the cholinergic neurons is critical in cognitive functions such as learning, memory, and attention. Moreover, the loss of the activity of these neurons is attributed to the loss of acetylcholine, which activates cholinergic receptors [163]. The knowledge of the cholinergic system as a pharmacological target is well established in the AD literature which makes it beyond the focus of this review. For more information about the system and its relevance to AD, the reader is directed to an excellent referenced review [168].

**GABAergic system**

Advances in the understanding of the γ-aminobutyric acid (GABA) neurotransmitter system and its remodeling in AD has directed focus toward maintenance of the system as a therapeutic target [169]. A central role for GABA is the regulation of the excitatory/inhibitory function of neurons, an essential process in learning and memory tasks. The GABA<sub>A</sub> receptor is an ionotropic receptor, which upon stimulation by a neurotransmitter, GABA, triggers Cl<sup>-</sup> influx into the cell. This leads to a hyperpolarization phase and change in membrane potential which leads to abrogation of excitatory stimulation [170]. The balance between the excitatory or inhibitory states downstream of GABA signaling is crucial for the plasticity of neurons and is related to learning and memory episodes [171]. The impairment of this system by Aβ has been shown to decrease cognitive function in mice [172]. Further, since Aβ causes synaptic failure through its depletion of synaptic vesicles and increases Ca<sup>2+</sup> concentration intracellularly, triggering the presynaptic neurotransmitter release leads to disrupted neuronal excitation [173, 174]. Levetiracetam, which acts by stabilizing the excitatory and inhibitory states of neurons, is currently enrolling in a phase II clinical trial in patients with MCI (NCT03461861, NCT03489044). While MCI patients possess a very different hippocampal pathology compared to AD patients, results from this trial may be of use to AD therapeutic development. It may, therefore, be possible to stabilize neuron signaling through regulating the GABAergic system. A clearer understanding of altered receptor expression in AD is still required to further cement the GABAergic system as a target, as it was shown that the GABA<sub>A</sub> receptor subunit expression changes in AD [175]. This incomplete understanding of the modulation of GABA receptors in the AD brain focuses future research towards an informative understanding of the GABA mechanisms underlying the remodeling of neurons and overall contribution to cognitive dysfunction. It is important to note that a single neurotransmitter imbalance would not explain the pathological features of AD that involves whole brain circuits and regions. Etazolate, a GABA<sub>A</sub> receptor modulator (Fig. 4), reached phase II trials but failed to progress beyond this point. Further development is needed to assess the drug’s implication in AD and its tolerability for extended treatment as off-target effects such as seizures and anxiety are a concern with GABA receptor modulators [176].

**N-methyl D-aspartate receptor**

Glutamate excitotoxicity is an underpinning of AD pathology. Convincing evidence shows the mutual interaction between Aβ and the NMDA receptor [177]. However, whether NMDA receptor activation leads to Aβ production, or Aβ oligomers binding and subsequently activating NMDA receptors is what contributes to AD pathology requires further understanding [178]. The glutamate cycle starts by synthesis of glutamate in pre-synaptic neurons. Glutamate is then released in the synaptic cleft where it either binds to post-synaptic receptors or it gets cleared from the synaptic cleft by astrocytes to be recycled back to neurons. This cycle is defective in different stages of AD pathology which leads to a state of extracellular glutamate accumulation, increased NMDA receptor activation, Ca<sup>2+</sup> influx and excitotoxicity [179]. Memantine (Fig. 10) is an uncompetitive NMDA antagonist which acts by

