Published in final edited form as:
Neurosci Lett. 2019 May 14; 701: 162–169. doi:10.1016/j.neulet.2019.02.011.

γ-Secretase and its modulators: Twenty years and beyond

Weiming Xia[a,b,*]

[a]Geriatric Research Education Clinical Center, Edith Nourse Rogers Memorial Veterans Hospital, Building 70, Room 202, Bedford, MA, 01730, United States

[b]Department of Pharmacology and Experimental Therapeutics, Boston University School of Medicine, Boston, MA, United States

Abstract

Twenty years ago, Wolfe, Xia, and Selkoe identified two aspartate residues in Alzheimer's presenilin protein that constitute the active site of the γ-secretase complex. Mutations in the genes encoding amyloid precursor protein (APP) or presenilin (PS) cause early onset familial Alzheimer's disease (AD), and sequential cleavages of the APP by β-secretase and γ-secretase/presenilin generate amyloid β protein (Aβ), the major component of pathological hallmark, neuritic plaques, in brains of AD patients. Therapeutic strategies centered on targeting γ-secretase/presenilin to reduce amyloid were implemented and led to several high profile clinical trials. This review article focuses on the studies of γ-secretase and its inhibitors/modulators since the discovery of presenilin as the γ-secretase. While a lack of complete understanding of presenilin biology renders failure of clinical trials, the lessons learned from some γ-secretase modulators, while premature for human testing, provide new directions to develop potential therapeutics. Imbalanced Aβ homeostasis is an upstream event of neurodegenerative processes. Exploration of γ-secretase modulators for their roles in these processes is highly significant, e.g., decreasing neuroinflammation and levels of phosphorylated tau, the component of the other AD pathological hallmark, neurofibrillary tangles. Agents with excellent human pharmacology hold great promise in suppressing neurodegeneration in pre-symptomatic or early stage AD patients.

Keywords

Alzheimer; Secretase; Modulator; Neuroinflammation

1. Introduction

Alzheimer's disease (AD) is by far the most prevalent cause of dementia in the elderly, and the disease may evolve over the course of decades. Pre-symptomatic AD subjects usually do not exhibit any phenotype before converting to the earliest clinically detectable stage, known as "mild cognitive impairment (MCI)". A certain percentage of MCI maintain their cognitive function throughout the remaining of their life, but a majority gradually converts from MCI to mild AD, moderate AD, and finally advanced stage AD. Overt cognitive decline from

*Correspondence address: Geriatric Research Education Clinical Center, Edith Nourse Rogers Memorial Veterans Hospital, Building 70, Room 202, Bedford, MA, 01730, United States. weiming.xia@va.gov, wxia@bu.edu.

initial clinical manifestation lasts for 10–15 years toward the later stages of a more devastating neurodegenerative process. During the disease progression, AD patients exhibit many clinical features, memory impairment being the most prevalent symptom at all stages.

2. Alzheimer pathology: Aβ and Tau

Amyloid β protein (Aβ) plays an important role in AD pathogenesis. At the molecular level, senile amyloid plaques and neurofibrillary tangles (NFT) are two neuropathological hallmarks of AD [1]. Mutations in genes encoding amyloid precursor protein (APP) or presenilin (PS) cause early onset familial AD (FAD), and one or two copies of the apolipoprotein E (apoE) ε4 allele is a major risk factor for lateonset sporadic AD. Aβ is generated by sequential cleavages of the APP by β- and γ-secretases. First, APP is proteolytically processed by β-secretase (BACE1) and generates a 12 kDa C-terminal stub of APP (C99); second, C99 is cleaved by γ-secretase to yield two major species of Aβ ending at residue 40 (Aβ40) or 42 (Aβ42) [2,3]. Genetic studies show that detrimental familial AD-linked missense mutations in APP or PS increase the ratio of 42 residue of Aβ (Aβ42) to a more common 40-residue of Aβ (Aβ40) and cause early onset AD, while a beneficial mutation in APP leads to decreased Aβ production and those carriers maintain intact cognitive function at advanced ages [4]. ApoE has three major isoforms, ApoEε2, ε3 and ε4. ApoEε4 allele is the strongest known risk factor for AD. Brains of sporadic AD patients carrying ApoEε4 allele were found to have increased density of Aβ deposits, limited capability to clear Aβ, and enhanced neuroinflammation [5].

