Topics in Medicinal Chemistry 24

Michael S. Wolfe *Editor*

Alzheimer's Disease II

Springer

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Michael S. Wolfe
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Alzheimer's Disease II

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Preface

Alzheimer's disease (AD) is a devastating neurodegenerative brain disorder that leads to progressive loss of memory and other higher cognitive functions and ultimately death. Age is the greatest risk factor for AD, and therefore longer life spans mean more cases. In the United States alone, over five million people have AD, and worldwide estimates are 20–30 million. These numbers are projected to rise dramatically in the coming decades, creating a crisis that could bankrupt healthcare systems and overwhelm society as a whole.

The need for effective prevention and treatment is urgent, and many laboratories around the world are engaged in research into the root causes and mechanisms of the disease and the discovery of potential therapeutics. Although important gaps remain in our understanding of the molecular basis of AD pathogenesis and progression, considerable progress has been made in this regard.

Two key pathological features are found in the AD brain: amyloid plaques and neurofibrillary tangles. The former is primarily composed of a 42-residue amyloid β-peptide (Aβ), while the latter are composed of the microtubule-associated protein tau. While the deposits themselves may only be disease markers, a large body of evidence, particularly from genetics, strongly suggests that these proteins are directly involved in pathogenesis and progression.

The process apparently begins with the overproduction or under-clearance of the Aβ, a proteolytic product generated through cleavage of the amyloid precursor protein by the sequential action of β- and γ-secretases. Assembly of the amphipathic Aβ peptide in the form of neurotoxic oligomers and other higher-order forms leads to synaptic toxicity and the triggering, through unknown mechanisms, of tau misfolding and aggregation.

This volume first provides an overview of AD biology and the prospects for developing therapeutics (chapter "Targets and Strategies Toward the Development of Alzheimer Therapeutics"). What follows are specific and detailed medicinal chemical strategies for AD drug discovery and the development of diagnostic tools.

Preventing the formation of neurotoxic Aβ through inhibition of β-secretase (chapter "The Design, Development, and Evaluation of BACE1 Inhibitors for the Treatment of Alzheimer's Disease") or modulation of γ-secretase (chapter "γ-Secretase Modulators as Aβ42-Lowering Pharmacological Agents to Treat Alzheimer's Disease") is a leading strategy. As pathological tau is hyperphosphorylated, the responsible kinases are considered important targets (chapter "Inhibitors of Tau-Phosphorylating Kinases"), and because pathological tau is not able to serve its normal function in stabilizing microtubules, small molecules that rescue this loss of function may have therapeutic benefit (chapter "Microtubule-Stabilizing Agents for Alzheimer's and Other Tauopathies"). Finally, early diagnosis of AD is critical for enrolling the right patients into clinical trials and intervening before disease progression has gone too far, and the development of PET imaging agents for amyloid plaques has led to several approved diagnostic agents (chapter "PET Imaging Agents for Alzheimer's Disease").

I would like to thank all the authors who contributed to this volume for their time, thought, and effort in putting together high-quality chapters on these important targets and strategies for AD prevention, treatment, and diagnosis. I also thank all those working on this difficult problem in human health. We all greatly hope that these efforts will speed the day when AD is a scourge of the past.

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Targets and Strategies Toward the Development of Alzheimer Therapeutics

Michael S. Wolfe

Abstract Although Alzheimer's disease (AD) is a devastating neurodegenerative disorder affecting tens of millions worldwide, there are no effective disease-modifying therapies and only a handful of symptomatic treatments. Much has been deciphered regarding the molecular basis of AD in the past 25 years, but serious gaps in understanding remain. Aggregation-prone proteins amyloid-β (Aβ) and tau are apparently central to disease pathogenesis and progression; however, the neurotoxic forms of both proteins and the connection between the two remain unclear. Genetic mutations in the precursor protein for Aβ and in a protease that produces Aβ from this precursor cause dominantly inherited early-onset AD. In contrast, an allelic variant of apolipoprotein E is a major risk factor for the much more common late-onset AD, and the encoded protein is critical for Aβ clearance from the brain. Tau is mutated in other forms of dementia, and tau pathology correlates better with neurodegeneration and progression than does Aβ pathology. A variety of therapeutic agents are advancing through the pipeline, many targeting Aβ and tau. New symptomatic treatments are still needed for those who have already progressed too far in the disease process. Advances in drug discovery, new diagnostic tools such as Aβ imaging agents, and improvements in clinical trial design increase the likelihood that effective agents that slow or halt the progression of AD will soon be realized.