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Fig. 9. Structures of clinically available acetylcholinesterase Inhibitors.
blocking the Ca\(^{2+}\) channel and trapping it in the open conformation, which leads to a decrease of intracellular calcium [180]. The elevation of Ca\(^{2+}\) levels and induction of neuronal death has been the central mechanistic hypothesis for AD for many years [181]. Through a number of known pathways, Ca\(^{2+}\) elevation can be toxic, which includes the activation of calpain, release of reactive oxygen species (ROS) and depolarization of the mitochondrial membrane that leads to reduced energy metabolism and cytochrome c release [182]. The calpain release leads to activation of kinases that are involved in tau hyperphosphorylation [183]. These cascades ultimately result in neuronal loss through apoptotic and necrotic pathways. Memantine also acts to block NMDA receptor channels, more potently than that achieved under normal conditions by Mg\(^{2+}\). Due to the prolonged blockage achieved by memantine, it delays depolarization during chronic excitotoxic insults and delays glutamate binding to the NMDA receptor [184]. Memantine’s differential activity toward diverse NMDA receptor subtypes needs to be elucidated more clearly to fully understand its beneficial effects in moderate to severe AD. Studies show that excessive glutamate concentration in the synaptic cleft overcomes the blockage of memantine and facilitates its dissociation from the Ca\(^{2+}\) channel, which makes the antagonism of the NMDA receptor temporary and not of an ultimate benefit [184]. Memantine is the only drug approved by the FDA for moderate to severe AD; studies have shown that in early stages of AD the drug has a slight benefit in improving cognitive function but does not halt the progression of the disease [185]. Perhaps the dysregulation of NMDA receptors is a consequence of \(\alpha\beta\) aggregation, or maybe, due to the dysfunction of the cholinergic neurons, the functional neurotransmission between neurons is compromised. Nevertheless, it may be considered a secondary mechanism in AD pathology [186]. The modulation of NMDA by \(\alpha\beta\) can modulate receptor \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) which is also a regulator of glutamate concentration in the synapses. Additionally, the modulation of AMPA by \(\alpha\beta\) has been implicated in synaptic dysfunction and neurodegeneration. The reader is directed to the review of Guntupalli et al. on \(\alpha\beta\)-mediated signaling that disturbs synaptic function by modulating AMPA trafficking and its connection to AD pathology [187].

Serotonin receptors

Targeting serotonin (5-HT) receptors for AD treatment has gained attention recently following studies that showed the association of serotonin signaling in lowering the burden of \(\alpha\beta\) plaques in animal models of AD [188]. These results were confirmed upon employment of the selective serotonin reuptake inhibitor (SSRI) citalopram (Fig. 11) in transgenic mice that resulted in 37% reduced \(\alpha\beta\) production in CSF [189]. Furthermore, cholinergic-serotonergic imbalance has been linked to the cognitive dysfunction characteristic of AD [190]. However, the main question that this data poses is whether the serotonergic dysfunction in AD is a consequence of the disease or an attributor, as \(\alpha\beta\) deposition has been shown to disrupt axon formation and monoaminergic neuron generation [191]. Controversially, the SSRI paroxetine showed no effect in \(\alpha\beta\) pathology following chronic treatment (Fig. 11) [192]. There are seven distinct classes of serotonin receptors based on their structural and functional characteristics. Two of these classes are the most studied as a therapeutic target for AD, 5-HT\(_4\)R and 5-HT\(_6\)R. The non-amyloidogenic
pathway of AβPP is activated by the promotion of 5-HT₄R [193]. On the other hand, the antagonism of the 5-HT₆R has been shown to enhance cognitive functions in AD [194]. However, when a selective antagonist to 5-HT₆R named Idalopirdine (Fig. 11) reached phase III clinical trials, it showed weak efficacy, and further studies on the drug were discontinued [195]. More recently, 5-HT₇R agonism has shown to improve neuron and synaptic plasticity impaired by Aβ. Further, its agonistic effect enhanced cognition and memory function by alleviating Aβ plaque accumulation and neuronal apoptosis [196]. The significance of serotonin receptors in AD is still under development, and its direct connection to pathology observed in AD requires further investigation.

**Histamine receptors**

The modulation of histamine receptors, specifically the H₃ receptor, is being recognized as a novel therapeutic target for CNS disorders, more specifically AD [197, 198]. The antagonistic effect of the H₃ receptor enhances the release of various neurotransmitters including acetylcholine, GABA, dopamine and noradrenaline [199]. This mechanism has given rise to the hypothesis of employing inverse agonists or antagonists of the H₃ receptor as promising therapeutics for the treatment of several neuropathological diseases. The effect of H₃ receptor antagonists has been shown to enhance cognitive function and affect downstream effectors that represent a potential method of therapeutic intervention in the treatment of AD [200]. More recently, a selective H₃ receptor antagonist, GSK239512 (Fig. 12), has entered clinical trials. However, in phase II it showed no improvements in cognitive functions or working memory [201]. Although preliminary results showed an excellent safety profile with positive effects on attention and memory [202], the drug was discontinued from GlaxoSmithKline’s development pipeline due to sedative side effects. Further investigation of the therapeutic role of this compound is needed, which may further define the role of the H₃ receptor in AD pathophysiology.

It remains unclear whether a single neurotransmitter or its receptor can be considered as a sole therapeutic target for AD. Combination therapy of neurotransmitter modulators may be an avenue to stabilize neuron function and reverse the neurodegeneration seen in AD [203]. However, it is not well understood if these modulators can be tolerated in long-term treatment. It seems that the slow degenerative process of AD requires chronic treatment and modulating the brain circuitry in this way may be an unsuccessful approach. The approved FDA drugs that target the modulation of neurotransmitters have failed to slow the progression of the disease which suggests directing the focus of disease-modifying therapies to pathological cellular targets that are affected in AD may be more effective [204].