The Aβ42 peptide has been the center of investigation and the target for therapeutic exploration. In AD, the protein subunit of the amyloid plaques, Aβ, does not occur as a single molecular species; many different Aβ-containing peptides have been detected in human cerebrospinal fluid (CSF) and/or brain [6,7]. The most common Aβ isoform in vivo is Aβ40, i.e., a peptide that begins at Asp1 and terminates at Val40 of the Aβ region of APP. Increased accumulation of Aβ42, a peptide that differs from Aβ40 by the inclusion of Ile41 and Ala42, is particularly associated with development of AD. The extra two hydrophobic amino acids of Aβ42 greatly enhance its aggregation propensity [8], leading to accelerated formation of small (low-n) Aβ oligomers (oAβ), larger intermediate assemblies like protofibrils, and eventually the typical ~ 8 nm amyloid fibrils found abundantly in neuritic plaques and amyloid-bearing micro vessels. Small, soluble oligomers of Aβ have been linked to neuronal toxicity and synaptic failure (for review, see [9]). The ratio of Aβ42/Aβ40, rather than the total amount of Aβ, has been shown to correlate with the age of onset of FAD [10] and with the amount of plaques in mouse models [11,12]. Aβ42 constitutes approximately 10% of total Aβ species [13] and is more prone to aggregation than Aβ 40 [8,14], Furthermore, Aβ40 may play an antagonistic role in preventing Aβ42 aggregation in vivo [11,12] and in vitro [15-17]. Expression of Aβ42, rather than Aβ40, in Drosophila and mice led to the formation of Aβ plaques [18,19]. Therefore, specific inhibition of γ-secretase activity for Aβ42 generation would be an appealing strategy for the treatment of AD [20,21].

The Aβ peptide is closely linked to a second AD pathological protein, tau. This intracellular hallmark of AD is the paired helical filament (PHF) in NFT containing hyperphosphorylated

tau. Mutation in the tau gene causes frontotemporal dementia with Parkinsonism linked to chromosome 17 (FTDP-17) [22]. Transgenic mice expressing mutant tau show close association of mutation to NFT formation and neurodegeneration [23,24]. Furthermore, Aβ has been shown to drive tau pathology in vivo [25]. The normal microtubule-associated tau gradually loses association with microtubules and hyperphosphorylated tau forms PHF which accumulate in neuronal cytoplasm as the major component of the NFT. Glycogen synthase kinase-3β (GSK3β) is one of several microtubule-associated kinases responsible for tau phosphorylation [26]. GSK3β has been found to phosphorylate a number of sites on tau that were identified by nanoelectrospray mass spectrometry, including the residue Thr181 to form pTau181 [27].

The significance of Aβ and tau as therapeutic targets is not only derived from pathological evidence of postmortem brain but also from biochemical analysis of cultured human neurons. This is largely based on the induced pluripotent stem cell (iPSC) technology that has been established to study Aβ, tau, and GSK3β in AD [28]. When iPSC lines from two normal subjects, two SAD (sAD1 and sAD2), and two FAD patients carrying a duplicated copy of the APP gene were established for human neuronal differentiation, those from two duplicate APP gene carriers and patient sAD2 showed very high levels of Aβ40, phosphortau(Thr 231) and active GSK3β [28]. Importantly, levels of Aβ, pTau and active GSK3β can be reduced by β-secretase inhibitors, indicating a direct relationship between γ-secretase substrate C99 and GSK3β activation/Tau phosphorylation. Other studies have demonstrated that iPSC-derived neuronal cells exhibit reduced Aβ levels in the presence of BACE1 inhibitor or γ-secretase inhibitor/modulators [29,30]. Thus, involvement of Aβ and tau in AD pathogenesis can be modeled in human neuronal cells amendable for testing of therapies targeting either Aβ or phosphorylated tau [31].

