CLINICAL INVESTIGATION

γ-secretase inhibitors for treating Alzheimer's disease: rationale and clinical data

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There are no drugs available for slowing down the rate of deterioration of patients with Alzheimer's disease (AD). With the aim of altering the natural history of the disease, the pharmaceutical industry has designed and developed several compounds inhibiting γ-secretase, the enzymatic complex generating β-amyloid (Aβ) peptides ($A\beta_{1-40}$ and $A\beta_{1-42}$), believed to be involved in the pathophysiological cascade of AD, from amyloid precursor protein (APP). This article briefly reviews the profile of γ-secretase inhibitors that have reached the clinic. Studies in both transgenic and nontransgenic animal models of AD have indicated that γ-secretase inhibitors, administered by the oral route, are able to lower brain Aβ concentrations. However, few data are available on the effects of these compounds on brain Aβ deposition after prolonged administration. γ-secretase inhibitors may cause abnormalities in the gastrointestinal tract, thymus, spleen, skin, and decrease in lymphocytes and alterations in hair color in experimental animals and in man, effects believed to be associated with the inhibition of the cleavage of Notch, a transmembrane receptor involved in regulating cell-fate decisions. Unfortunately, two large Phase III clinical trials of semagacestat in mild-to-moderate AD patients were prematurely interrupted because of the observation of a detrimental cognitive and functional effect of the drug. The pejorative effects of semagacestat in AD patients may be due to its lack of selectivity on APP processing. The compound could inhibit the processing of one or more substrates of γ-secretase important for cognition. It has been also noted that semagacestat causes the accumulation of a neurotoxic peptide (CTFβ or C99) resulting from the block of the γ-secretase cleavage activity of APP. New more selective γ-secretase inhibitors are being developed with the hope of overcoming these limitations.

Keywords: β-amyloid • γ-secretase inhibitors • Alzheimer's disease • Notch processing

With an aging population and the increase in disease incidence, it is mandatory to develop disease-modifying therapies for Alzheimer's disease (AD). In fact, AD is the most common type of dementia in clinical and autopsy surveys, associated with progressive cognitive decline and profound neuronal loss. The 2010 figures suggested 5.3 million of estimated AD cases in the USA [1], with >26 million patients having AD worldwide, and an expected increase to more than 106 million by 2050 [2]. Currently, clinicians use the term AD to refer to a clinical entity that typically presents with a characteristic progressive amnestic disorder with subsequent appearance of other cognitive, behavioral, and neuropsychiatric changes that impair social function and activities of daily living [3]. The initial presentation can also be atypical, with non-amnestic focal cortical cognitive symptoms [4]. Since the first description of the disease by Alois Alzheimer in the early 20th Century, much work has been undertaken to identify the molecular basis of the disease. The majority of AD cases are

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sporadic compared with fewer than 5% of familial cases that are caused by autosomal dominant inheritance of mutations in presenilin 1 (PS-1), presenilin 2 (PS-2), or amyloid precursor protein (APP) [5]. At autopsy, the most frequent pathological alterations in the brains of AD patients include extracellular deposits in specific brain regions, for example, neuritic or senile plaques (SPs), mainly composed of aggregates of a peptide with 40 or 42 amino acid residues known as β-amyloid (Aβ). In fact, from a neuropathological view, AD involves aberrant protein processing and is characterized by the presence of both intraneuronal protein clusters composed of paired helical filaments of hyperphosphorilated tau protein (neurofibrillary tangles), and the extracellular SPs. These two lesions represent the neuropathological hallmarks of the disease, and their observation during postmortem examination is still required for the diagnosis of AD. These pathological lesions first appear in the entorhinal regions of the hippocampus and then become widespread. The Aβ peptide is the result of the metabolic processing of a complex transmembrane glycoprotein known as APP. APP may be metabolically processed according to two pathways. In the so-called non-amyloidogenic pathway, the α-secretase enzyme cleaves APP within the Aβ sequence and releases extracellularly the soluble N-terminal fragment, soluble APP (sAPP) α, that appears to exert neuroprotective activity. In the amyloidogenic pathway, the β-secretase enzyme releases sAPPβ plus a 12 kDa protein fragment (C99 or CTFβ), which in turn is cleaved by the γ-secretase enzyme giving way to Aβ (Figure 1). The correlation among Aβ histopathologic lesions, brain cell death and cognitive deficiency in AD represents the so called 'amyloid cascade hypothesis' of the disease, conceptualized in 1991 by Hardy and Allsop [6]. The updated version of this theory says that the oligomeric forms of $A\beta_{1-42}$ are the main cause of neuronal death in AD [7,8].

Drugs targeting β-amyloid for the treatment of AD

At present, AD is treated with drugs that only provide symptomatic relief [9]. Many pharmaceutical companies are pursuing different paths to develop treatments for AD that aim to reverse or prevent the disease. Over the last 15 years, with astonishing advances in our understanding of the AD neurobiology, a number of therapeutics targeting Aβ has been investigated [9–11]. Based on the amyloid cascade hypothesis, drugs that can prevent production, aggregation, and deposition of Aβ are thought to be promising therapeutics for AD [12]. For potential AD treatment targeting the aggregation and deposition of Aβ, several clearance facilitators by active and passive immunotherapy approaches are under investigation in clinical trials [13,14], while brain penetrant inhibitors of Aβ aggregation have been identified and one of such compounds, PBT-2, has produced encouraging neuropsychological results in a recently completed Phase II study [15,16].

Much attention has been focused on the inhibition or modulation of activities of α-, β-, and γ-secretases as disease-modifying therapies based on pathological mechanisms [17–20]. Unfortunately, the most biologically attractive of these proteases, β-secretase or β-site APP-cleaving enzyme 1 (BACE-1; memapsin-2 and Asp-2), that regulates the first step of the amyloidogenic APP metabolism, was found to be particularly problematic to block and only few compounds (CTS21166 and LY2811376) have reached clinical testing so far [20]. Compounds that stimulate α-secretase, the enzyme responsible for the non-amyloidogenic metabolism of APP, are also being developed one of them, EHT-0202, has recently started a Phase II study [20]. Conversely, several inhibitors of γ-secretase, the protease that regulates the last metabolic step generating Aβ, have been identified [18,19]. In particular, γ-secretase is an unusual aspartyl protease that intramembranously cleaves a wide range of type I membrane proteins in addition to APP [21,22]. γ-secretase complex is composed of four components that are required for the enzymatic activity: presenilin (PS), Aph-1, Pen-2 and nicastrin [22–25]. Two mammalian PS homologues exist, PS-1 and PS-2, and they show a high degree of homology (67%) and functional redundancy. At present, more than 175 mutations in *PSEN1* gene have been identified in aggressive early-onset familial AD (FAD), and most of them elevate the $A\beta_{1-42}$:$A\beta_{1-40}$ ratio and interfere with the processing of APP and other γ-secretase substrates [26,27]. Extensive cellular, molecular, and biochemical analyses revealed that PS functions as a catalytic center of γ-secretase [24]. Thus, the inhibition of the catalytic unit of the γ-secretase enzymatic complex appears to be a logical strategy for contrasting Aβ accumulation in the brain of AD patients and indeed many potent, orally active and brain penetrant compounds have been synthesized and developed [18–20,28]. Unfortunately, γ-secretase cleaves many other important substrates other than APP [29], one of the most relevant from toxicological point of view being Notch. Thus, γ-secretase inhibitors block the processing of other proteins and this is the main cause of toxicity in preclinical testing and represents a major source of concern in clinical trials.

A considerable advance in the field of γ-secretase-based drugs took place after the discovery that some small organic molecules [30] and mainly some commonly used nonsteroidal anti-inflammatory drugs (NSAIDs) selectively lowered $A\beta_{1-42}$ in cell culture and transgenic animal models, independently of cyclooxygenase activity [31,32]. Therefore, the (R)-enantiomers that have low