Keywords Alzheimer's disease, Amyloid, Neurodegeneration, Tau

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Abbreviations

5-HT	5-Hydroxytryptamine or serotonin
AD	Alzheimer's disease
ApoE	Apolipoprotein E
APP	Amyloid-β protein precursor
Aβ	Amyloid-β protein
BACE	β-Site APP-cleaving enzyme
CDK	Cyclin-dependent kinase
CSF	Cerebrospinal fluid
CTF-β or –β	C-Terminal fragment generated by β- or β-secretases
FAD	Familial Alzheimer's disease
FDA	Food and Drug Administration
GSK	Glycogen synthase kinase
MARK	Microtubule affinity-regulating kinase
MCI	Mild cognitive impairment
NMDA	*N*-Methyl-D-aspartate
PET	Positron emission tomography
PS1 or PS2	Presenilin-1 or presenilin-2
SAD	Sporadic Alzheimer's disease
USP	Ubiquitin-specific protease

1 Introduction

Alzheimer's disease (AD) is a devastating neurodegenerative disorder that leads to progressive memory deficiencies and cognitive impairment and ultimately to death within 5–20 years from onset of symptoms. Although many factors such as genetics and lifestyle affect an individual's risk of getting AD, the greatest risk factor by far is age: AD is rare before age 65 and increases dramatically in the years afterward, with a ~40% chance of having AD by age 85. Over 5 million people in the United States and perhaps over 30 million worldwide have AD, with these numbers expected to increase dramatically as demographic changes result in greater numbers of elderly [1].

Much has been learned in the past three decades about the biological basis of the disease, revealing potential means of developing biomarkers for diagnosis, risk assessment, clinical trial enrollment, and drug target engagement, as well as suggesting targets for drug discovery for prevention and treatment. Despite this progress, only a few drugs for AD have been approved, and these are symptomatic treatments that do not affect the underlying progressive neurodegeneration and for this reason soon become ineffective. Indeed, no drugs have been approved for AD since 2003; every clinical candidate brought forward since the approval of the NMDA receptor blocker memantine has failed, and these failures number in the hundreds. The possible reasons for these failures are many, but taken together the inability to identify effective therapeutics suggests insufficient knowledge of disease mechanisms. This overview chapter will summarize the current state of understanding of the biology of the disease along with targets and strategies being pursued to discover effective AD therapeutics, presenting the progress as well as the challenges.

2 AD Presentation and Progression

AD begins with subtle problems with memory, learning, and executive function, termed mild cognitive impairment (MCI). As the disease progresses over the course of years, these problems become more pronounced, with the patient increasingly less able to function without help. Ultimately, family and friends are overwhelmed by the challenges of helping the AD patient meet his or her basic daily needs, preventing the wandering off without notice, and managing the disruptions in the sleep-wake cycle, with professional care in the form of a nursing home being the typical fate for late-stage AD patients. This pattern of disease presentation and progression is the consequence of the relentless loss of synapses and neurons in regions of the brain responsible for learning, memory, and cognition, beginning in the entorhinal cortex, spreading into the hippocampus and the frontal cortex and temporal lobes [2].