3. Targeting γ-secretase with inhibitors: from the end to the beginning?

Aβ targeted therapies are being actively pursued in preclinical and clinical studies for treatment of AD. These therapies are based on the amyloid cascade hypothesis, which postulates that Aβ peptides form neurotoxic species, trigger a pathological cascade and ultimately lead to neurodegeneration and dementia [32,33]. γ-Secretase, along with the β-secretase, have become the prime target for this purpose. γ-Secretase is composed of PS1, Presenilin Enhancer-2 (Pen-2), anterior pharynx defective-1 (Aph-1), and Nicastrin (Nct). PS1 carries two aspartate residues constituting the active site of γ-secretase [34], and Pen-2 is a small protein of 101 amino acids with two TM domains [35,36]. Many studies have demonstrated that overexpression of all four components results in increased γ-secretase activity, both in mammalian cells [37-42] and in yeast [43]. Purified PS1 and Pen-2 are sufficient to carry out γ-secretase cleavage of its substrates in vitro; PS1 itself has proteolytic activity [44,45], while Pen-2 promotes the conversion of PS1 from zymogen to the active protease [44,46].

A large number of potent γ-secretase inhibitors (GSIs) have been developed. Two GSIs, LY-450139 (Semagacestat) and BMS-708163 (Avagacestat), are among those tested in clinical trials. LY-450139 is known to block the cleavage of APP and Notch, another γ-secretase substrate [47], and subjects receiving LY-450139 presented with worsening of

clinical measures of cognition and the ability to perform activities of daily living [48,49]. Because perturbed Notch signaling has been implicated in cancer formation, inhibition of Notch signaling by LY-450139 could be one of the culprits causing the undesired clinical outcomes [50].

More selective GSIs targeting APP over Notch were developed, like allosteric GSIs and BMS-708163. Allosteric GSIs (AGSI) displays specificity against Aβ42 production over Aβ40, Aβ38 and Notch1 cleavage [51]. These AGSIs bind to an allosteric site within γ-secretase rather than the APP substrate. Furthermore, AGSIs affect γ-secretase activity for both Aβ40 and Aβ38 production similarly and therefore lack the interconnected effect of the γ-secretase modulators (GSM) (see below) in which decreased Aβ42 resulted in increased Aβ38 generation [52]. Clearly, these AGSIs represent a class of inhibitors that are distinct from the Notch-sparing GSIs that have no selectivity for Aβ40 and Aβ42 [53-55].

BMS-708163 is a potent GSI that showed impressive γ-secretase inhibition with 50% inhibition concentrations (IC50) of 0.27 and 0.30 nM for Aβ42 and Aβ40, and 58 nM for Notch, respectively, thus representing a 193-fold selectivity for APP over Notch [56]. Pharmacokinetic (PK)-pharmacodynamic (PD) analysis of BMS-708163 in male dogs revealed plasma concentration of BMS-708163 of ~0.5 μM at 3h post-dosing (hpd) and brain concentration of~0.75 μM at 5 hpd, with a sustained decrease of brain Aβ40 by 50%. However, a separate study indicates that only 3–7 fold selectivity exists for APP over Notch [57], and clinical outcomes [58,59] seem to be discrepant with reported 193-fold selectivity for APP over Notch cleavage [56].

Clinical trials of all GSIs have ended prematurely, raising the question on our understanding of their target, the γ-secretase/presenilin. Development of compounds to target γ-secretase and reduce Aβ production is complicated by the fact that γ-secretase/presenilin has critical biological function, and γ-secretase mediates the final proteolytic cleavage of Notch [60,61] and many substrates. There are over 100 γ-secretase substrates that are type I membrane proteins and have diverse functions. Notch is one of the most interesting and important substrates with diverse functions. Notch has a large extracellular domain, a single transmembrane (TM) domain and an intracellular domain. Notch is proteolyzed in the trans-Golgi as part of its maturation process into a heterodimeric cell surface receptor, then undergoes a second proteolysis upon ligand activation, leading to shedding of the extracellular domain of the receptor. The remaining membrane-bound C-terminal stub, like APP C-terminal fragment, is cleaved by γ-secretase at two sites (in the middle of its TM domain and at a residue close to the interface of the membrane and cytoplasm) to release the Notch-1-β peptide (Nβ, similar to Aβ of APP) and Notch intracellular domain (NICD), which translocates to the nucleus where it regulates gene expression [61-63]. Notch ICD signaling is critical to a wide variety of cell fate determinations during embryonic development and adulthood.