**STRATEGIES THAT IDENTIFY SENSITIVE TARGETS IN AD**

The focus of this section is to discuss the current targets being studied for AD treatment and their relevance in AD pathology. A sensitive target is defined as a protein that upon accumulation of Aβ in the brain or directly at the target, a modification in the protein function is induced which subsequently leads to neuron dysfunction. Restoring the target function is where this strategy aims to treat AD pathology. Figure 13 summarizes the targets discussed and their

![Fig. 13. Summary of discussed sensitive targets and their effect on Aβ accumulation, tau formation, and inflammation. Aβ, amyloid-β; AβPP, amyloid-β protein precursor; ApoE, Apolipoprotein E; ABAD, Aβ-binding alcohol dehydrogenase; ACAT1, Acyl-CoA cholesteryl acyltransferase 1; β₂-AR, β₂ Adrenergic receptor; 12/15 LO, 12/15 Lipoxigenase; PDEs, Phosphodiesterases; SIRT1, Sirtuin 1 histone deacetylases.](image-url)
relevance to AD pathology. Table 3 summarizes emerging targets that are being studied in vitro and in vivo to restore the function of sensitive targets thereby, restoring neuron health from the effects of Aβ-induced toxicity.

Membrane receptor targets

One of the mechanisms by which Aβ oligomers initiate their toxicity is by interaction with membrane receptors and disrupting their function either directly and/or by initiating toxic downstream events (reviewed in [205]). Three emerging membrane receptors are discussed in this review. The first is the amylin receptor which still requires further investigation, as the mechanism by which the receptor is affected is yet to be determined. Whether Aβ binds to the amylin receptor or activation of the receptor by amylin is the driving mechanism of Aβ-mediated toxicity is still under investigation. Second is the p75 neurotrophin receptor (p75NTR) which has been shown to be elevated in AD patients [206]. The exact mechanism by which binding of Aβ to p75NTR would induce cell death is still under investigation. However, several postulated mechanisms include the activation of the c-Jun N-terminal kinase (JNK) pathway [207, 208], which translocates NF-κB and activates p53 [209]. This translocation results in the activation of caspase-9, caspase-8, and caspases-3/7 which leads to apoptosis [208–210]. Another mechanism is activation of the p21-activated kinase which leads to impairment of metabotropic glutamate receptor 7 (mGluR7) regulation of NMDARs, leading to disturbance in Ca2+ hemostasis [211]. A small molecular ligand of p75NTR, LM11A-31 (Fig. 14), when studied in a murine model of AD has been shown to reduce tau phosphorylation and misfolding, and prevent cognitive deficits and neurite degeneration [212]. Binding of Aβ to p75NTR has also been shown to mediate activated microglia to secrete proinflammatory factors, TNF-α and IL-1β [210]. These data strongly suggest that Aβ-p75NT interaction leads to apoptosis as well as tau pathology. The third receptor of interest is the β2 Adrenergic receptor (β2-AR) which is a G-protein-coupled receptor (GPCR) that is important for various pathological functions in the brain, including synaptic plasticity regulation, learning and memory [213, 214]. Whether its activation is a consequence of Aβ toxicity or of a protective mechanism against Aβ is still under investigation. Hence, further studies are needed to identify the exact role of β2-AR in Aβ-induced neurotoxicity.

Nuclear targets

The attraction toward nuclear targets is supported by their regulation of several gene expressions which can elicit a neuroprotective and/or anti-inflammatory response. Two emerging nuclear targets are discussed in this review: the peroxisome proliferator-activated receptor gamma (PPARγ) and Liver X receptors (LXR). These nuclear genes function as master regulators of the transcription of several genes including ApoE4. Reports pertinent to PPARγ suggest a molecular mechanism by which it could play a role in improving cognitive and memory function [215, 216]. However, the drawbacks of using PPARγ agonists include their associated side effects as well as their inability to cross the BBB [94, 217]. Hence further studies are warranted for understanding the molecular mechanism of PPARγ receptor modulation and perhaps identifying a more specific target that is regulated by PPARγ. On the other hand, the study of agonists that activate LXRs and can selectively bind the brain nuclear receptor is still to be fully elucidated in in vivo models of AD. Further, translation to AD patients is hampered by the prospect of adverse reactions. Increased plasma LDL-cholesterol and decreased circulating neutrophils were reported in a phase I clinical trial of BMS-852927, a novel LXRβ-selective compound, in an atherosclerosis study [218]. These adverse reactions were not predicted even by nonhuman primate models and remains unclear whether it was an off-target effect or LXR-mediated. The aim is to find an LXR activator that 1) shows selectivity to the LXR isoform that will have the anti-inflammatory response; 2) selectively bind to brain nuclear receptors; and 3) be transrepression-selective LXR agonists. These later aspects make the development of LXR activating agents challenging for AD treatment [219].