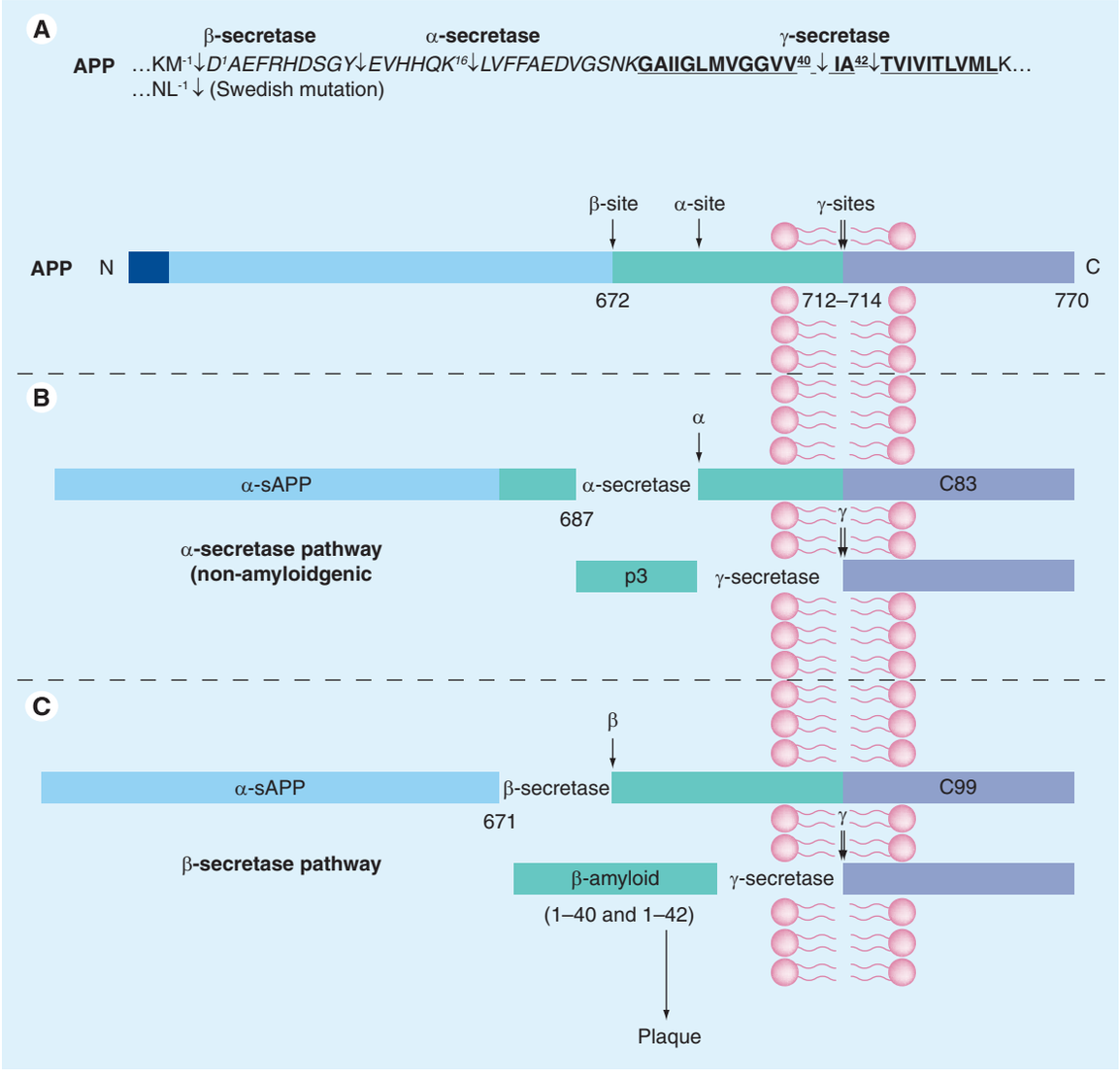

Figure 1. Overview of amyloid precursor protein processing. (A) Panel shows the APP region comprising the transmembrane sequence (underlined, bold) and the sequences of β-amyloid $(A\beta)_{1-40}$ (D^1-V^{40}) and $A\beta_{1-42}$ (D^1-A^{42}) peptides. The β-secretase cleaves at D^1 and Y^{10}. The α-secretase cleaves at Lys^{16}, and the γ-secretase cleaves at Val^{40} and/or Ala^{42}. Below the sequence is a representation of APP with the residue numbers of interest in β- and γ-secretase cleavage. **(B)** Represents the nonamyloidogenic α-secretase pathway in which sAPPα and C83 are generated. Subsequent hydrolysis by the γ-secretase produces a p3 peptide that does not form amyloid deposits. **(C)** Represents the amyloidogenic pathway in which cleavage of APP by the β-secretase to liberate sAPPβ and C99 is followed by γ-secretase processing to release Aβ peptides ($A\beta_{1-40}$ and $A\beta_{1-42}$) found in plaque deposits. Cleavage by γ-secretase of both C83 and C99 generates the APP intracellular domain.
APP: Amyloid precursor protein; sAPP: Soluble APP.
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cyclooxygenase inhibitory activity still retain the ability to reduce $A\beta_{1-42}$ [31,33]. NSAIDs shift the cleavage of APP to promote shorter Aβ species, in particular $A\beta_{1-38}$, but do not inhibit the cleavage of other γ-secretase substrates [31,34,35]. In particular, a subset of US FDA-approved NSAIDs (e.g., sulindac sulfide, ibuprofen and indomethacin) directly modulate γ-secretase activity to selectively decrease the secretion of $A\beta_{1-42}$ accompanied by an increase in $A\beta_{1-38}$ generation, whereas the generation of APP intracellular domains (AICDs) was not significantly affected [31,33,36]. Interestingly, some NSAID- and lipid metabolism-related compounds

(e.g., enofibrate) caused a significant increase in $A\beta_{1-42}$ levels accompanied by a decrease in $A\beta_{1-38}$ generation [37,38]. It has been also proposed that NSAID-like γ-secretase modulators interact with the substrate (APP) rather than with the enzyme (γ-secretase) [39,40]. Based on these initial observations, a new class of drugs called γ-secretase modulators with both NSAID-like and non-NSAID structure has been developed in the last 5 years [41,42].

The present review article focuses on the profile of γ-secretase inhibitors that have reached the clinic and will discuss the pharmacological and clinical issues of this new class of anti-AD compounds both in terms of safety and efficacy. Therefore, the review will not include other anti-AD drugs targeting Aβ, for example, γ-secretase modulators [18–20,41,42], active and passive immunization [13,14], α-secretase activators [20], β-secretase inhibitors [20], and Aβ aggregation inhibitors [15,16]. We review studies from the primary English literature on γ-secretase inhibitors published before March 2011. Studies were identified through the PubMed database of NCBI by author and the following keywords: drugs targeting β-amyloid; γ-secretase inhibitors; dementia syndromes and AD.

Structure of γ-secretase complex as a potential therapeutic target in AD

γ-Secretase is an intramembranous multiprotein complex and an aspartyl protease that resides and cleaves within the lipid bilayer many type-I proteins with critical roles in neuronal function. In fact, γ-secretase complex belongs to a group of proteases called intramembrane cleaving proteases (I-CLiPs) that are membrane embedded enzymes. These enzymes hydrolyze transmembrane substrates and the residues essential to catalysis reside within the boundaries of the lipid bilayer [43]. Historically, clues regarding the molecular identity of γ-secretase are first obtained in genetic studies on FAD. After the finding that mutations in the APP gene at the chromosome 21 are minor in total FAD pedigree, PS-1 and PS-2 genes were identified as major causative genes for FAD [5]. As seen above, at present, over 175 point mutations in PS-1 and PS-2 genes have been linked to FAD [26,27]. In cell-based assays and transgenic mice, these FAD-linked mutations resulted in increased production of $A\beta_{1-42}$, the highly self-aggregating and neurotoxic form of Aβ, or an increased $A\beta_{1-42}$:$A\beta_{1-40}$ ratio [44–46]. On the contrary, cells derives from PS knockout mice lost γ-secretase activity, suggesting that PS is a pivotal component of γ-secretase activity [23,24,47]. AD is believed to be caused by a progressive cerebral accumulation of Aβ, and the γ-secretase activity, which consists of both PS-dependent and PS-independent activities [48–52], determines the length of Aβ and therefore controls the $A\beta_{1-42}$:$A\beta_{1-40}$ ratio [53]. As seen above, this enzyme was shown to consist of four protein components: PS-1 or PS-2 (which contains the catalytic domain), nicastrin (which may serve to dock substrates), Aph-1, and Pen-2 in a 1:1:1:1 ratio [54] (Figure 2). PS-1 and PS-2 are nine-transmembrane domain (TMD) proteins that must undergo endoproteolysis to generate active N- and C-terminal fragments, each of which contains an active site aspartate, and that remain closely associated [55,56]. The cleavage occurs in the large cytoplasmic loop between TMD6 and TMD7 within a short hydrophobic domain that is believed to dive into the membrane [24]. This endoproteolysis is believed to be an autoproteolytic event. Nicastrin is a type I membrane glycoprotein with a large lumenal domain involved in the assembly, maturation and activation of the γ-secretase complex [57–59]. It is hypothesized that the free N-terminus of the γ-secretase substrates first binds to the ectodomain of nicastrin [59,60], which may facilitate its interaction with the docking site on PS which is followed by relocation to the active site on PS where it is cleaved. However, whether the extracellular region of nicastrin plays a role in substrate recognition remains controversial [59,60]. Pen-2 is the smallest component (≈10 kDa) of γ-secretase, it encodes a two-TMD hairpin-like protein with both ends in the lumen, and is thought to be required for the stabilization of the PS fragments in the γ-secretase complex [61,62]. Aph-1 is a seven-TMD protein (≈20 kDa) with a cytosolic C-terminus whose function in γ-secretase is currently unclear [62,63]. In humans, there are two paralog APH-1 genes (APH-1A and B) but three variants of the Aph-1 protein (Aph-1a with two splice variants, S and L respectively, and Aph-1b) [64], which differentially incorporate in different γ-secretase complexes [65,66]. Aph-1a is the major isoform present in γ-secretase complexes [67]. Given that there are two PSs and three Aph-1 proteins, at least six different complexes exist with potentially different biological functions [65–69]. Consistent with this notion, complexes containing different Aph-1 or PS proteins have been shown to display distinct but overlapping γ-secretase activities, and there is potential to target specific complexes for AD therapeutics [66,68–71]. Initially, nicastrin and Aph-1 form a subcomplex and subsequently PS is incorporated to form a heterotrimeric subcomplex [72]. The addition of Pen-2 results in a mature complex and allows the activation of the complex by endoproteolysis of PS [73,74]. It is still debated the exact stoichiometry of the complex and whether oligomerization can occur *in vivo* [75]. However, this phenomenon is unlikely to have a significant impact on the activity because it has been reported that purified γ-secretase can be fully active in a monomeric form [75]. The structure elucidation of the γ-secretase complexes

has been complicated by the lack of crystal structure of γ-secretase [22]. However, the first high resolution (12 Å) structure of γ-secretase have been recently obtained, and according to this low-resolution map obtained by electron microscopy studies [76–78], human γ-secretase has an spherical structure with three potential interior cavities [77,78] or, alternatively, one interior central pore [76]. These cavities are open to either the extracellular space or the cytosol as well as an almost continuous surface groove at the membrane region that could be a substrate entry site.

Since the initial studies that demonstrated that PS-1-dependent γ-secretase is essential for the processing of APP and the Notch receptor [23,79], more than 70 type-I integral membrane proteins have been shown to be cleaved by γ-secretase, some of them with critical cellular functions [22]. However, despite the fact that are known to be cleaved by γ-secretase, the physiological function of these proteolytic events is poorly understood [22]. γ-Secretase displays poor substrate specificity, but a functional γ-secretase cleavage has been clearly demonstrated for some substrates. Notch proteolysis by γ-secretase generates an intracellular domain (NICD) which is essential for many cell differentiation events and neurite outgrowth [79,80]. Proteolysis of N-cadherin leads to degradation of the transcriptional factor CBP, and cleavage of ErB4 inhibits astrocyte differentiation by interacting with repressors of astrocyte gene expression [81,82]. Cleavage of APP generates an AICD, although its role in signal transduction remains controversial [83]. The long list of substrates processed by γ-secretase has clear implications for the development of new therapies for AD and, in particular, for the search of γ-secretase-based drugs. The challenge in AD research has been thus far to find a γ-secretase inhibitor able to selectively lower Aβ but without interfering with the cleavage of other important substrates. In fact, interference with the cleavage of substrates with important cellular functions, such as Notch, has been shown to be associated with serious adverse effects in animal models [84,85].