The cytosolic ICDs from known γ-secretase substrates represent a unique library of signaling molecules. Like Notch, they are generated by γ-secretase/PS1 cleavage of substrates [64,65]. These ICDs have different physiological functions linked to regulation of transcription of downstream genes, such as ICDs of alcadeins, CD44, DCC, Notch, Delta,

Jagged, E- and N-cadherin, receptor-like protein tyrosine phosphatases, and leukocyte-common antigen related protein. They are involved in a variety of cellular pathways including regulation of cell fate and death, neurite outgrowth, transcriptional regulation, cell-cell adhesion, regulation of ion conductance, and neurotrophin signaling [64,65]. A key concern with GSI is their lack of selectivity among these γ-secretase substrates, e.g., GSIs have shown Notch-related toxicity in rats, including interference with maturation of B- and T-lymphocytes and gastrointestinal tract toxicity [66,67]. A similar requirement of γ-secretase for neuronal survival was found in zebrafish [68,69]. Treating zebrafish with a potent γ-secretase inhibitor, DAPT, causes Notch phenotypes with defects in somitogenesis and neurogenesis [70-73]. The DAPT treated embryos exhibit suppression of Notch phenotypes after injection of Notch intracellular domains (NICD) mRNA [70].

In humans, a PS1 mutation that causes almost complete loss of γ-secretase activity was found in familial AD cases [74]. In mice, double conditional knockout (KO) of PS1 and its homolog PS2 showed progressive loss of synapses, dendrites and neurons, accompanying reduction of NMDA receptor mediated responses and synaptic levels of NMDA receptors [75,76]. Conditional KO of another γ-secretase component, nicastrin, in adult mice displays similar age-dependent cortical neuronal loss likely occurring through apoptosis [77]. With new knowledge of presenilin biology and γ-secretase substrates, it was apparent that development of GSIs for AD needed to be replaced with alternative approaches, such as γ-secretase modulators (GSM).

4. Modulating γ-secretase and neuroinflammation: one stone two birds?

GSMs theoretically have a “regulated” inhibition of γ-secretase activity that could reduce Aβ42 production without obliterating Notch signaling [78], unlike non-selective inhibition of γ-secretase and unwanted side effects for AD therapy caused by GSIs. GSMs only modulate the γ-secretase cleavage site of APP instead of the downstream ε-cleavage site [79]. Competition studies indicate that GSMs have distinct binding sites [80-82]. Cross-linking probes have independently identified PS1-NTF as a specific target of some potent GSMs, while APP is targeted by R-flurbiprofen [83]. These studies have demonstrated that the enzyme γ-secretase itself can be modulated, rather than inhibited, and in such a way that can lower the ratio of Aβ42/40 [80-82]. Therefore, GSMs that have less of an effect on Notch or other substrates will cause fewer adverse side effects.

The first GSM was identified from the discovery of non-steroidal anti-inflammatory drugs (NSAIDs). An amyloid reducing GSM that also suppresses inflammation is desirable. Inflammatory response is an invariable characteristic of AD pathogenesis, in part triggered by Aβ. During AD onset and progression, microglial cells and astrocytes are activated, and cytokines like TNFα are secreted by microglia [84]. In 3X Tg AD model, TNFα is upregulated as Aβ pathology appears, at about 3 months of age [85]. Most cytokines are expressed at very low levels in the healthy brain, and neuroinflammation can be detected years before neurons die. Previous studies have shown that Aβ can bind to scavenger receptors expressed on microglia like CD36 [86] and Scara1 [6], which enter microglia and activate inflammation. When microglia cells engulf extracellular aggregates like Aβ, they trigger inflammasomes (such as NOD-like receptor family pyrin domain-containing 3

(NLRP3)) and activate caspases, and promote IL-1β release [87]. This pathway was validated in AD transgenic mice where NLRP3 was shown to contribute to AD like pathology in mouse brains [88]. Aβ generation and inflammatory response are thus concurrent events associated with Aβ clearance. Accordingly, genetic mutations found in the microglial receptor TREM2 (triggering receptor expressed on myeloid cells 2) triple a person's risk for AD [89,90] and increased expression of CD33, which functions to suppress Aβ uptake and clearance, modifies AD risk [91,92]. Systems analysis of hundreds AD brain reveals changes in network related to immunologic molecules and microglial cells, including microglial protein TYROBP that binds TREM2 and may regulate CD33 [93].