Cellular targets

One other mechanism by which Aβ oligomers can initiate toxicity is by their action on intracellular targets, in fact, to some researchers this is considered the more reasonable hypothesis in the assumption that Aβ oligomers are initially formed intracellularly. Four emerging cellular targets that show potential to be modifying targets for AD therapy are discussed below. First is Sirtuin histone deacetylases 1 (SIRT1) which is highly expressed in neurons, has been linked to neuronal plasticity and cognitive function, as well as protection against
<table>
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<tr>
<th>Target</th>
<th>Proof of concept</th>
<th>References</th>
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<tr>
<td>Amylin receptor</td>
<td>Amylin shares similar biophysical and physiological properties with Aβ and has a similar effect on potassium conduction in rat cholinergic basal forebrain neurons.</td>
<td>[273–279]</td>
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<td>In TgCRND8 mice, overexpressing AβPP, the expression of amylin receptors is shown to be elevated in the same region of the brain where Aβ plaques form. Further, the administration of an amylin receptor antagonist to these mice has restored long-term potentiation between synapses.</td>
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<td></td>
<td>The amylin receptor peptide antagonists AC253 and AC187 attenuate expression of proapoptotic genes induced by Aβ in human fetal neurons and restore cognitive function of spatial memory and learning in a TgCRND8 mouse model.</td>
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<td></td>
<td>Amylin injection reduces Aβ accumulation, improve cognitive function in murine models of AD and increases Aβ efflux through the BBB in vitro model.</td>
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<td>p75 neurotrophin receptor (p75NTR)</td>
<td>In vitro, the binding of Aβ to p75NTR has been hypothesized as a critical factor in the degeneration of cholinergic neurons. The survival of neurons was found to be dependent on p75NTR when Aβ oligomers were delivered to the brains of wild-type but not p75NTR deficient mice. The removal of the neurotrophin-binding domain of the receptor showed attenuation of neuronal loss in AD transgenic mice. Together, these reports have identified p75NTR as a protein binding receptor for Aβ and resulted in its definition as a novel therapeutic target for AD. A small molecule p75NTR ligand, LM11A-31 (Fig. 14), has been shown to reduce tau phosphorylation and misfolding, and prevent cognitive deficits and neurite degeneration in a murine model of AD. The p75NTR receptor has also been shown to interact with BACE and promotes its activity towards producing Aβ in mouse cortical neurons; this may suggest a vicious cycle where Aβ increases its own production through binding to p75NTR.</td>
<td>[211, 212, 280–285]</td>
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<td>β2 Adrenergic receptors (β2-AR)</td>
<td>In AD transgenic mice, studies demonstrate selective binding of Aβ to β2-AR over β1-AR and which mediates a downstream event that leads to tau phosphorylation via the induction of cAMP and protein kinase A (PKA) signaling that leads to the activation of the JNK pathway which is responsible for tau phosphorylation. This binding is accompanied by internalization and degradation of the receptor in primary cortical neurons. Further, binding of Aβ enhances AMPA receptor activity which contributes to the distribution of Ca2+ hemostasis. Activation of the receptor leads to the increase of Aβ production through the activation of γ-secretase activity in AD mouse models, conversely, activation of the receptor has been shown to prevent Aβ-induced inhibition of long-term potentiation (LTP) in the hippocampus in vitro and induce neurogenesis, dendrite ramification and spine generation in AβPP transgenic mice.</td>
<td>[286–293]</td>
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<td>Peroxisome proliferator-activated receptor gamma (PPARγ)</td>
<td>The significance of PPARγ was highlighted when its knockdown was correlated to the regulation of seven other genes. Wherein ABCA7, APOE, CASS4, CELF1, PTK2B, and ZCWPW1, were upregulated and one, DSG2, was downregulated. These genes are shown to be dysregulated in late-onset AD, most notably, the upregulation of ApoE which induces Aβ clearance. In Tg2576 transgenic mice, PPARγ agonists improve memory and learning functions, and cause a significant reduction of both Aβ and tau in cerebral blood fluid.</td>
<td>[215, 216, 294–297]</td>
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<td>Liver X receptors (LXR)</td>
<td>Two LXRs has been identified in mammals, LXRα and LXRβ, in which their presence in the brain is of relevance to AD due to their function in cholesterol homeostasis, inducing the expression of ApoE and clearance of Aβ, along with reducing inflammation.</td>
<td>[298–303]</td>
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(Continued)
Table 3  
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<th>Target</th>