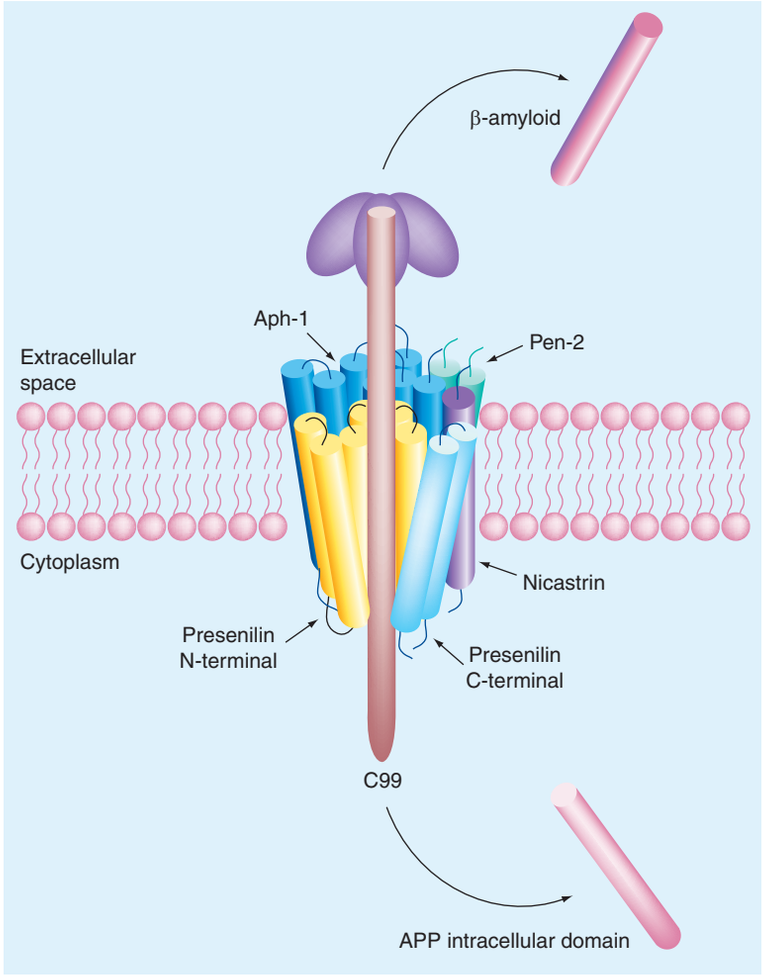

Figure 2. γ-secretase enzymatic complex and of its processing of the C99 substrate.

Aph-1: Anterior pharynx-defective-1; APP: Amyloid precursor protein; Pen-2: Presenilin enhancer-2.

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γ-secretase inhbitors in clinical development for AD treatment

Based on a substantial cellular, molecular, and biochemical body of evidence, the inhibition of the catalytic unit of the γ-secretase enzymatic complex may be therefore an attractive and valid therapeutic target for drug intervention in AD, counteracting Aβ accumulation [86]. In particular, as seen above, some properties make γ-secretase complex a highly interesting but challenging target [22]. In fact, γ-secretase is an unconventional aspartyl protease that resides and cleaves its substrates within the lipid bilayer. Furthermore, AD is believed to be caused by a progressive cerebral accumulation of Aβ, and γ-secretase cleaves APP to release Aβ. Finally, γ-secretase processes a wide range of type I membrane proteins, some of them with critical cellular functions [22]. Transition state analogue (TSA) inhibitors (e.g., L-685,458 and 31-CIII), compounds designed to interact with the active site of γ-secretase the protease, were found to bind directly to PS1 N-terminal fragment–C-terminal fragment heterodimer [87,88], which is the biologically active form. TSA inhibitors also block the cleavage of other γ-secretase substrates, including the Notch receptor [89]. However, these TSA are used only for the discovery stage because of instability and inefficacy *in vivo* [19]. Importantly, discovery and chemical biological application of these TSA led to the conclusions that PS is a catalytic subunit in the

γ-secretase complex, that is aspartyl protease. A number of structurally diverse γ-secretase inhibitors have been described in addition to the classical TSA. In fact, dipeptidic γ-secretase inhibitors, the potent and cell-permeable compounds, also inhibit Notch signaling. In 2001, Elan and Eli Lilly firstly reported the *in vivo* inhibition of brain Aβ with *N*-[*N*-(3,5-difluorophenacetyl)-L-alanyl]-*S*-phenylglycine t-butyl ester (DAPT), a peptidomimetic γ-secretase inhibitor [51,90]. Nevertheless, data on the cognitive effects of single and prolonged administration of γ-secretase inhibitors in animal models of AD are scanty, the only published study being one on DAPT [91]. In this study, single oral doses of DAPT (100 mg/kg) reversed the contextual fear-conditioning deficit of Tg2576 mice only when administered before training. Peak inhibitions of approximately 30% in soluble brain $A\beta_{1-40}$ and $A\beta_{1-42}$ levels were measured at 4 and 8 h, respectively, after dosing [91]. Several other nonpeptidic, orally available, γ-secretase inhibitors have been synthesized [86]. Historically, the first γ-secretase inhibitor publicly reported to reach the clinic is a compound synthesized at Bristol-Myers Squibb and the former SIBIA Neurosciences (BMS-299897) [92]. Human testing of BMS-299897 started in 2001 but clinical data have never been fully described. The long-lasting lack of information on its clinical development may indicate that it has been abandoned [92]. The benzodiazepine analog LY-411575 and benzolactam semagacestat (LY-450139), developed by Eli Lilly, are highly potent γ-secretase inhibitors that have been tested extensively *in vivo* to assess SP deposition in transgenic animals [93–96]. At least five other γ-secretase inhibitors (PF-3084014, GSI-953, BMS-708163, MK-0752 and ELND006) reached the clinic (Table 1 & Figure 3). For only one compound (semagacestat) clinical data have been fully published. Most of the information on the other compounds derives from congress communications.

■ Semagacestat (LY-450139)

Semagacestat (LY-450139) (Figure 3) is the most well known γ-secretase inhibitor that has reached clinical testing, and it is only 3-fold selective in inhibiting APP and Notch cleavage (APP IC_{50} = 15 nM, Notch EC50 = 49 nM) [96]. Among dipeptidic γ-secretase inhibitors, the benzoazepinone derivative semagacestat is a compound developed at Eli Lilly that has been widely tested *in vitro* and *in vivo*. In experimental animals, the effects of semagacestat on Aβ levels in brain, cerebrospinal fluid (CSF) and plasma were well characterized in transgenic mice [97], nontransgenic mice [98], guinea pigs [99] and dogs [100]. In particular, in PDAPP transgenic mice expressing the 'Indiana' mutation (Val717Phe) of human APP (APPV717F), administration of single oral doses caused a dose-dependent reduction in hippocampal Aβ (maximum effect >40% reduction at 1 mg/kg), hippocampal $A\beta_{1-42}$, cortical Aβ, and cortical $A\beta_{1-42}$. Inhibition was sustained for up to 12 h following a dose of 5 mg/kg. Efficacy was comparable with multiple doses (twice-daily for 7 days), suggesting that the target was not desensitized [97]. In a comparative study of single-dose semagacestat in PDAPP and nontransgenic mice (dosed at 3 and 10 mg/kg orally, respectively), $A\beta_{1-40}$ levels were significantly decreased (~60%) in the plasma, CSF and hippocampus of PDAPP mice. In nontransgenic mice, however, there was a transient reduction in plasma $A\beta_{1-40}$ levels followed by a significant increase (~50%) at 9 h postdose; levels returned to baseline by 12–16 h postdose. $A\beta_{1-40}$ levels were significantly reduced (40–50%) in the CSF and hippocampus at 1.5 h postdose, with a return to baseline by 12 h [98]. Nevertheless, the drug failed to show a statistically significant effect on brain plaque deposition in chronic studies in transgenic mice expressing mutated human APP_{V717F} (PDAPP mice) [101]. In fact, long-term dosing of semagacestat (3, 10 or 30 mg/kg once-daily orally for 5 months) was also assessed in PDAPP mice. $A\beta_{1-40}$ levels were significantly reduced in the cortex and hippocampus (53 and 37%, respectively) by the highest dose; $A\beta_{1-42}$ levels in the cortex were also reduced (32%). Cortical and hippocampal levels of $A\beta_{1-40}$ and $A\beta_{1-42}$ in animals treated at the lower doses did not significantly differentiate from control; interestingly, there was a numerical trend of increase in both $A\beta_{1-40}$ and $A\beta_{1-42}$ levels (30 and 25%, respectively) in the cortex of mice treated at 3 mg/kg once-daily. Total plasma Aβ was dose-dependently decreased; maximum inhibition (60%) was observed at the 30 mg/kg once-daily dose 3 h after the last dose with the effect persisting for approximately 6 h. Consistent with the mechanism of action, the concentrations of the carboxy-terminal fragment of APP in the cortex and hippocampus were dose-dependently increased by semagacestat treatment. Quantitative analysis of Aβ immunohistochemistry data did not demonstrate significant changes in total plaque burden between treatment groups. However, the median plaque burden was 43 and 48% lower in the cortex and hippocampus, respectively, of treated animals compared with controls [101]. More importantly, no data are available on the cognitive or behavioral effects of the drug in animal models of AD [102]. The lack of cognitive effects of semagacestat in animals could be linked to the fact that the drug has neurotoxic effects *in vivo*. A study employing *in vivo* two-photon imaging showed that dendritic spines get irreversibly lost in the cerebral cortex of wild-type mice after only 4 days of treatment with semagacestat (30 mg/kg subcutaneously). The same experiments

Table 1. γ-secretase inhibitors in clinical development for the treatment of Alzheimer's disease[†].