Several classes of GSMs have been developed [94,95]. As discussed above, one class of GSMs includes a subset of NSAID-like carboxylic acids that specifically block cleavage of the γ-secretase substrates in the middle of their TM domains without affecting generation of the ICD of several type I transmembrane proteins, including APP, ErbB-4, and Notch [96]. These GSMs inhibit Aβ42 production with a concurrent increase of Aβ38 and no effect on Aβ40 production or Notch processing. Dosing cultured cells and transgenic mice revealed that NSAIDs directly modulate the γ-secretase complex [78,97-101], independent of their inhibitory effects on cyclooxygenase (COX) and Rho activity in the Rho-Rock pathway [102].

Another GSM that suppress neuroinflammation is CHF5074. In cultured cells, CHF5074 exhibits IC50s of 18.4 and 3.6 μM for Aβ40 and Aβ42, respectively (6-fold selectivity for Aβ42). In HEK293 cells expressing Notch substrate, Notch cleavage by γ-secretase was not inhibited by CHF5074 at 5 μM [103,104]. In 10-month old Tg2576 mice (expressing the Swedish mutant of APP), steady state brain and plasma concentrations of CHF5074 reached 6.4 μM and 228 μM, respectively, with a ~50% reduction in both the number of plaques and the area occupied by plaques in brain was observed. This corresponded to a ~50% reduction of total brain Aβ and Aβ42 (49% and 42%, respectively). A third reduction of Aβ was found in a second transgenic mouse line expressing both Swedish and London mutant APP after chronic exposure with CHF5074, with brain and plasma drug exposure at 3 and 281 μM respectively [104]. CHF5074 showed reversal of contextual memory deficit and restoration of hippocampal neurogenesis potential [105].

CHF5074 is not only a GSM but also a neuroinflammation modulator [106,107]. Ross et al. reported that subjects on CHF5074 showed linear decreases in levels of inflammatory marker TNFα and soluble CD40 ligand in CSF [106]. Apparently, CHF5074 is the first GSM that shows positive cognitive outcomes in humans with clear decrease in CSF TNFα. TNFα is a major marker of neuroinflammation in AD. It is produced in glial cells and neurons during normal aging and in patients suffering brain trauma, neurodegenerative disorder or excitotoxic insults, and TNFα has detrimental effects on synaptic transmission and plasticity [108]. It is not specific for AD, as most neurodegenerative diseases are accompanied by a cytokine-mediated inflammatory response. Binding of TNFα to Tumor necrosis factor receptor 1 (TNFR1) leads to activation of NFκB and MAPK pathways, and induction of death signaling [109]. CHF5074 is devoid of anticyclooxygenase (COX) and Notch-interfering activities in vitro [110]. It is likely that CHF5074 has a direct anti-inflammatory effect that is mediated by its interaction with the γ-secretase complex.

Although these compounds were no longer pursued in clinical trials, it is an attractive approach to develop a class of GSMs that may reduce Aβ42 and neuroinflammation markers. Apparently, Aβ deposition and neuroinflammation play critical roles in AD onset and progression, and the challenge is to identify highly potent compounds targeting both pathways.

5. γ-Secretase modulators: a loss of pharmacology?

A lack of translation from in vitro to in vivo pharmacology is an unresolved issue among most GSMs. R-flurbiprofen was tested in clinical trials, however, it did not achieve statistical significance on either of its primary endpoints – cognition or activities of daily living. R-flurbiprofen is a weak GSM with an IC50 for Aβ reduction at approximately 300 μM [100]. Due to its poor brain penetration, it was unlikely to have lowered brain Aβ42 levels in the clinical studies. Treatment of monkeys at 100 mg/kg of ibuprofen or humans with 800 mg single dose did not reveal any changes of Aβ in plasma; CSF Aβ from ibuprofen dosed monkeys did not show any changes [111].

Among 100 CHF5074 treated subjects with MCI, apoEε4 carrier improved on several cognitive measures over the initial three months of treatment [106,107]. During the open label extension period of more than one and half year, apoEε4 carriers maintained their improved cognition and even score better on verbal memory and tests of attention and executive function, compared to baseline performance. Non-carriers' cognitive abilities have remained stable for almost two years [106,107]. The clinical outcome from apoEε4 carriers versus non-carriers could be explained by findings that apoEε4 carriers usually have more neuroinflammation compared to non-carriers [5], thus it was easier to achieve any anti-neuroinflammatory benefit of CHF5074. However, the IC50 for Aβ42 was at 3.6 μM in cultured cells, indicating that CHF5074 is a weak GSM [103,104].