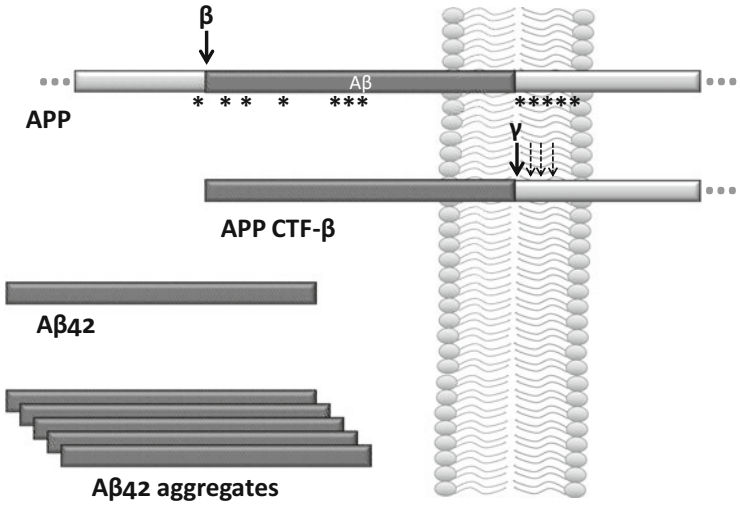

Fig. 1 Proteolytic processing of APP to Aβ, with subsequent aggregation of Aβ42

3 Pathology

Two pathological hallmarks of AD were first described by Alois Alzheimer over a century ago. Silver staining of brain sections taken from a 55-year-old woman who died after developing presenile dementia revealed extra-neuronal deposits of what was dubbed amyloid as well as intraneuronal deposits called neurofibrillary tangles in nerve cell bodies and processes. Decades later, in the 1980s, the extra-neuronal deposits were found to be composed of a 4 kDa peptide called the amyloid-β protein (Aβ) [3], and the intraneuronal deposits were determined to be composed of a 352–441-residue protein called tau [4, 5]. A third pathological feature also became recognized, neuroinflammation, an apparent consequence of over-activation of non-neuronal cells such as microglia and astrocytes [6].

3.1 Amyloid

Aβ is a 38–43-residue secreted peptide and the product of proteolytic processing of the Aβ precursor protein (APP), a type I integral membrane protein of unknown function [7, 8]. The N-terminus of Aβ is formed through cleavage in the lumenal/extracellular region of APP by the membrane-tethered aspartyl protease β-secretase [9], to release the APP ectodomain into the lumenal/extracellular space and leave a 99-residue membrane-bound protein stub (APP CTF-β) (Fig. 1). The C-terminus of Aβ is generated through the action of γ-secretase, an unusual membrane-embedded aspartyl protease complex [10], cutting APP CTF-β within its single

transmembrane domain and releasing the APP intracellular domain (AICD) as the other proteolytic product. Alternative processing of APP by α-secretases ADAM-10 and ADAM-17 [11, 12] within the Aβ sequence precludes Aβ production, with the 83-residue protein remnant (APP CTF-α) subsequently cut by γ-secretase to produce an N-terminally truncated form of Aβ called p3. Although the 40-residue form of Aβ (Aβ40) is the primary proteolytic product, the minor 42-residue variant (Aβ42) is much more aggregation prone [13] and is the principle protein found in the cerebral amyloid deposits of AD [14].

Dominantly inherited missense mutations in the gene encoding APP cause familial AD (FAD), with an age of onset before age 60 [15, 16]. These mutations are found in and around the small Aβ region of the much larger APP. Mutations near the β-secretase cleavage site increase proteolysis by the protease, leading to increased production of all forms of Aβ. Mutations within the Aβ region increase the aggregation propensity of Aβ. Mutations within the transmembrane of APP increase the proportion of Aβ42/Aβ40. Thus, all FAD-causing mutations in APP increase the tendency of Aβ to aggregate. Other dominant mutations that cause early-onset FAD are found in the multi-pass membrane proteins presenilin-1 (PS1) [16, 17] and presenilin-2 (PS2) [16, 18], proteins that are the catalytic component of the γ-secretase complex [10, 19]. These mutations also increase the proportion of Aβ42/Aβ40 [10]. Cleavage of APP CTF-β by γ-secretase, however, is more complicated than originally thought. Initial proteolysis at the ε cleavage site near the membrane-cytosolic boundary releases AICD and produces 48- or 49-residue Aβ (Aβ48 or Aβ49), which is sequentially trimmed generally every three amino acids by a carboxypeptidase function of the γ-secretase complex [20, 21]. Thus, there are two pathways to secrete Aβ: Aβ49→Aβ46→Aβ43→Aβ40 and Aβ48→Aβ45→Aβ42→Aβ38, with this last cleavage releasing a tetrapeptide instead of a tripeptide. Most presenilin mutations increase the proportion of ε cleavage giving Aβ48, thereby increasing Aβ42/Aβ40 [22]. These mutations also dramatically reduce the carboxypeptidase function of the γ-secretase complex [23].

Although FAD is clearly associated with altered Aβ production, the much more common late-onset sporadic AD (SAD) is associated with decreased clearance of Aβ [24]. Degradation of Aβ in the brain has been attributed to the metalloprotease neprilysin [25] and insulin-degrading enzyme [26]. Thus, a common feature of AD is increased levels of aggregation-prone forms of Aβ, either through altered production or clearance. For this reason, Aβ has been the major target for AD drug discovery, either directly through antibodies or aggregation inhibitors or indirectly through the secretases or Aβ-degrading enzymes.