ClinicalTrials.gov Identifier	Compound (company)	Pros	Cons	Status	Ref.
NCT00594568 NCT00762411 NCT01035138	Semagacestat (LY-450139; Eli Lilly)	Decreased production of newly synthesized Aβ in the CSF of healthy humans	Dose-dependent cognitive and functional decay of AD patients, increased skin cancer in AD patients, neurotoxic (i.e., reduces dendritic spine density) in mice, lack of data on behavioral effects in animal models of AD	Discontinued	[201] [202] [203]
NCT00878189	PF-3084014 (Pfizer)	Notch sparing, good brain penetration, long-lasting effects on Aβ levels in animals, no rebound effect on plasma Aβ in animals	Lack of data on brain plaque deposition in transgenic mice and on behavioral effects in animal models of AD, unfavorable pharmacokinetic and pharmacodynamic profile in humans	Discontinued for AD	[204]
	Begacestat (GSI-953) (Wyeth)	Notch sparing, good brain penetration, improved memory in a transgenic mouse model of AD	Lack of data on brain plaque deposition in transgenic mice, no decreased Aβ levels in CSF of AD patients, rebounds on plasma $Aβ_{1–40}$	Phase II trial	
NCT00810147 NCT00890890	BMS-708163 (Bristol-Myers Squibb)	Notch sparing, decreased Aβ levels in CSF of healthy volunteers	Lack of data on brain plaque deposition in transgenic mice and on behavioral effects in animal models of AD, poor tolerability in AD patients	Phase II trial (in progress)	[205] [206]
NCT00645333 NCT00756717	MK-0752 (Merck)	Decreased $Aβ_{1-40}$ levels in CSF of healthy volunteers	Inhibits Notch cleavage, causes significant gastrointestinal toxicity in humans	Discontinued for AD	[207] [208]
	ELND-006 (Elan)	Notch sparing, good brain penetration, decreased brain Aβ burden in transgenic mice	Produces late rebounds in plasma Aβ in animals, Lack of data on behavioral effects in animal models of AD	Phase I trial	

[†]See **Figure 3** for chemical structures.
Aβ: β-amyloid; AD: Alzheimer's disease; CSF: Cerebrospinal fluid.

carried out in APP-deficient mice suggested that APP-cleavage products (probably an accumulation of C-terminal fragments), are critically involved [103].

A Phase I study evaluated the safety and tolerability and biomarker responses to single oral doses of semagacestat 60, 100, or 140 mg in 31 healthy male and female volunteers (≥40 years) [104]. No clinically significant adverse events or laboratory changes were observed in this study. A dose-proportional increase in drug exposure was observed in plasma and in CSF with an estimated CNS penetration of 8%. Peak drug plasma levels occurred at 1 h and then declined with a half-life of 2.5 h. A dose-dependent decrease in plasma $Aβ_{1–40}$ levels was also demonstrated with maximum inhibition (-73%) at 6 h after the administration of the 140-mg dose. A rebound effect on plasma $Aβ_{1–40}$ levels was observed at 8–12 h after administration and lasted for at least the 24 h. CSF concentrations of Aβ were unchanged 4 h after drug administration [104]. In a second Phase I study, semagacestat was administered to 37 healthy men and women (≥45 years) for up to 14 days at doses of 5, 20, 40 and 50 mg once-daily [105]. Two subjects in the 50 mg dose group developed possibly drug-related adverse events and discontinued treatment. The first subject had significant increases in serum amylase and lipase and complained of moderate abdominal pain. The other subject reported diarrhea that was positive for occult blood. The plasma half-life of semagacestat was found to be approximately 2.5 h and peak plasma concentrations were achieved approximately 1 h after administration. The 50-mg dose caused a maximal 40% reduction in total plasma Aβ that returned to baseline within 8 h. After returning to baseline, plasma Aβ levels increased to approximately 300% of baseline values at 15 h before slowly

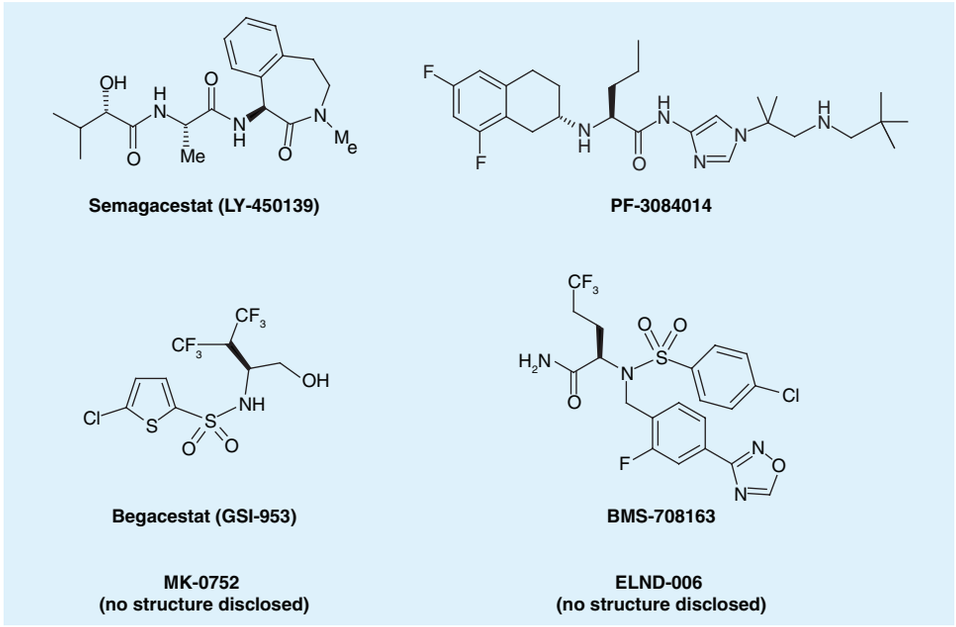

Figure 3. γ-secretase inhibitors that have reached clinical development.

declining again. At lower doses, smaller and shorter decreases in plasma Aβ were observed, although the subsequent plasma Aβ increases were similar. No significant changes in CSF Aβ levels were detected [105].

Semagacestat has been evaluated also in AD patients in Phase II studies [106,107]. In a first randomized, placebo-controlled trial, 70 patients received the drug for 6 weeks (30 mg once-daily for 1 week followed by 40 mg once-daily for 5 weeks) [106]. Six patients taking semagacestat reported diarrhea. A 76-year-old man on semagacestat had gastrointestinal bleeding associated to a Barrett esophagus, a clinical condition characterized by the conversion of normal squamous cells into abnormal specialized columnar cells. Approximately 4 months after discontinuing treatment, the patient developed endocarditis and approximately 1 month thereafter, died. In the semagacestat-treated group, circulating CD69, T lymphocytes, eosinophils, and serum concentrations of potassium and inorganic phosphorus showed statistically significant changes, although these findings were reported as 'clinically irrelevant'. Plasma $A\beta_{1-40}$ concentrations of patients taking the compound decreased significantly by 38% compared with baseline. The lowest concentration was achieved approximately 3 h after administration of a 40-mg dose and returned to baseline approximately 7 h postdose. $A\beta_{1-40}$ concentrations in CSF did not decrease significantly.

Another study evaluated the safety, tolerability, and Aβ response to semagacestat in 51 AD patients treated for 14 weeks [107]. Patients were randomized to receive placebo (n = 15) or semagacestat (n = 36). Patients on semagacestat received 60 mg once-daily for 2 weeks, then 100 mg once-daily for 6 weeks, and then either 100 or 140 mg once-daily for 6 additional weeks. Forty-three patients completed the study. There were seven cases of skin rashes and three reports of hair color change in the drug treatment groups. There were three adverse event-related discontinuations, including one transient bowel obstruction. Compared with placebo, $A\beta_{1-40}$ plasma concentrations were reduced by 58% in the 100-mg group and 65% in the 140-mg group. No significant reduction was seen in CSF Aβ levels. No differences were seen in cognitive or functional measures between placebo-and semagacestat-treated patients.

A recent study using a stable isotope-labelled aminoacid ($^{13}C_6$-leucine) in 20 healthy volunteers has shown that semagacestat (100, 140 and 280 mg) is able to acutely lower Aβ in CSF [108]. CSF was collected hourly with a lumbar catheter. A dose-dependent decrease in levels of newly-generated Aβ was observed; this was statistically significant at all doses versus placebo. The mean decrease in Aβ generation was 47, 52 and 84% over a 12-h period with doses of 100, 140 and 280 mg, respectively. There was a significant inverse relationship between semagacestat concentrations in the CSF and the amount of newly generated Aβ in CSF ($r = -0.646$; $p = 0.002$). Furthermore, the AUC of CSF Aβ (measured by ELISA) tended to decrease in this time period, with significance from placebo achieved in the 280-mg group (48.2% decrease). Interestingly, there was a rebound effect on CSF $A\beta_{1-42}$ concentrations at later times (24–36 h); compared with placebo, a twofold increase was observed at 30 h with the 280-mg dose. Clearance of Aβ was unaffected by semagacestat.

With the aim of verifying whether semagacestat was able to slow the Aβ deposition and neurodegeneration, in March 2008, Eli Lilly initiated its first Phase III trial, called the IDENTITY trial, that was a randomized, double-blind, placebo-controlled, parallel-assignment, multicenter clinical trial (NCT00594568; H6L-MC-LFAN) in patients with mild-to-moderate AD (expected n = 1,500). Patients were treated with semagacestat (100 or 140 mg orally, once-daily) for 21 months, with the option of enrolling in an open-label extension trial for further treatment. Patients taking symptomatic treatments for AD were permitted to continue treatment.

The trial incorporated a 'randomized delayed start' design, which means that patients initially assigned to the placebo arm will be administered semagacestat sometime before the end of the 21-month period to assess the effects on disease progression. The primary outcome measures of efficacy are the Alzheimer's Disease Assessment Scale-Cognition (ADAS-cog) for cognition and the Alzheimer's Disease Cooperative Study – Activities of Daily Living scale (ADCS-ADL) for functionality. Secondary end points included Aβ levels in plasma and CSF and other brain biomarkers determined by neuroimaging. Eli Lilly initially estimated the trial would be completed by March 2012 [102,201].

A second Phase III trial, called IDENTITY-2 (NCT00762411; H6L-MC-LFBC), was also started in patients with mild-to-moderate AD (expected n = 1100). Patients would be treated with semagacestat (60 mg orally once-daily, titrated to 140 mg orally once-daily) for 21 months, with the option of enrolling in an open-label extension trial for further treatment. Patients taking symptomatic treatments for AD were permitted to continue treatment. The trial also incorporated a randomized delayed start design, similar to IDENTITY. The primary outcome measures of efficacy is the ADAS-Cog scale for cognition and the ADCS-ADL scale for functionality. Secondary end points include other dementia rating scales, Aβ levels in plasma and CSF and other brain biomarkers determined by neuroimaging. The dose titration based on patient tolerability was designed to provide a more 'real-world simulation' of semagacestat. Eli Lilly initially estimated the trial would be completed by March 2012 [102,202]. Finally, in December 2009, Eli Lilly launched an open-label extension called IDENTITY XT (NCT01035138) for AD patients who completed one of the two semagacestat Phase III double-blind studies, IDENTITY or IDENTITY-2 (H6L-MC-LFAN or H6L-MC-LFBC), with an estimated enrolment of 1700 patients and an initial estimated study completion by January 2014 [203].