Another class of non-NSAID derivative GSMs include Eisai's E-2012 [112,113] and NeuroGenetics' Compound 4 (Cpd 4) [114]. While Cpd4 directly interacts with PS and Pen2, the binding protein for E-2012 is not clear. When brain exposure was over 100-fold of IC50, Cpd 4 inhibited all three Aβ peptides (Aβ38, 40 and 42) in animals under chronic treatment [114], which is similar to Eisai's E-2012 that inhibits both Aβ40 and Aβ42 [112,113]. In addition, a number of GSMs were reported by Merck [115-117], and among them, one GSM showed 70% Aβ42 reduction when brain GSM exposure reached 7.8 μM (~400-fold of IC50) [117].

More potent GSMs with IC50 at sub-μM have been reported [118-120]. GSM-10h is a NSAID-derived GSM with an in vitro IC50 of 0.8 μM. In a transgenic mouse line expressing mutant APP and PS1, GSM-10h brain and plasma levels at 6 hpd reached 54.7 μM and 32.9 μM, respectively, which were 40–70 fold of IC50, but brain Aβ42 was reduced by about 20% [121]. There was a concomitant>30% increase in Aβ38, with no effect on Aβ40 [122]. In rats, GSM-10h caused a dose-dependent decrease in the level of Aβ42, but not Aβ40, in brain, CSF, and plasma [123]. An analogue of GSM-10h, GSM-1, carries an in vitro IC50 at 0.35 μM, causes a dose dependent decrease of Aβ42 and an increase of Aβ38 in mice expressing Swedish mutant APP [80,124].

Among the GSMs with IC50 at sub-μM, EVP-A, EVP-B and JNJ-40418677 show similar potency. EVP-A and EVP-B (EnVivo, later Forum Pharmaceuticals) showed in vitro IC50 for reduction of Aβ40 and Aβ42 at 0.24 μM and 0.14 μM, respectively. In rats, a brain concentration of 2.7 μM EVP-A produced no reduction of Aβ, while a brain concentration of 10 μM (40–70 fold above the IC50) of EVP-B produced a 20–30% reduction of brain Aβ [125]. A better compound EVP-0015962 showed a similar IC50 of 0.12 μM in stable human cells, a 4-fold higher IC50 of 0.49 μM in neuronal cells, and no effect on Notch processing. Dose dependent reduction of Aβ42 was observed in rat models. Brain exposures at 2.8 μM and 8.3 μM (5- and 17-fold of IC50) led to a 22% and 38% reduction in brain Aβ42 respectively. Chronic dosing at 20 and 60 mg/kg/day in APP transgenic mice for 6 months led to a lowering of brain plaque load of 81% and 95% respectively [126,127].

A GSM with a similar potency to the EnVivo compounds, JNJ-40418677, selectively inhibited Aβ42 production with IC50s in neuroblastoma cells and primary rat cortical neuronal cultures of 0.20 μM and 0.18 μM, respectively [128]. A lack of effect of JNJ-40418677 on α-and β-secretase was confirmed by visualizing unchanged APP CTFα and CTFβ. In cell-free APP and Notch assays in vitro, JNJ-40418677 did not affect the AICD generation at 100 μM and NICD at 10 μM. Although the difference in its effect on AICD and NICD generation is not clear, a 50-fold selection for Aβ42 inhibition over NICD inhibition was achieved [128]. In mice, four hours after a single oral dose, both brain and plasma GSM exposures achieved 17 μM (85-fold of IC50), with a brain/plasma ratio of 1. Between 2 and 24 h, Aβ42 levels were significantly reduced, and total Aβ levels were not changed in the brain. Chronic dosing of JNJ-40418677 in Tg2576 mice for 7-months at doses of 20,60 and 120 mg/kg/day led to corresponding dose dependent effect. When brain exposure was at 2-fold of IC50 (0.42 μM), no effect on Aβ levels was found. When the brain exposure reached 12-fold of IC50 (2.4 μM) or higher, a significant reduction of Aβ42 was observed, and all three Aβ peptides, Aβ38, 40, and 42, were reduced. The Aβ reduction correlated with a significant reduction in the numbers of plaques that contained Aβ38, Aβ40 and Aβ42 [128]. This is similar to “Notch sparing” GSI, BMS-708163, that reduces levels of CSF Aβ38, 40 and 42 [129]. Therefore, chronic dosing of JNJ-40418677 in animals led to a complete inhibition of all Aβ peptides, a feature like that of GSI.