<th>Proof of concept</th>
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<tr>
<td>LXR</td>
<td>The agonistic effect of LXR in brain capillary endothelial cells result in elevated levels of ApoE with an increase in the expression of ATP-binding cassette transporter A1 (ABCA-1). This dual elevation in protein production is of importance as lipidation of ApoE is required for its function to clear Aβ which is influenced by ABCA-1. Investigation of the LXR agonist, TO90131, in vitro and in vivo showed reduction in BACE expression and attenuation in Aβ-induced inflammation through inhibition of NF-κB signaling.</td>
<td>[220–226, 304–310]</td>
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<td>Sirtuin 1 histone deacetylases (SIRT1)</td>
<td>Immunoblotting and immunohistochemical analysis of the hippocampus and entorhinal cortex showed a stepwise reduction of SIRT1 expression which corresponds to brain regions affected during AD progression from early to late stages. Activating SIRT1 using Resveratrol, Leptin or Curcumin (Fig. 3) in vitro has been shown to promote neuronal survival, decrease Aβ-induced neuronal apoptosis through the SIRT1–ROCK1 signaling pathway and inhibit BACE expression and Aβ production through inhibiting the activation and transcriptional activity of NF-κB. Activation of SIRT1 in vitro using Cilostazol (Fig. 15) inhibited Aβ-induced tauopathy. In p25 transgenic mice, a model of AD and tauopathies, activating SIRT1 by Resveratrol, a SIRT1 activating molecule, showed enhancement in learning function and protection against neurodegeneration</td>
<td>[220–226, 304–310]</td>
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<td>12/15Lipoxygenase (12/15-LO)</td>
<td>The deletion of 12/15-LO in Tg2576 transgenic mice resulted in reduction of Aβ production through modulation of BACE. Triple transgenic mice treated with PD146176 (Fig. 16), a selective and specific inhibitor of 12/15-LO, showed lower Aβ levels, decrease tau neuropathology, increased synaptic integrity and autophagy activation. In transgenic tau mice, the pharmacological inhibition of 12/15-LO showed significant memory improvement along with restoring synaptic integrity and reduction of tau pathology via a CDK5-dependent mechanism.</td>
<td>[227, 228, 311–313]</td>
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<td>Phosphodiesterases (PDEs)</td>
<td>In vitro, the PDE4 inhibitor, Etazolate (Fig. 4), has been linked to αβPP processing through modulating GABA&lt;sub&gt;A&lt;/sub&gt; receptor activity. Inhibition of PDE5 by Tadalafil or Sildenafil, specific PDE5 inhibitors (Fig. 17), resulted in a decrease of Aβ production and improvement in cognitive function in transgenic mice. Combined inhibition of PDE4 and PDE5 or inhibition of PDE2 showed improvement in the cognitive function of AD transgenic mice. The small molecule PDE9 inhibitor PF-04447943 (Fig. 17), successfully reached clinical trials, but achieved no success as it did not show improvement in cognitive function compared to placebo.</td>
<td>[81, 229–233, 314–318]</td>
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<td>Acyl-CoA cholesterin acyltransferase 1 (ACAT1)</td>
<td>AD transgenic mice with genetic ablation of ACAT showed an increase in hydroxycholesterol levels in the brain and attenuated Aβ pathology. A small molecule inhibitor of ACAT decreased Aβ accumulation in the brain. In triple transgenic mice, activation of autophagy combined with the inhibition of ACAT activity increased Aβ clearance and reduced tau content.</td>
<td>[234, 235, 319–325]</td>
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<td>Amyloid-Binding Alcohol Dehydrogenase (ABAD)</td>
<td>Immunofluorescence images showed the co-localization of ABAD with Aβ aggregates in the cerebral cortex of human AD brain tissues and murine models of AD. Further, enzyme levels have also been shown to be elevated in these neurons. In vitro inhibition of ABAD enzyme activity in SH-SY5Y cells using the novel inhibitor, AG18051 (Fig. 18), showed restoration of estradiol levels following Aβ injury. Neurons cultured from mAβPP/ABAD double transgenic mice showed mitochondrial dysfunction associated with higher ROS generation, as well as elevated cell death compared to control neurons which were associated with increased DNA fragmentation.</td>
<td>[239, 241, 242, 245]</td>
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neurodegenerative diseases that are associated with cognitive decline [220, 221]. The pathways of SIRT1 are affected in neurons that are susceptible to age-related diseases, and its activity is of importance to synaptic plasticity [222]. The mechanism by which SIRT1 can regulate the production of Aβ has been linked to the Rho-dependent kinase 1 (ROCK1) pathway, in which SIRT1 increases AβPP processing through α-secretase [223, 224]. Activation of SIRT1 in vitro using Cilostazol (Fig. 15) inhibited Aβ-induced tauopathy [225]. Together, SIRT1 activation combined with several other potential mechanisms discussed in the referenced review have highlighted SIRT1 as a novel therapeutic target for AD [226].