However, on August 17, 2010, Eli Lilly announced the end of the clinical development of semagacestat. In fact, preliminary results from the two Phase III trials showed that semagacestat worsened clinical measures of cognition and the ability to perform activities of daily living compared with placebo. In addition, data showed semagacestat was associated with an increased risk of skin cancer compared with those who received placebo. The detrimental effects of semagacestat appeared to be dose-dependent [109]. These negative findings in more than 2600 patients lead the company to interrupt the development of semagacestat, although the two Phase III studies plus the open-label extension IDENTITY XT are still ongoing in double-blind conditions to follow up the cognitive and clinical conditions of the patients [109]. It has been recently disclosed that the detrimental effects of semagacestat on cognition and on activity of daily living of the AD patients are dose-dependent and it has been hypothesized that lack of selectivity of semagacestat on the processing of other γ-secretase substrates and the accumulation of the neurotoxic precursor of Aβ (the carboxy-terminal fragment of APP, or CTFβ) may play a role [109,110]. It has been also argued that γ-secretase inhibitors should be employed in the very early stages of the disease progression (patients with mild cognitive impairment or patients with 'prodromal' AD) when neuronal loss is still limited. Thus, the inclusion of patients with mild-to-moderate AD in the semagacestat Phase III trials could also explain the negative outcome of these studies.

■ PF-3084014

PF-3084014 (Figure 3) is a novel aminotetraline derivative, potent, Notch-sparing, γ-secretase inhibitor in development at Pfizer. In a cell-free assay, PF-3084014 appears to be a potent, noncompetitive but reversible inhibitor of human γ-secretase activity with an IC_{50} of 6.2 nM [111]. In a whole-cell assay, PF-3084014 displays an IC_{50} of 1.3 nM. In fetal thymus organ culture assay, PF-3084014 appears to be a weak inhibitor of Notch signaling with an IC_{50} of 1915 nM. The APP to Notch selectivity ratio is 1473. In guinea-pigs, dose-response inhibition of total Aβ levels was observed in plasma, CSF and brain after subcutaneous administration (0.03–10 mg/kg). At the highest dose (10 mg/kg), Aβ levels were reduced by 70% in brain and plasma, and by 50% in CSF, which was maintained at 30 h postdose. No late rebound effects on plasma Aβ were observed. Drug levels in the brain were similar to that measured in plasma. Studies in young (plaque-free) Tg2576 transgenic mice showed that brain, CSF, and plasma levels of Aβ were inhibited dose-dependently following doses of 1–18 mg/kg. At the highest dose (18 mg/kg), Aβ levels were reduced by 78% in brain, 72% in CSF and 92% in plasma. $Aβ_{1–40}$ was most potently inhibited in all compartments. $Aβ_{1–42}$ showed approximately 20% less reduction than $Aβ_{1–40}$ in all compartments [111]. However, dose-dependent increases in $Aβ_{11–40}$ and $Aβ_{1–43}$ were seen at doses that potently inhibited $Aβ_{1–40}$ and $Aβ_{1–42}$. In addition, PF-3084014, like previously described γ-secretase inhibitors, preferentially reduced $Aβ_{1–40}$ relative to $Aβ_{1–42}$ [111].

In healthy volunteers enrolled in a Phase I study, single doses were associated with mild and self-limiting adverse events, no apparent clinically relevant changes in vital signs and no significant ECG

trends [112]. Doses of 1–120 mg were safe and well tolerated, and the maximum tolerated dose was not identified. Mean pharmacokinetic variables with the single 120 mg dose included an oral clearance of approximately 16 ml/min/kg, an half-life of approximately 19 h, a mean C_{max} of approximately 21 nM and an average steady-state concentration of approximately 4 nM [112]. However, an analysis of pharmacokinetic/pharmacodynamic data from a study of multiple oral dosing in humans led to the decision to end development of the compound for AD [113]. Plasma drug and Aβ concentrations collected from 18 healthy volunteers given 40 or 90 mg once-daily for 14 days were used to model the relationship between these concentrations. Pharmacokinetic/pharmacodynamic profiles for these doses were used to extrapolate the profile at higher doses. This population pharmacokinetic/pharmacodynamic analysis yielded the finding that exposure levels needed to reach the prespecified area above the effect curve target for plasma inhibition of $Aβ_{1–40}$ on day 14 relative to day 1 were two- to three-fold higher than exposure limits based on animal toxicology data. As a result, much higher doses than those previously used would be need to attain a high probability of technical success [113]. PF-3084014 has now entered clinical study as an anticancer agent. A Phase I study in patients with advanced solid tumors and leukemia, with an estimated enrolment of 60, is currently recruiting patients (NCT00878189) [204].

■ Begacestat (GSI-953)

Begacestat (GSI-953) (Figure 3) is a novel thiophene sulfonamide γ-secretase inhibitor in development at Wyeth/Pfizer that selectively inhibits cleavage of APP over Notch. This compound inhibits Aβ production with low nanomolar potency in both cellular ($Aβ_{1–40}$ EC_{50} = 14.8 nM, $Aβ_{1–42}$ EC_{50} = 12.4 nM)) and cell-free (IC_{50} = 8 nM) assays [114]. The compound determines CTFβ elevation in cellular assay with an EC_{50} = 6.6. nM. Cellular assays of Notch cleavage reveal that this compound is 17-fold selective for the inhibition of APP cleavage (Notch EC_{50} = 208.5 nM) [114]. In Tg2576 mice, a 100 mg/kg doses of begacestat markedly reduced $Aβ_{1–40}$ and $Aβ_{1–42}$ levels in both CSF (maximum ≈ 90% inhibition) and in brain (maximum ≈60% inhibition). At 30 mg/kg, brain Aβ levels were significantly reduced for 24 h with maximum effects between 4 and 6 h. The minimal efficacious doses were 1 mg/kg on brain $Aβ_{1–40}$ and 2.5 mg/kg on brain $Aβ_{1–42}$. Importantly, this compound has been reported to attenuate dose-dependently contextual memory deficit in Tg2576 transgenic mice with the effect being significant at 10 mg/kg [114]. In mice, the drug showed a very good blood–brain barrier penetration with brain-to-plasma exposure ratios ranging from 1.1 to 1.3. Interestingly, CSF drug levels were lower than those measured in plasma (5–10%) [114].

In a first-in-man study in healthy human volunteers, oral administration of single doses of begacestat (3–600 mg) produced dose-dependent transient reductions in plasma $Aβ_{1–40}$ levels with maximum inhibition of 40% with 600 mg at 2 h. Rebounds in plasma $Aβ_{1–40}$ levels were visible at later times [114]. Other studies have described the pharmacokinetic, pharmacodynamic and tolerability profile of begacestat after single oral administration in young subjects and in AD patients [115]. Begacestat was well-tolerated and no dose-limiting adverse events were observed, and it was rapidly absorbed in both young subjects and AD patients (T_{max} = 1–2 h). Drug plasma levels increased proportionally to the dose. Plasma elimination half-life was also similar in the two populations (7–8 h). Drug concentrations in CSF were tenfold lower than in plasma in both young and AD subjects [115]. For both young healthy subjects and AD patients, a biphasic pattern of plasma $Aβ_{1-40}$ concentrations was observed, with an initial reduction below baseline for approximately 4 h, followed by a second phase of increased concentrations above baseline lasting up to 48 h before returning to baseline. Maximum inhibition in plasma $Aβ_{1–40}$ concentrations was observed at 2 h with a 28% reduction in AD subjects and 33% reduction in young subjects. Notably, initial reductions in plasma concentrations were less pronounced for $Aβ_{1–42}$ than $Aβ_{1–40}$. Maximum reductions in plasma $Aβ_{1–42}$ were only 7% in AD subjects and 17% in young subjects. Subsequent increases in $Aβ_{1–42}$ above baseline were similar to those of $Aβ_{1–40}$ in magnitude and duration. No significant effects of the drug on CSF $Aβ_{1–40}$ levels were observed in either AD patients or young volunteers [115].

A translational medicine study was subsequently performed on begacestat, comparing pharmacokinetic/pharmacodynamic biomarker relationships in Tg2576 mice and humans [116]. It was found that a 10 mg/kg dose in Tg2576 mice produces different drug exposures and inhibitory effects on Aβ in plasma ($AUC_{0–3}$ = 5951 ng h/ml, 9–20% Aβ inhibition), brain ($AUC_{0–3}$ = 9338 ng h/ml, 22–33% Aβ inhibition) and CSF ($AUC_{0–3}$ = 350 ng h/ml, no Aβ inhibition). In AD patients receiving a 450 mg dose, drug exposures and Aβ inhibitory effects in plasma ($AUC_{0–3}$ = 2334 ng h/ml, 28% Aβ inhibition) and CSF ($AUC_{0–3}$ = 240 ng h/ml, no Aβ inhibition) were correlated with the Tg2576 results and suggested that the exposure in human brain would lead to $AUC_{0–3}$ = 2400 ng·h/ml, which should produce brain Aβ inhibition similar to what was

observed in plasma [116]. No clinical trials of begacestat in AD patients are presently registered in the ClinicalTrials.gov website.

■ BMS-708163

A potent, Notch-sparing, γ-secretase inhibitor in development at Bristol-Myers Squibb is the benzene sulfonamide BMS-708163 **(Figure 3)**, in which an oxadiazolylbenzyl group is attached to the sulfonamide nitrogen. *In vitro*, the drug shows a 193-fold selectivity versus Notch cleavage ($A\beta_{1-40}$ IC_{50} = 0.3 nM and Notch EC_{50} = 58 nM) [117]. Studies in rats have shown that BMS-708163 dose-dependently decreases CSF and brain $A\beta_{1–40}$ levels without causing Notch-related gastrointestinal and lymphoid toxicity [60]. In dogs receiving 2 mg/kg orally, a decrease in brain and CSF $A\beta_{1–40}$ levels were observed for at least 24 h and peak inhibition approximately 75% in CSF and 50% in the cortex [117].