The aggregation of Aβ is closely correlated with AD. However, the amyloid plaques do not correlate well spatially with neurodegeneration [2]. This has led to the focus on soluble Aβ oligomers as candidate toxic entities responsible for synaptic and neuronal dysfunction [27]. Synthetic Aβ oligomers as well as natural Aβ oligomers, secreted from cells or extracted from transgenic AD mouse brain or human AD brain, have been shown to affect dendritic spines, synaptic signaling, and electrophysiological responses, including long-term potentiation associated with memory formation [28]. The search for possible receptors for oligomeric Aβ

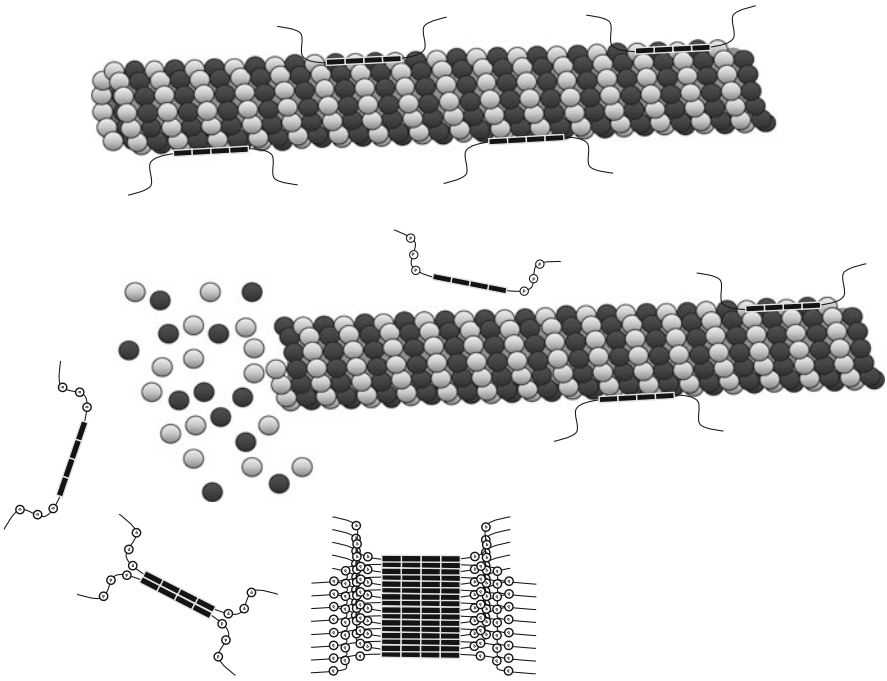

Fig. 2 Destabilization of microtubules upon tau protein release and phosphorylation. Hyperphosphorylation is correlated with tau aggregation and neurofibrillary tangles

has led to the identification of the cellular prion protein as a leading candidate [29], with evidence for downstream effects on NMDA receptors and activation of kinases that phosphorylate tau [30]. Sizes ranging from dimers and trimers [31] to dodecamers [32] and to protofibrils [33] have been suggested as toxic forms of Aβ, but at this time there is no consensus on which of these, if any, is most likely. Indeed, it has been suggested that there is no single toxic species but rather that a toxic "Ab soup" may be responsible [34].

3.2 Tau

Tau, the other aggregation-prone protein in AD, is normally associated with microtubules (Fig. 2) [35]. Although tau can help stabilize microtubules, this function is apparently redundant with other proteins, as knockout of tau is not lethal [36]. Crossing tau knockout mice with APP transgenic AD mouse models does not lead to altered Aβ production or deposition but results in attenuation of age-dependent learning and memory deficits [37], suggesting that tau is downstream of Aβ in AD pathogenesis. Dominantly inherited mutations in tau do not cause AD, but they do result in tau pathology, neurodegeneration, and other forms

of dementia [38, 39]. These and other findings suggest that Aβ is one of a variety of factors that can trigger pathological tau aggregation and consequent neurodegeneration.