The second cellular target is 12/15Lipoxygenase (12/15-LO) which catalyzes the oxidation of arachidonate substrates to form 12- and 15-hydroxyeicosatetraenoic acid (12-HETE and 15-HETE) and linoleic acid to form 13-hydroxyoctadecadienoic acid. This action provides the enzyme the ability to regulate anti-inflammatory effects during the innate and adaptive immune response [227]. A selective and specific pharmacological inhibitor of 12/15-LO, PD146176 (Fig. 16), has been shown to lower Aβ levels, decrease tau neuropathology, increase synaptic integrity and activate autophagy [228]. These reports, and others, suggest the direct involvement of 12/15-LO in AD pathology, more particularly playing a role in ensuring the synaptic integrity of neurons which makes it an attractive target for drug development.

The third cellular target is the phosphodiesterases (PDEs), that hydrolyze the second messengers cyclic adenosine monophosphate (cAMP) and/or cyclic guanosine monophosphate (cGMP). Thus, they regulate the second messenger signaling intracellularly as well as by absolute and temporal regulation of cyclic nucleotides [229–231]. The localization of PDEs in the cell make them significant regulators of cellular function, with effects including receptor trafficking and neurotransmission and as such, are involved in several neurodegenerative disorders (reviewed in [232]). A selection of representative PDE inhibitors being tested for the treatment of AD is depicted in Fig. 17. Prickaerts et al. summarized the performance of PDE inhibitors in clinical trials and concluded that the first trials in AD patients with PDE3, 4, or 9 inhibitors did not provide an informative conclusion [233]. Further, small molecules developed as inhibitors have lacked selectivity. This is compounded by the fact that the specific subtypes of PDE that are candidates for therapeutic intervention in AD are yet to be determined [233].

The fourth cellular target is Acyl-CoA cholesterin acyltransferase 1 (ACAT1), which converts free cholesterol to cholesteryl esters by transferring the fatty acyl group of fatty acyl-CoA to the 3β-hydroxy moiety of cholesterol [234]. These cholesteryl esters are elevated in AD-affected brain regions [235]. The
benefit of inhibiting ACAT is well established in animal models. However, testing in ApoE mutant models, where cholesterol transport is hindered, will be of importance to further validate the target.