Studies in healthy young subjects have indicated that BMS-708163 is well tolerated up to 400 mg after single administration and up to 150 mg once-daily after multiple doses for 28 days [118]. After oral administration, BMS-708163 appears to be quickly absorbed (T_{max} = 1–2 h), to produce systemic exposure proportional to the dose (up to 200 mg) and to be slowly eliminated (terminal half-life ≈40 h). The effects of BMS-708163 on CSF Aβ levels in humans were evaluated after both single and multiple oral doses. At 12 h after a single administration, doses of 200 and 400 mg to young subjects produced 37 and 40% reductions, respectively in CSF $A\beta_{1–40}$ levels versus baseline. The corresponding inhibitory values for CSF $A\beta_{1–42}$ levels were 32% and 34%, respectively. Exposure for BMS-708 in CSF was much lower (<1%) than in plasma [119]. After multiple administrations for 4 weeks (50–150 mg once-daily), steady-state trough CSF $A\beta_{1–40}$ levels were reduced dose-dependently compared with baseline values [120]. A 24-week dose-range (25, 50, 100 and 125 mg once-daily) finding Phase II study in 209 mild-to-moderate AD patients has been completed in June 2010 but data have not been released (NCT00810147) [205]. A 104-week Phase II study in patients with prodromal AD was started in May 2009 and is still recruiting patients (NCT00890890) [206]. Initially, the study was planned to last 52 weeks and later was amended to prolong treatment to 104 weeks. Dosing regimens were initially 50, 100 and 125 mg once-daily. Later, the two upper doses were likely discontinued based on the results of a previous 6-month dose-range finding study in mild-to-moderate AD patients in which poor tolerability and detrimental effects on cognition were observed at 100 and 125 mg once-daily doses [121]. The estimated primary completion date for the study on prodromal AD patients is December 2012 [206].

■ MK-0752

MK-0752 (no structure disclosed) is a potent γ-secretase inhibitor (IC_{50} = 50 nM) that Merck is developing and that does not distinguish between APP and Notch. Doses of 30 or 60 mg/kg orally to rhesus monkeys produced high plasma levels (C_{max} of 32 or 88 μM and an AUC of 477 or 858 μM h, respectively) but unfortunately spleen and ileum toxicity due to inhibition of Notch signaling [122]. A Phase I study evaluated the safety, tolerability, pharmacokinetics and pharmacodynamics of single oral doses (110–1000 mg) of MK-0752 in 27 healthy young men [123]. The drug was generally well tolerated. Drug plasma levels increased proportionally to the dose, peaked at 3–4 h and then declined with a half-life of approximately 20 h. MK-0752 CSF levels were similar to unbound plasma concentrations, suggesting a good penetration of the drug into the CNS. MK-0752 doses of 500 mg significantly inhibited for 12 h $A\beta_{1–40}$ concentrations in CSF with a peak inhibitory effect of 35%. After 1000 mg, CSF $A\beta_{1–40}$ inhibition was sustained over 24 h. Plasma $A\beta_{1–40}$ concentrations also showed a dose-dependent decrease but were followed by a later rebound over baseline levels.

Increasing evidence implicates the Notch pathway in normal T-cell lymphopoiesis and the pathogenesis of several human malignancies. Although MK-0752 was initially developed as a treatment for AD, in mid 2005, Phase I trials in patients with advanced breast cancer and in patients with T-cell acute lymphoblastic leukemia (T-ALL) were initiated and results were presented in June 2006 [124]. Indeed, there is increasing interest in the applicability of γ-secretase inhibitors to the treatment of cancer. MK-0752 has been shown to inhibit γ-secretase-mediated cleavage of Notch with an IC_{50} of 55 nM. Prolonged activation of the Notch signal transduction pathway occurs in more than 50% of patients with T-ALL and is important in the pathogenesis of the disease. Preclinical studies indicate that pharmacologic inhibition of γ-secretase activity suppresses T-ALL cell growth and induces apoptosis by preventing cleavage of Notch, thus preventing prolonged activation in downstream pathways. Unfortunately, studies with MK-0752 in pediatric and adult patients with T-ALL and acute myeloid leukemia reported that drug was associated with gastrointestinal toxicity and fatigue without substantive clinical activity [124]. An intermittent dosing schedule appears to reduce toxicity while demonstrating adequate target inhibition, warranting further evaluation [125]. In February 2008, the Phase I/II trial of MK-0752 in combination with docetaxel began in 30 patients with locally advanced or metastatic breast cancer (NCT00645333). The primary outcome was dose limiting toxicity. At that time, the estimated study completion date was March 2012 [207]. In May 2008,

an open-label, uncontrolled pilot study was initiated in 20 women with early stage, estrogen receptor-positive breast cancer to establish the safety and tolerability of MK-0752 in the presurgical setting in combination with tamoxifen or letrozole (NCT00756717). The study was expected to complete in late 2010 [208].

ELND-006

Recently, Elan Pharmaceuticals started the development of another novel γ-secretase inhibitor, ELND-006. The chemical structure of ELND-006 is still undisclosed. Like BMS-708163, ELND-006 is claimed to be an 'APP-selective' γ-secretase inhibitor. These compounds are thought to interact with γ-secretase outside the catalytic site, but the precise binding site and mode of action is not known. The potency and *in vivo* characterization of the agent after oral administration was recently described. In cellular and enzymatic assays, ELND-006 displayed some selectivity for APP, inhibiting APP and Notch cleavage with IC_{50} values of 0.34 and 5.3 nM, respectively, and inhibiting Aβ production and Notch signaling in cells with IC_{50} values of 1.1 and 81.6 nM, respectively [126]. It is not know if this *in vitro* selectivity is high enough to translate in *in vivo* selectivity. Brain/plasma ratios exceeded one in both rodents and nonhuman primates, indicating a very good brain penetration [126,127]. After oral administration (0.3–30 mg kg) to wild-type mice, PDAPP mice, wild-type rats or wild-type guinea pigs, ELND-006 was associated with significant reductions in CSF Aβ [126]. In cynomolgus monkeys, an oral dose regimen of 0.3 mg/kg once-daily for 13 weeks produced a decrease of brain Aβ levels of at least 25% for approximately 24 h [127]. ELND-006 concentrations in plasma needed to reduce Aβ in brain were consistent across species. Similarly to other γ-secretase inhibitors, ELND-006 administration determined late rebounds in plasma Aβ levels in both rodent and nonhuman primate [126,127]. Studies in PDAPP transgenic mice indicated that treatment with 12.5 mg/kg once-daily for 13 weeks significantly reduced hippocampal amyloid burden and brain Aβ levels but not of dystrophic neurites [128]. More prolonged treatment with the same dose significantly reduced both plaque burden and dystrophic neuritis but not brain Aβ levels measured by ELISA [128]. According to Elan, ELND-006 is currently being evaluated in Phase I studies.

Future perspective

The exact mechanisms leading to AD are largely unknown and this limits the identification of effective disease-modifying therapies [129]. In the last 15 years, most of the efforts of the pharmaceutical industry has been directed against the production and the accumulation of Aβ [9–11]. Unfortunately, up to now, these efforts have not produced effective therapies. Between the several failures observed in the last 5 years, those on γ-secretase inhibitors appear particularly disappointing both in terms of safety and efficacy [28]. γ-secretase inhibitors block proteolysis of Notch by inhibiting cleavage between Gly-1743 and Val-1744 at a site (termed site 3 or S3) that lies near the cytoplasmic side of the lipid bilayer [89]. Physiological cleavage of Notch leads to release of the NICD, a protein fragment that is translocated to the nucleus where it regulates transcription of target genes involved in cell development and in differentiation of adult self-renewing cells. The inhibitory effects of γ-secretase inhibitors on Notch activation in embryonic and fetal development may not be of concern for the treatment of AD patients. However, it is known that Notch signaling plays an important role in the ongoing differentiation processes of the immune system [130], gastrointestinal tract [131], and epidermis [132]. Treatment of mice with γ-secretase inhibitors can cause severe gastrointestinal toxicity and compromise the proper maturation of B- and T-lymphocytes [84,133]. Notch signaling is also present in the mature brain where its activation influences structural and functional plasticity including processes involved in learning and memories [134]. Mice heterozygous for a null mutation in the gene encoding Notch1 display deficits in spatial learning [135] and mice overexpressing Notch1 antisense mRNA have approximately 50% of the normal levels of Notch protein in the hippocampus and do not display long-term potentiation (LTP) in response to high-frequency stimulation [136]. Thus, the detrimental cognitive and functional effects of semagacestat observed in the interrupted Phase III clinical trials may be ascribed to its interference activity on Notch signaling. The increased rate of skin cancer observed with semagacestat in AD patients could be also linked to the drug inhibitory activity on Notch1 signaling that may have a role as tumor suppressor in certain type of nonmelanoma skin cancer [132]. Skin cancer could be also due to excessive inhibition of PS-1 function since it has been shown that PS1 depletion leads to skin tumorigenesis through a catenin-mediated mechanism [137].

Another possible reason for the faster clinical decline of semagacestat-treated AD patients could be linked to the ability of the drug to accumulate the neurotoxic C-terminal fragment of APP (CTFβ or C99) in response to the block of γ-secretase activity [101]. Indeed, an *in vivo* study indicated that semagacestat is neurotoxic in mice [103]. This study employed *in vivo* two-photon imaging and showed that dendritic spines get irreversibly lost in the cerebral cortex of wild-type mice after only 4 days of treatment with semagacestat (30 mg/kg subcutaneously). The same experiments carried out in APP-deficient mice suggested that

APP-cleavage products (probably an accumulation of C-terminal fragments), are critically involved [99]. Recent studies have shown that a number of proteins regulate γ-secretase activity, including GSAP [138], the member of the p24 cargo protein family TMP21 [139], and the orphan GPR3 [140], and p53 [141]. These proteins represent potential therapeutic target for the treatment of AD because their inhibition appear to affect Aβ production without affecting the cleavage of Notch. However, even Notch-sparing γ-secretase inhibitors (PF-3084014, BMS-708163, begacestat) could have neurotoxic effects due to their inhibitory activity on AICD release from either C-terminal fragments of APP (C83 in the non-amyloidogenic pathway and C99 in the amyloidogenic pathway). Indeed, recent studies suggest that AICD generated by C99 (via γ-secretase cleavage) regulates nuclear transcription whereas AICD generated by C83 (via α-secretase cleavage) is degraded before reaching the nucleus [142]. Thus, pharmacological inhibition of C99-derived AICD generation may have important patophysiological consequences in gene expression, apoptosis, and cytoskeletal dynamics [143]. In fact, β-secretase inhibitors, acting on the first step in the process of cleavage of membrane-bound APP due to BACE-1 that forms sAPPβ and C99 peptide, appear to be promising drugs to prevent and treat AD also through the inhibition of C99-derived AICD generation [20].