Tau protein in the neurofibrillary tangles of AD is hyperphosphorylated, which has led to efforts to identify the responsible kinases and search for selective inhibitors [40, 41]. Although the assumption is that the increased phosphorylation of tau is important for AD pathogenesis, whether the relationship between tau hyperphosphorylation and neurodegeneration is one of cause and effect or only correlative is unclear. Aggregation of tau in the form of filaments dramatically decreases tau turnover and may therefore simply allow more time for kinases to phosphorylate the protein. Even if hyperphosphorylation is part of the pathogenic process, identifying the specific sites that are most important and the responsible kinases has been fraught with complications, especially as phosphorylation of a given site can affect phosphorylation of another site, and more than one kinase is often capable of phosphorylating a given site [42].

Despite the above critique of the role of hyperphosphorylation in AD pathogenesis, it is important to point out that phosphorylation of tau is critical to regulating its interaction with microtubules [43], and dissociation from microtubules increases the tendency of the protein to aggregate [42] (Fig. 2). Moreover, hyperphosphorylation may play a role in AD pathogenesis due to dissociation of tau and consequent destabilization of microtubules. Although knockout of tau in mice is not lethal and does not lead to neurodegeneration [36], compensation may take place in the context of deletion from conception onward. Thus, it would be important to determine the effects of conditionally knocking out the tau gene in the adult mouse brain. Furthermore, even if loss of microtubule-stabilizing function alone does not cause neurodegeneration, it may be a secondary contributor to the primary insult of tau aggregation. For this reason, microtubule-stabilizing agents have been sought for the potential treatment of AD and other "tauopathies" [44].

The aggregation of tau is also closely linked to AD pathogenesis, with neurofibrillary tangle pathology correlating better with neurodegeneration than amyloid pathology does [2]. Moreover, seeds of aberrant forms of tau are now thought to spread tau dysfunction and pathology from neuron to neuron through synapses, that is, through neuronal networks [45, 46]. This mechanism of transmission from neuron to neuron is reminiscent of how prion diseases manifest themselves in the brain. Indeed, the idea that many proteins that misfold and aggregate in neurodegenerative diseases spread through prion-like means is gaining advocates [47]. If true, this could potentially provide new means of intervening pharmacologically.

3.3 Neuroinflammation

The activation of non-neuronal cells, specifically microglial and astrocytes, along with the release of reactive cytokines is also a hallmark of AD pathology [6]. Related to macrophages, microglial are immune cells of the brain, responsible

for scavenging for debris, damaged neurons, and infectious agents. Amyloid plaques are among the brain debris engulfed through phagocytosis by microglial. Chronic over-activation of microglial leads to a state called neuroinflammation, which involves release of pro-inflammatory cytokines such as interferon-γ and interleukin-1 and subsequent tau phosphorylation and neuronal dysfunction. Astrocytes are support cells for neurons, providing critical nutrients and even eliciting signaling through release of the neurotransmitter glutamate. Reactive astrocytes are also found near amyloid plaques and can take up and degrade Aβ.

4 Pathogenesis and Risk Factors

The leading model for AD pathogenesis is the amyloid hypothesis [28], which posits that elevation of aggregation-prone forms of Aβ, either through altered production as in FAD or altered clearance as in SAD, results in the formation of toxic Aβ, perhaps soluble oligomers, that disrupt normal synaptic and neuronal function, trigger changes in tau, and elicit neuroinflammation. These effects ultimately lead to neurodegeneration and over years and decades to the clinical manifestation of AD.

The primary risk factor for AD is age. Incidence of AD before age 60 is rare and associated with dominantly inherited FAD mutations in APP and presenilins. After age 60, the risk increases dramatically, so that by age 85 the chance of having AD is ~40–50% [1]. For late-onset SAD, the most important genetic risk factor is apolipoprotein E: a single copy of the E4 allele increases the risk of AD three- to fourfold, and two copies increase the risk 12–15-fold [48]. These findings suggest that ApoE may be a worthwhile target for drug discovery. Diabetes and cardiovascular disease are also important AD risk factors, raising hopes that dietary and lifestyle changes may reduce lifetime risk to AD [49, 50].

5 Animal Models

Transgenic mice have been by far the primary animal models developed for AD. These have been engineered to overexpress FAD mutant forms of human APP and PS1, leading to age-dependent deposition of amyloid plaques and deficiencies in learning and memory [51]. However, these mice have demonstrated neither tau pathology nor frank neurodegeneration, leading to concerns about whether the observed cognitive deficits are elicited through bona fide AD mechanisms and not simply the consequence of gross overexpression of these proteins. To elicit tau pathology, APP/PS1 transgenic mice have been crossed with mice overexpressing human tau with point mutations that cause the AD-related frontotemporal dementia [52]. This cross has no effect on the amyloid production and deposition, but does increase tau pathology and exacerbate cognitive deficits.