**Mitochondrial targets**

The significance of the mitochondrial targets is supported by studies showing that mitochondrial dysfunction is an early stage of AD pathology [236–238]. One emerging target for AD localized in the mitochondria is the most characterized Aβ binding protein, Amyloid-Binding Alcohol Dehydrogenase (ABAD), which was identified by Yan et al. in a yeast two-hybrid screen for Aβ binding molecules [239]. The mitochondrial enzyme is an energy regulator, and through its oxi-reductase activity, ABAD can break down several substrates containing alcohol, ketone, or aldehyde functional groups and utilize them as an energy source for neurons [240, 241]. One of the most significant findings was the colocalization of ABAD in the same regions of the brain that are affected early in AD. Immunofluorescence images showed the co-localization of ABAD with Aβ aggregates [242]. Enzyme levels have also been shown to be elevated in the neurons of AD patients as well as murine models of AD [242]. The conversion of estradiol to estrone is regulated by ABAD, estrone is a less potent estrogen hormone than estradiol, and the levels of the latter are a determinant of neuronal health [243]. Reports show that a consequence of Aβ binding to ABAD is a conformational change in the enzyme which leads to elevated activity to degrade estradiol [241, 244, 245]. Estradiol has been shown to reduce tau hyperphosphorylation through phosphorylating GSK3β which leads to its deactivation [246–248]. Moreover, estradiol decreases AβPP processing through BACE, hence reducing Aβ production [249, 250]. *In vitro* inhibition of ABAD enzyme activity in SH-SY5Y cells using the novel inhibitor, AG18051 (Fig. 18), showed restoration of estradiol levels following Aβ injury [245]. Neurons cultured from mAβPP/ABAD double transgenic mice showed mitochondrial dysfunction associated with higher ROS generation, as well as elevated cell death compared to control neurons which were associated with increased DNA fragmentation [251]. Two other proteins have been linked to ABAD overexpression in AD. The first is peroxiredoxin-2 (Prdx-2), which is an antioxidant and is elevated subsequent to Aβ binding to ABAD. The latter correlates with the increase of expression of Prdx-2 that has been shown in AD patients and transgenic mice of AD. However, due to elevated levels of CDK-5 in the cytosol, Prdx-2 is phosphorylated and deactivated [252]. The second protein is endophilin-1 (Ep-1), which is essential for synaptic function as it regulates synaptic vesicle endocytosis, mitochondrial function, and receptor trafficking [253]. The upregulation of Ep-1 is a direct response to Aβ-ABAD interaction [254]. The upregulation of Ep-1 in pre-synaptic neurons has been shown to increase glutamate release in the synapses, interfering with normal neurotransmitter signaling and disturbing neuronal circuitry [255]. Moreover, activation of the stress kinase JNK by Ep-1 leads to neurotoxicity in AD [256, 257]. Together, these findings have defined ABAD as a novel target for Aβ-induced toxicity. Thereafter, several inhibitors have been identified which are in their nascent stages of development which are summarized in the referenced review [258]. The mitochondrial enzyme ABAD regulates synaptic function through modulating Ep-1 expression and several reports have demonstrated that synaptic dysfunction is correlated with mitochondrial oxidative stress. Therefore, these findings define a connection between ABAD and the early dysfunction of both mitochondria and synapses in AD [259, 260].

**OTHER TARGETS**

Neuroinflammation plays a critical role in AD and has recently been recognized as a significant contributor to the neurodegeneration seen in AD. Targets that can be modulated to reduce neuroinflammation represent a novel approach for disease intervention. The reader is directed to referenced reviews which explain how the astrocytes and microglia, two components of glial cells, modulate their function during AD pathology and their interaction with Aβ which contributes to AD via an at presently poorly defined neuroinflammatory mechanism [261–263]. However, this nascent area of research still requires comprehensive study to understand the communication and crosstalk between neurons and glial cells. Other targets of
interest that are still in early stages of exploitation as potential therapeutic avenues, including activating transcription factor 4 (ATF4) [264], protein kinase RNA-like endoplasmic reticulum kinase (PERK) [265], triggering receptor expressed on myeloid cells-1 (TREM1) [266], Ca\textsuperscript{2+}, permeable \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (CP-AMPARs) [267], Calcitonin gene-related peptide (CGRP) [268], Adenosine \(A_{2A}\) receptors (\(A_{2A}\) R) [269–271] and DJ-1/encoded in a causative gene of familial Parkinson’s disease (PARK7) [272], have recently garnered increased attention in the literature. However, their significance in AD is still to be determined.