Two excellent GSMs with clear pharmacological effect across rats, dogs, monkeys, and human subjects are BMS-932481 and BMS-986133 with IC50 at 6.6 and 3.5 nM to reduce Aβ42, respectively. Both GSMs exhibit dose- and time-dependent activity in vivo by decreasing Aβl-42 and Aβ1-40 levels while increasing Aβ1-38 and Aβ1-37 [130,131]. Although the mechanism and central activity of these GSMs translate across preclinical species and humans, insufficient margin for human safety prevents further testing for efficacy of Aβ lowering in AD patients [131].

A unique GSM, BPN-15606, exhibited an IC50 of 7 nM and 17 nM to reduce Aβ42 and Aβ40 from cultured cells, with a concomitant increase of Aβ38 and no change in total Aβ [132]. BPN-15606 binds to an allosteric site within the γ-secretase complex and does not affect Notch cleavage at 25 μM. Dose dependent decreases of plasma, brain and CSF Aβ were found in both mice and rats. Chronic dosing of transgenic mice with BPN-15606 significantly reduced accumulation of Aβ plaques in both the hippocampus and cortex. Like

previous reported BACE inhibitors [133], BPN-15606 treatment of 3-dimensional neuronal culture decreased total tau and phosphorylated pThr181 tau [132]. Based on in vivo pharmacokinetic profile of BPN-15606, sub micromolar plasma exposures of BPN-15606 expect to achieve a significant lowering of Aβ42 in human brain, thus requiring much lower doses than those reported for BMS-932481 and BMS-986133.

6. Detecting efficacy of γ-secretase modulators: in 20 years?

It is widely accepted that future AD therapies need to start at an earlier stage, as the onset of disease may occur 15–20 years before the appearance of clinical symptoms [134]. One of major factors contributing to the failure of GSIs and GSMs could be the timing of treatment, i.e., patients at mild to moderate stage might be too late for Aβ reducing therapies as neuronal damage is extensive and irreversible. To test asymptomatic patients at very early stages of disease, biomarkers are needed to identify those subjects for clinical trials. Alternative approaches have been pursued in AD patients carrying FAD mutations (e.g., Dominantly Inherited Alzheimer's Network). The Alzheimer's Prevention Initiative (API) was created for clinical trials of Aβ vaccine in pre-symptomatic members from an extended Colombian family carrying a PS1 mutation. Specific GSMs reversing familial mutant PS1/γ-secretase activity may be ideally positioned for those subjects [135]. Anti-Amyloid Treatment in Asymptomatic AD Trial (A4) with brain amyloid imaging has enrolled over a thousand asymptomatic subjects. Therapeutic development with brain imaging and cognitive function as efficacy readouts has been pursued [136], and new Aβ-reducing approaches might be effective in patients with MCI and in pre-symptomatic AD patients. With recent development of GSMs such as BPN-15606 [132] or endogenous cholesterol metabolite cholestenoic acid [137], it does not take 20 years to wait for AD patients converting from pre-symptomatic to symptomatic stages while testing efficacy of GSMs. Advancements in brain imaging and fluid biomarkers will greatly facilitate the discovery of disease modifying therapeutics for AD.

Acknowledgements

This study was supported by the award I21BX003807 and IO1 BX003527 from the Biomedical Laboratory Research and Development Service of the Veterans Affairs Office of Research and Development (WX) and the Cure Alzheimer's Fund (WX). The views expressed in this article are those of the author and do not represent the views of the US Department of Veterans Affairs or the US Government.

Abbreviation:

AD	Alzheimer's disease
FAD	familial AD
GSI	γ-secretase inhibitor
GSM	γ-secretase modulator
GSK3β	glycogen synthase kinase-3β
KO	knockout

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