Furthermore, the detrimental effects of semagacestat in AD patients could be linked to the higher inhibitory potency displayed by the drug on $A\beta_{1-40}$ production compared $A\beta_{1-42}$ [99], a characteristic shared with other γ-secretase inhibitors. On the contrary, β-secretase inhibitors have demonstrated to reduce both $A\beta_{1-40}$ and $A\beta_{1-42}$ in the brain of transgenic AD mice Tg2576 [144]. Recent studies in double transgenic mice have shown that $A\beta_{1-40}$ inhibits amyloid deposition while $A\beta_{1-42}$ increases it [145]. In addition, increasing $A\beta_{1-40}$ levels protected transgenic mice from the premature death. The protective properties of $A\beta_{1-40}$ with respect to amyloid deposition suggest that drugs preferentially targeting $A\beta_{1-40}$ may actually worsen the disease course. Studies in guinea-pigs [99] and in normal men [108] have indicated that semagacestat may cause rebound effects on $A\beta_{1-42}$ levels in the CNS. Whatever is the mechanism of the detrimental effects of semagacestat on cognition in AD patients, it has to be pointed out that no studies have been published showing positive cognitive or behavioral effects of the drug in animal models of AD, neither after acute or chronic administration [102]. This is a problem shared by other γ-secretase inhibitors in clinical development: there are no studies showing that the chronic administration of these drugs produce positive effects on memory in animal models of AD.

Finally, we may also need to revise or to reconsider the amyloid cascade hypothesis of AD. Two key observations resulted in the original formulation of this hypothesis [6]. First, the discovery of Aβ as the most important molecular constituent of the SPs [146] drew attention to the importance of these amyloid peptides in AD. Second, mutations of the APP gene and, subsequently, of the PS genes (PS-1 and PS-2) were directly linked to cases of FAD [5]. Hence, the presence of Aβ within SPs was regarded as the residue of the effect of these pathogenic gene mutations and which, via the accumulation of toxic and insoluble Aβ peptides, led to cell death and dementia. Since the pathological phenotype of FAD is similar, apart from age of onset, to that of the more common sporadic late-onset AD [147], it was assumed that a similar mechanism, via genetic risk factors and/or environmental factors, could explain the pathogenesis of all cases of AD [148]. Moreover, a genetic support for a role of Aβ in AD involves also the apolipoprotein E (APOE) gene. The APOE ε4 allele represents an important genetic risk factor for familial and sporadic late-onset AD, as well as for autosomal-dominant forms of FAD [5]. In fact, among the ApoE isoforms, the apoE4 isoform is more effective than ApoE3 in promoting Aβ deposition and its conversion to a fibrillar form, which could trigger Aβ nucleation and plaque formation [149]. ApoE may also be involved inAβ clearance, as the binding of ApoE to Aβ actually reduces Aβ toxicity in cell cultures. These findings confirmed the pathological cross-talk between ApoE and Aβ in the amyloid cascade hypothesis [149]. Indeed, the recent negative clinical results on semagacestat pose doubts on the hypothesis that Aβ is the key pathologic factor affecting AD process. The semagacestat failure also echoes a previous observation that immunization with pre-aggregated $A\beta_{1-42}$ (AN1792) resulted in almost complete clearance of SPs from the brain of patients with AD but did not alter disease progression [150]. It has been argued that the accumulation of Aβ in the brain of AD patients is simply a downstream manifestation of the disease rather than its cause [151]. SPs may represent a defensive mechanism in response to a neuronal damage process [152]. On the other hand, the updated version of the amyloid cascade hypothesis says that SPs may not be the main contributor to neuronal death, as there are consistent evidences that soluble oligomeric forms of Aβ are strongly neurotoxic [8]. Indeed, recent evidence has implicated oligomeric Aβ and Aβ-derived diffusible ligands (ADDLs) in cognitive decline [153,154]. Electrophysiological studies have shown that addition of oligomeric Aβ/ADDLs to hippocampal slices results in an inhibition of LTP, a cellular model of learning and memory [155]. These

results were corroborated *in vivo* via demonstration of deficits in learning and memory performance following injection of oligomeric Aβ/ADDLs directly into the hippocampi of living rats [155,156].

The reason for the presence of Aβ in the normal brain and its physiological role is not fully understood but it may regulate neuroplasticity [157]. Electrophysiological studies in rodent hippocampal preparations have shown that endogenously released Aβ peptides positively regulate the release ability of synapses without altering postsynaptic function or intrinsic neuronal excitability [158]. Other *in vivo* studies have shown that intra-hippocampal injections of picomolar concentrations of $A\beta_{1-42}$ monomers and oligomers to normal mice cause a marked increase of hippocampal LTP and enhancement of reference and contextual memory [159]. More recently, it has been shown that low doses of Aβ enhance memory retention and acetylcholine production in the hippocampus of normal mice [160]. Blocking endogenous Aβ with antibodies or decreasing Aβ expression with antisense directed at APP, all resulted in impaired learning in a spatial memory test [160]. Interestingly, $A\beta_{1-42}$ facilitated induction and maintenance of LTP in hippocampal slices, whereas antibodies to Aβ inhibited hippocampal LTP [160]. All these studies indicate that in normal healthy young animals the presence of Aβ is important for normal synaptic function and for normal learning and memory. Thus, it may be plausible that indiscriminate and complete inhibition (with γ-secretase inhibitors) or removal (with anti-Aβ antibodies) of endogenous Aβ could be detrimental in AD patients rather than beneficial. Interestingly, a significant proportion (20–30%) of cognitively intact individuals shows a significant amount of pre- or postmortem amyloid [161,162].

Collectively, these observations may question the hypothesis that Aβ is the key pathologic factor affecting AD process. It has been recently proposed that a decline in brain metabolic activity or synaptic activity is the underlying cause of the disease [163]. Decreased metabolic activity, which can be consequent to decreased synaptic activity, increases β-secretase expression or activity which, in turn, increases Aβ deposition as a secondary response [163]. If this is true we should expect other failures with other γ-secretase inhibitors even if APP-selective, especially those for which no proofs of neuronal rescue and attenuation of memory deficit have been documented in preclinical models of AD. Recent studies have also pointed out that other pathways, including tau protein [164] and oxidative stress [165] may play a pivotal role in the disease process. The recent introduction of new diagnostic criteria of AD based on specific cognitive patterns and reliable biomarkers [166] may open a new paradigm of therapeutic intervention based on the distinction of two preclinical states of AD in which individuals are free of cognitive symptoms [167]. One group is formed of 'asymptomatic subjects at risk for AD' with biomarker evidence of AD pathology. The other group is formed of 'presymptomatic AD subjects' carrying genetic determinants which eventually will develop the disease [167]. New drugs should be tested in these two populations of 'asymptomatic' or 'presymptomatic' subjects rather than in AD patients. Very recently, also the National Institute on Aging and the Alzheimer's Association charged a workgroup with the task of revising the 1984 criteria for AD dementia [168], developing criteria the for the symptomatic predementia phase of AD (mild cognitive impairment [MCI] due to AD) [169], and defining the preclinical stages of AD for research purposes and toward earlier intervention at a stage of AD when some disease-modifying therapies may be most efficacious [170]. In particular, for MCI due to AD, the workgroup developed core clinical criteria that could be used by healthcare providers without access to advanced imaging techniques or CSF analysis, and research criteria that could be used in clinical research settings, including clinical trials, incorporate the use of biomarkers based on imaging and CSF measures [169]. Therefore, the stage of the mild-to-moderate AD patients, in which most clinical progression trials have been run, is relatively late in disease course, where irreversible damage to the brain may have already occurred. Newly-designed clinical trials that access patients earlier in disease are in process [171], and new diagnostic criteria recognizing preclinical or prodromal/predementia AD will enhance the ability to more fairly test the amyloid cascade hypothesis of AD in patients that may still have the capacity to respond to treatment. This approach may increase chances of success in delaying this devastating disease or slowing down the rate of deterioration of AD patients.

Financial & competing interests disclosure

BP Imbimbo is an employee at Chiesi Farmaceutici and is involved in the development of CHF5074. He is listed between the inventors of a number of Chiesi Framaceutici's patents of anti-Alzheimers disease drugs, including γ-secretase modulators. This work was supported by the Ministero della Salute, IRCCS Research Program 2009–2011, Line 2: 'Malattie complesse'. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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Executive summary

Background

- The two principal neuropathological hallmarks of Alzheimer's disease (AD) are senile plaques and neurofibrillary tangles, the former being mainly composed of amyloid-β peptide (Aβ).
- Aβ is generated from its precursor, amyloid precursor protein, by the sequential action of β- and γ-secretases.

Drugs targeting amyloid β for treating Alzheimer's disease

- Drugs that can prevent production, aggregation, and deposition of Aβ are thought to be promising therapeutics for AD.
- Passive immunotherapy approaches are under investigation in clinical trials, while brain penetrant inhibitors of Aβ aggregation have also been identified.
- Increasing attention has been focused on inhibition or modulation of activities of α-, β-, and γ-secretases as disease-modifying therapies for AD.

γ-secretase complex as a potential therapeutic target in Alzheimer's disease

- Inhibition and modulation of γ-secretase to reduce the amount of Aβ in the brain, the pivotal enzyme that generates Aβ, are plausible therapeutic options against AD.
- γ-Secretase complex is composed of four components that are required for the enzymatic activity: presenilin , anterior pharynx-defective-1, presenilin enhancer-2 and nicastrin.
- Extensive cellular, molecular, and biochemical analyses revealed that presenilin functions as a catalytic center of γ-secretase.

Clinical development of γ-secretase inhibitors for Alzheimer's disease treatment

- Several potent orally γ-secretase inhibitors have been developed and are under clinical investigation, of these semagacestat is the most publicly documented.
- Unfortunately, γ-secretase inhibitors may cause significant toxicity in man, mainly ascribed to the inhibition of Notch processing and to the accumulation of the neurotoxic precursor of Aβ.
- Two large Phase III clinical trials of semagacestat in mild-to-moderate AD patients were prematurely interrupted because of detrimental cognitive and functional effects of the drug.
- New Notch-sparing γ-secretase inhibitors are being developed with the hope of overcoming the previous setbacks.
- A refined test of the 'amyloid cascade hypothesis' in AD progression trials should include patients earlier in disease course, employing recently amended diagnostic criteria for AD.