Such AD animal models have been used extensively to test candidate AD therapeutics. However, the translation of promising preclinical candidates to the clinic has not been successful, raising serious concerns about the validity of the animal models, particularly of the transgenic mice that highly overexpress aggregation-prone proteins. The concern about artifacts due to overexpression has led to efforts to generate knock-in mice that express disease-causing mutant forms of APP, PS1, or tau under the control of endogenous promoters, with levels and spatiotemporal distribution that is physiological [53–55].

6 Clinical Trials

The design of clinical trials for AD experimental therapeutics is challenging [56]. First, these trials can be quite lengthy, depending on the goal. For symptomatic treatments, 6-month trial is often sufficient, whereas testing for disease modification requires at least 18 months and hundreds of subjects during phase II and thousands of subjects during phase III. This is because the underlying neurodegeneration – the fundamental aspect that is to be modified – is a slow process; extensive time and large numbers of subjects are needed to distinguish a statistically significant effect between treatment and placebo.

Second, identifying those suitable for enrollment requires tests for memory and cognition that are spaced apart in time to determine that the disease is progressive. This is still not sufficient for distinguishing AD from other dementias; biomarkers such as amyloid PET imaging are required for a clearer diagnosis [57]. The use of amyloid imaging agents is particularly critical for testing the efficacy of anti-Aβ therapeutic candidates: the enrollment of non-AD dementia subjects has interfered with the clear interpretation of many previous trials for AD clinical candidates.

Third, biomarkers are needed not only to enroll the right subjects but as a measure of drug effectiveness in engaging its target. In addition to PET imaging for Aβ deposition, CSF Aβ42 is a biomarker [58]. Counterintuitively, CSF levels of this peptide are reduced in AD, presumably due its deposition in the brain. Elevated total tau and phosphorylated tau in the CSF are also considered AD biomarkers. Fourth, the optimal point of intervention is critically important but can be difficult to ascertain. For instance, amyloid pathology can begin 25 years before the onset of symptoms [59], and it is unclear if enrolling presymptomatic subjects 1–2 years prior to the emergence of cognitive deficits will be sufficient for testing anti-Aβ therapeutics.

7 Therapeutic Targets and Strategies

There are many potential points of therapeutic intervention for AD, both for symptomatic and disease-modifying agents. Candidate symptomatic treatments often target a neurotransmitter or its receptors, examples being cholinesterase inhibitors or 5-HT receptor subtype agonists or antagonists. Candidates for disease modification may target Aβ production, aggregation, or clearance; tau aggregation, loss of microtubule-binding function, or interneuronal transmission; and glial cell over-activation.

7.1 Symptomatic Treatment

Cholinesterase inhibitors such as donepezil (Aricept) have been a mainstay of AD therapy for nearly 20 years [60]. These agents boost acetylcholine levels at synapses, allowing for stronger cholinergic signaling. This is important because many of the neurons lost in AD critical to learning and memory are cholinergic. Cholinesterase inhibitors, however, do not affect the underlying neurodegeneration and are thus symptomatic treatments. An advantage of these agents is that they boost acetylcholine levels that are naturally released from the presynaptic terminal; that is, they potentiate the signal that is normally produced in time and space in the brain. However, cholinesterase inhibitors typically become ineffective within 6 months to a year, as more and more cholinergic neurons are lost.

Another strategy for cholinergic stimulation is developing muscarinic and nicotinic agonists to target acetylcholine receptor subtypes [61–63]. Although this could lead to chronic stimulation of these receptors, which could result in intolerable side effects, positive allosteric modulators are typically sought, allowing greater sensitivity or responsiveness to the endogenous neurotransmitter. In this way, spatial and temporal control over receptor activation and synaptic firing is maintained. 5-HT6 receptor antagonists have also been considered for symptomatic treatment [64], primarily as an add-on with donepezil. Blocking this serotonin receptor subtype enhances acetylcholine and glutamate function. Modulation of glutamate receptors also provides symptomatic treatment; this is the basis of the approved AD drug memantine, which blocks excess signaling from the NMDA glutamate receptor subtype [60, 65].