**PERSPECTIVE**

Since the identification of the pathological hallmarks A\(\beta\) and tau, enormous efforts have been devoted toward identifying a disease-modifying target for the successful treatment of AD. These efforts collectively have faced common challenges while posing many outstanding questions. Firstly, much evidence points to the fact that the oligomer form of A\(\beta\) is the toxic form, yet what the effect of the other forms of A\(\beta\) monomer, dimers, and fibrillar aggregates have in relation to AD is not well understood. Do these forms have a pathological function in the normal brain? Secondly, diagnostic tests have come a long way since the change in the diagnostic criteria in 2011 [8, 326]. However, a reliable biomarker is still lacking. Thirdly, an *in vitro* or *in vivo* model that fully recapitulates AD pathology is still not available. This is mainly due to the lack of a full understanding of the disease pathology, raising the question if targets for experimental therapeutics that failed phase III trials were fully elucidated. Different sources and different concentrations of A\(\beta\) seem to have various influences on experimental models of AD. There is also the chasm that currently exists between translating successful treatments in animal models of AD to human clinical trials [327, 328]. These challenges have introduced innovative models for AD such as the *in vitro* induced pluripotent stem cell (iPSC) models which are in early development [329]. One such advantage of iPSCs are efforts directed at establishing a 3D triculture system that mimics the three main pathological hallmarks of AD, A\(\beta\), tau, and neuroinflammation, in one model system [330]. Another aspect is the apparent negative correlation between A\(\beta\) plaques and cognitive dysfunction; up to 40% of older adults with no signs of cognitive dysfunction have accumulated A\(\beta\) plaques in the brain [331]. This observation, combined with the fact that all investigational new drugs that target A\(\beta\) in some way have failed in clinical trial, brings into question the validity of the A\(\beta\) hypothesis as the foundation for AD pathology. On the other hand, one of the most common explanations given for the failures seen in clinical trials has been that it might be already too late to treat patients when they have developed advanced symptoms of the disease and that trials should be run in people with milder, earlier symptoms. However, most of the recent monoclonal antibody-phase III clinical trial failures were in prodromal AD patients, and the questions that this presents are how early in the disease can we intervene, and how can one diagnose patients before any symptoms present? To date we cannot. Therefore, whether the A\(\beta\) hypothesis can still be considered the leading theory for the progression of AD can be debated. The mutations in the A\(\beta\)PP and PSEN genes that lead to the formation of the toxic A\(\beta\) oligomers have been linked to early onset of AD [332]. Although the interaction between ApoE and A\(\beta\) is not completely understood, mutation in the ApoE gene is linked with the onset of AD [102]. Further, when injecting A\(\beta\) oligomers into the hippocampus of mice, AD pathology is induced [333, 334]. Moreover, A\(\beta\) interacts with neurons and glial cells to induce neuroinflammation and tau phosphorylation [334, 335]. The injection of A\(\beta\) has also been linked to behavioral impairment [336–339]. This impairment has similarly been reported in A\(\beta\)PP transgenic mice [340, 341]. More interestingly, when seeding familial and sporadic AD neural progenitor cells in a novel 3D culture model, A\(\beta\) aggregation and plaques were formed that led to an increase in tau pathology after 10 to 14 weeks [342]. This model has also showed the induction of neuroinflammation of increased chemokines and cytokines, showing that A\(\beta\) may still be the leading cause of AD [330]. However, the unsuccessful outcomes of A\(\beta\)-targeted therapy ask serious questions as to the validity of the A\(\beta\) hypothesis. Representative of this is the continued failure of the secretase modulators that target amyloid formation [100] and immunotherapies that fail to reverse AD pathology, most recently Crenezumab and Aducanumab, which have been withdrawn from clinical trials due to being ineffective in meeting their primary endpoints (NCT02670083, NCT03114657 and NCT03639987). Other antibodies, Solanezumab and Bapineuzumab, have also failed clinical trials [61, 66]. The accumulation of A\(\beta\) has been shown...
to occur in healthy individuals who are cognitively normal suggesting a function related to the normal aging process [331].

On the other hand, the progression and severity of AD shows greater correlation to neurofibrillary tangle accumulation [343]. Although unfortunately, tau-targeted therapies have also failed clinical trials, including inhibiting tau phosphorylation using the TIDEglusib, which showed no clinical benefit in AD patients [136]. Further, inhibiting tau aggregation using a methylene blue derivative, LMTM, has also failed phase III trial in the treatment of mild to moderate AD patients [139]. Thus, the available evidence poses the question if Aβ is indeed the correct target to focus drug discovery efforts around. Given the continued high-profile failures of small molecule and biological experimental therapeutics it may not be. However, this must be tempered with the fact that our understanding of the full AD cascade is not complete, perhaps we are missing a vital piece of the puzzle that may allow for the successful identification of therapeutics in the future.

PROSPECTIVE

While Aβ-targeted therapy has not led to effective treatment, it does not necessarily diminish the involvement of Aβ in AD pathology. Perhaps the accumulation of Aβ in the brain reaches a threshold, which varies between individuals, that triggers formation of neurofibrillary tangles which accumulate, leading to neurodegeneration and neuroinflammation. As such, it may not be possible to reverse this pathology by simply clearing Aβ from the brain. Therapeutic intervention would also require the reversal of cellular processes that are disrupted by Aβ accumulation. This may include reversing the “sensitive targets” function, discussed in this review, or inhibiting neurofibrillary tangles with a number of immunotherapies against tau currently undergoing clinical trials (NCT02579252, NCT02880956, and NCT03289143), the results of which are eagerly awaited. These aspects highlight the importance of revisiting the science underlining our current understanding of AD to identify previously overlooked proteins, signaling pathways or processes that may contribute to disease pathophysiology. One such process gaining traction is the extent of involvement of neuroinflammation in AD [344, 345]. Another emerging hypothesis is the involvement of the microbiota in the progression of AD [346].

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S166

A. Morry and P.C. Trippier / Current and Emerging Pharmacological Targets