7.2 Targeting Aβ

Targeting Aβ with small molecules can be indirect, by preventing its production through β- or γ-secretase, or direct, by blocking its aggregation. Many candidates targeting amyloid, directly or indirectly, have been in clinical trials; however,

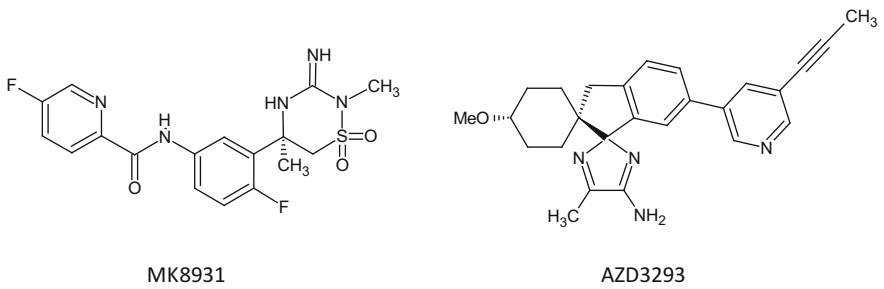

Fig. 3 BACE1 inhibitors in late-stage clinical trials

nothing has yet emerged as an approved drug. One reason for this may be what was mentioned earlier, that Aβ deposition begins many years, even decades before the onset of symptoms [59]. Clinical trials have typically enrolled subjects with MCI or early AD. Even a prevention trial, enrolling subjects predicted to show symptoms within the span of the trial, may be too late for an anti-Aβ therapeutic. The process may be tau driven and Aβ independent by this point, so intervening at the level of Aβ accomplishes little. Ongoing prevention trials with agents targeting Aβ are running 5 years, and perhaps there will be some clear benefits of intervening at this stage, several years prior to expected onset of symptoms.

7.2.1 β-Secretase Inhibitors

At present, β-secretase is the top anti-Aβ target for small-molecule therapeutics, with several compounds in the clinic, some in late-stage trials (Fig. 3), including long-term prevention trials [66]. The enzyme β-secretase, also known as β-site APP-cleaving enzyme 1 or BACE1 [67–71], is encoded by a single gene, and knockout of the BACE1 gene in mice eliminates Aβ production in the brain [72–74]. Moreover, crossing BACE1 knockout mice with APP transgenic mice rescues learning and memory deficits [75]. BACE1 knockout mice are viable and fertile; however, they show deficiencies in Schwann cell and oligodendrocyte sheathing of neurons [76] and develop cognitive, emotional, and synaptic problems [77], perhaps due to the inability to cleave the alternative substrate neuregulin-1 [76]. However, this occurs upon knockout of BACE1 from conception. Conditional knockout of the gene in adult mice would provide a better indication of the safety of BACE1 inhibition in elderly AD patients.

The β-secretase enzyme has been crystallized and its high-resolution structure determined, both alone and in complex with small-molecule inhibitors [66, 78]. This has allowed structure-based design and refinement of β-secretase inhibitors with high potency [66]. The biggest obstacle to β-secretase inhibitor development, however, has been brain penetration and retention. Potent inhibitors are often large molecules that do not penetrate the brain readily, and even when they do traverse the blood–brain barrier, they are often ejected by P-glycoprotein

[79]. These obstacles, however, have been largely overcome in recent years, with a number of compounds entering into human trials. Among these clinical candidates, Eli Lilly's LY2811376 could effectively block Aβ production in CSF in healthy volunteers; however, retinal toxicity observed in rodents led to halting human trials [80]. At present, it is unclear if mechanism-based toxicity will be a problem, as β-secretase has a number of other substrates besides APP and neuregulin-1 [81, 82].

7.2.2 γ-Secretase Modulators

The other protease responsible for Aβ production, γ-secretase, has also been a top target for the development of AD therapeutics [83]. However, as mentioned earlier, knockout of components of the γ-secretase complex blocks cleavage of Notch receptors, preventing essential signaling for cell differentiation, resulting in an embryonic lethal phenotype [84, 85]. Notch signaling is also critical for many cell differentiation events in adulthood. Because of this, inhibitors of γ-secretase cause serious side effects such as immunosuppression, skin lesions, and gastrointestinal distress [86–88]. Efforts to titrate the dose of γ-secretase inhibitors to effectively lower CNS Aβ levels while avoiding the toxicities due to deficient Notch signaling failed in advanced human trials, even with γ-secretase inhibitors that purportedly possessed selectivity for blocking proteolysis APP over that of Notch [88, 89].

For this reason, the focus with respect to γ-secretase as a target has shifted to modulators [90]. These are compounds that lower the aggregation-prone Aβ42 selectively, by specifically stimulating the carboxypeptidase trimming of Aβ42 to Aβ38 [91]. At effective concentrations, total Aβ is not lowered, and Notch proteolysis is not affected. These compounds can be very safe and given at high doses [92, 93]. However, potency and brain accessibility have been problems, and at present no γ-secretase modulator is in advanced clinical trials. It should be mentioned that the success of these agents entirely depends on Aβ42 being the pathogenic entity in AD. As mentioned earlier, this matter has not been entirely settled.

7.2.3 Aggregation Inhibitors

The identification of compounds that directly bind to Aβ and Aβ assemblies to prevent aggregation has been a long-studied strategy for the development of AD therapeutics [94]. The challenge of this approach over blocking the secretases is one of stoichiometry, as inhibiting an enzyme can prevent the production of many Aβ molecules, while direct binding requires higher levels of compound, with increased potential for off-target effects. Nevertheless, substoichiometric levels of A-β-binding compounds have been reported to prevent aggregation [95]. A critical issue here is that the compounds should not prevent deposition of Aβ while increasing levels of potentially neurotoxic Aβ oligomers. Other problems with this approach have been demonstrating selectivity and brain penetration. Although

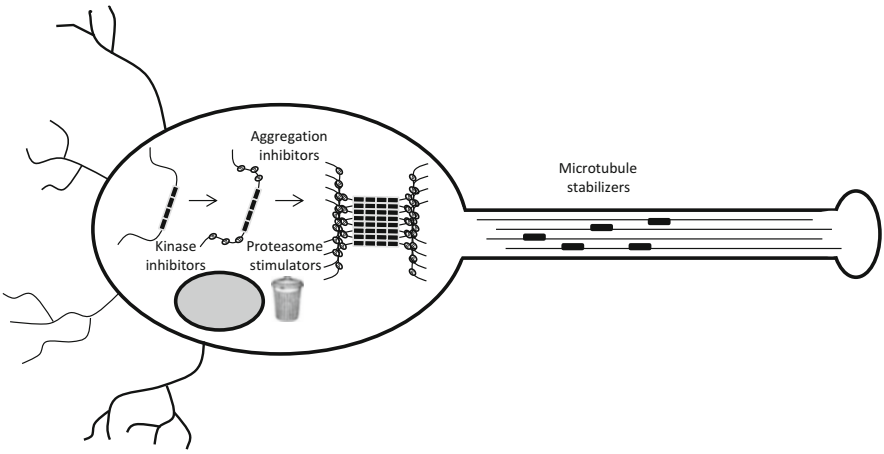

Fig. 4 Strategies for developing therapeutics targeting tau

many compounds have been reported to inhibit Aβ aggregation, at present none are in advanced human trials.

7.3 Targeting Tau

The deposition of tau in the form of filaments found in neurofibrillary tangles is another canonical feature of AD pathology. As mentioned earlier, tau is critical for the development of learning and memory deficits in APP transgenic mice [37]. This and other evidence suggest that tau is downstream of Aβ in the AD pathogenic process. As with Aβ, however, the pathogenic form of tau is not known, which makes it challenging to develop a strategy for developing effective therapeutics that target this protein. Nevertheless, several approaches have been pursued over the years, described below (Fig. 4).

7.3.1 Aggregation Inhibitors

Although the toxic form of tau is not known, its aggregation is closely coupled to pathogenesis, not only for AD but for other neurodegenerative diseases collectively called "tauopathies" [96]. As with Aβ aggregation inhibitors, the search for tau aggregation inhibitors faces challenges of identifying compounds that are potent, selective, and accessible to the brain. Nevertheless, the dye methylene blue appears to have these properties [97], although the compound has also been reported to have other effects such as enhancing protein degradation through the proteasome and autophagy and regulating gene expression [98–100]. In vivo, methylene blue can prevent tau aggregation as well as cognitive deficits in tau transgenic mice