Bibliography

Papers of special note have been highlighted as:

- ▪ of interest
- ▪▪ of considerable interest

1 Alzheimer's Association. Alzheimer's disease facts and figures. *Alzheimers Dement.* 6(2), 158–194 (2010).

2 Brookmeyer R, Johnson E, Ziegler-Graham K, Arrighi HM. Forecasting the global burden of Alzheimer's disease. *Alzheimers Dement.* 3, 186–191 (2007).

3 Cummings JL. Alzheimer's disease. *N. Engl. J. Med.* 351(1), 56–67 (2004).

4 Alladi S, Xuereb J, Bak T *et al.* Focal cortical presentations of Alzheimer's disease. *Brain* 130(pt 10), 2636–2345 (2007).

5 Seripa D, Panza F, Franceschi M *et al.* Non-apolipoprotein E and apolipoprotein E genetics of sporadic Alzheimer's disease. *Ageing Res. Rev.* 8(3), 214–236 (2009).

6 Hardy J, Allsop D. Amyloid deposition as the central event in the aetiology of Alzheimer's disease. *Trends Pharmacol. Sci.* 12(10), 383–388 (1991).

7 Lesne S, Koh MT, Kotilinek L *et al.* A specific amyloid-β protein assembly in the brain impairs memory. *Nature* 440(7082), 352–357 (2006).

8 Shankar GM, Li S, Mehta TH *et al.* Aβ protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. *Nat. Med.* 14(8), 837–842 (2008).

9 Panza F, Solfrizzi V, Frisardi V *et al.* Beyond the neurotransmitter-focused approach in treating Alzheimer's disease: drugs targeting β-amyloid and tau protein. *Aging Clin. Exp. Res.* 21(6), 386–406 (2009).

10 Frisardi V, Solfrizzi V, Imbimbo PB *et al.* Towards disease-modifying treatment of Alzheimer's disease: drugs targeting β-amyloid. *Curr. Alzheimer Res.* 7(1), 40–55 (2010).

11 Mangialasche F, Solomon A, Winblad B, Mecocci P, Kivipelto M. Alzheimer's disease: clinical trials and drug development. *Lancet Neurol.* 9(7), 702–716 (2010).

12 Barten DM, Albright CF. Therapeutic strategies for Alzheimer's disease. *Mol. Neurobiol.* 37(2–3), 171–186 (2008).

13 Lemere CA, Masliah E. Can Alzheimer disease be prevented by amyloid-β immunotherapy? *Nat. Rev. Neurol.* 6(2), 108–119 (2010).

▪ **Influential review article that provided an update on the active and passive β-amyloid (Aβ) immunotherapies currently in clinical trials.**

14 Panza F, Frisardi V, Imbimbo BP *et al.* Bapineuzumab: anti-β-amyloid monoclonal antibodies for the treatment of Alzheimer's disease. *Immunotherapy* 2(6), 767–782 (2010).

15 Lannfelt L, Blennow K, Zetterberg H *et al.* PBT2–201-EURO study group. Safety, efficacy, and biomarker findings of PBT2 in targeting Aβ as a modifying therapy for Alzheimer's disease: a phase IIa, double-blind, randomised, placebo-controlled trial. *Lancet Neurol.* 7(9), 779–786 (2008).

16 Faux NG, Ritchie CW, Gunn A *et al.* PBT2 rapidly improves cognition in Alzheimer's disease: additional Phase II analyses. *J. Alzheimers Dis.* 20(2), 509–516 (2010).

▪ ***Post hoc* analysis of the Phase II trial examining PBT2, confirming the positive cognitive effects of this zinc/copper ionophore in Alzheimer's disease patients.**

17 Ghosh AK, Gemma S, Tang J. β-secretase as a therapeutic target for Alzheimer's disease. *Neurotherapeutics* 5(3), 399–408 (2008).

18 Imbimbo BP. Therapeutic potential of γ-secretase inhibitors and modulators. *Curr. Top Med. Chem.* 8(1), 54–61 (2008).

19 Tomita T. Secretase inhibitors and modulators for Alzheimer's disease treatment. *Expert Rev. Neurother.* 9(5), 661–679 (2009).

20 Panza F, Solfrizzi V, Frisardi, V *et al.* Disease-modifying approach to the treatment of Alzheimer's disease: from α-secretase activators to γ-secretase inhibitors and modulators. *Drugs Aging* 26(7), 537–555 (2009).

21 McCarthy JV, Twomey C, Wujek P. Presenilin-dependent regulated intramembrane proteolysis and γ-secretase activity. *Cell Mol. Life Sci.* 66(9), 1534–1555 (2009).

22 Guardia-Laguarta C, Pera M, Lleó A. γ-secretase as a therapeutic target in Alzheimer's disease. *Curr. Drug Targets* 11(4), 506–517 (2010).

23 De Strooper B, Saftig P, Craessaerts K *et al.* Deficiency of presenilin-1 inhibits the normal cleavage of amyloid precursor protein. *Nature* 391(6665), 387–390 (1998).

24 Wolfe MS, Xia W, Ostaszewski BL, Diehl TS, Kimberly WT, Selkoe DJ. Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and γ-secretase activity. *Nature* 398(6727), 513–517 (1999).

▪ **This study showed that mutation of either of two conserved transmembrane aspartates (Asp-257 or Asp-385) in preselinin 1 abolishes γ-secretase activity, documenting the fact that the presenilins are the 'catalytic unit' of the γ-secretase complex.**

25 Yu G, Nishimura M, Arawaka S *et al.* Nicastrin modulates presenilin-mediated Notch/GLP-1 signal transduction and βAPP processing. *Nature* 407(6800), 48–54 (2000).

26 Bentahir M, Nyabi O, Verhamme J *et al.* Presenilin clinical mutations can affect γ-secretase activity by different mechanisms. *J. Neurochem.* 96(3), 732–742 (2006).

27 Lleo A, Berezovska O, Growdon JH, Hyman BT. Clinical, pathological, and biochemical spectrum of Alzheimer disease associated with PS-1 mutations. *Am. J. Geriatr. Psychiatry*, 12(2), 146–156 (2004).

28 Panza F, Frisardi V, Imbimbo BP *et al.* Review: γ-secretase inhibitors for the treatment of Alzheimer's disease: the current state. *CNS Neurosci. Ther.* 16(5), 272–284 (2001).

29 Lleo A. Activity of γ-secretase on substrates other than APP. *Curr. Top Med. Chem.* 8(1), 9–16 (2008).

30 Petit A, Bihel F, Alvès da Costa C, Pourquié O, Checler F, Kraus JL. New protease inhibitors prevent γ-secretase-mediated production of $A\beta_{40/42}$ without affecting Notch cleavage. *Nat. Cell Biol.* 3(5), 507–511 (2001).

▪ **Described the first nonpeptidic potential inhibitors of γ-secretase that inhibited Aβ production without inhibiting Notch cleavage (JLK inhibitors).**

31 Weggen S, Eriksen JL, Das P *et al.* A subset of NSAIDs lower amyloidogenic Aβ42 independently of cyclooxygenase activity. *Nature* 414(6860), 212–216 (2001).

▪▪ **Seminal paper suggesting that nonsteroidal anti-inflammatory drugs directly affect Aβ pathology in the brain by reducing $A\beta_{1-42}$ peptide levels independently of cyclooxygenase activity and that this $A\beta_{1-42}$-lowering activity could be optimized to selectively target the pathogenic $A\beta_{1-42}$ species.**

32 Sagi SA, Weggen S, Eriksen J, Golde TE, Koo EH. The noncyclooxygenase targets of non-steroidal anti-inflammatory drugs, lipoxygenases, peroxisome proliferator-activated receptor, inhibitor of kB kinase, and NFkB, do not reduce amyloid-β 42 production. *J. Biol. Chem.* 278(34), 31825–31830 (2003).

33 Eriksen JL, Sagi SA, Smith TE *et al.* NSAIDs and enantiomers of flurbiprofen target γ-secretase and lower Aβ42 *in vivo*. *J. Clin. Invest.* 112(3), 440–449 (2003).

34 Peretto I, Radaelli S, Parini C *et al.* Synthesis and biological activity of flurbiprofen analogues as selective inhibitors of β-amyloid$_{1-42}$ secretion. *J. Med. Chem.* 48(18), 5705–5720 (2005).

35 Lleó A, Berezovska O, Herl L *et al.* Nonsteroidal anti-inflammatory drugs lower $A\beta_{42}$ and change presenilin 1 conformation. *Nat. Med.* 10(10), 1065–1066 (2004).

36 Takahashi Y, Hayashi I, Tominari Y *et al.* Sulindac sulfide is a noncompetitive γ-secretase inhibitor that preferentially reduces $A\beta_{42}$ generation. *J. Biol. Chem.* 278(20), 18664–18670 (2003).

37 Kukar T, Murphy MP, Eriksen JL *et al.* Diverse compounds mimic Alzheimer disease-causing mutations by augmenting $A\beta_{42}$ production. *Nat. Med.* 11(5), 545–350 (2005).

38 Czech C, Burns MP, Vardanian L *et al.* Cholesterol independent effect of LXR agonist TO-901317 on γ-secretase. *J. Neurochem.* 101(4), 929–936 (2007).

39 Kukar TL, Ladd TB, Bann MA *et al.* Substrate-targeting γ-secretase modulators. *Nature* 453(7197), 925–929 (2008).

40 Richter L, Munter LM, Ness J *et al.* Amyloid β 42 peptide ($A\beta_{42}$)-lowering compounds directly bind to Aβ and interfere with amyloid precursor protein (APP) transmembrane dimerization. *Proc. Natl Acad. Sci. USA* 107(33), 14597–14602 (2010).

41 Wolfe MS. γ-secretase modulators. *Curr. Alzheimer Res.* 4(5), 571–573 (2007).

42 Peretto I, La Porta E. γ-secretase modulation and its promise for Alzheimer's disease: a medicinal chemistry perspective. *Curr. Top Med. Chem.* 8(1), 38–46 (2008).

43 Wolfe MS, Kopan R. Intramembrane proteolysis: theme and variations. *Science* 305(5687), 1119–1123 (2008).

44 Borchelt DR, Thinakaran G, Eckman CB *et al.* Familial Alzheimer's disease-linked presenilin 1 variants elevate $A\beta_{1-42/1-40}$ ratio *in vitro* and *in vivo*. *Neuron* 17(5), 1005–1013 (2008).

45 Citron M, Westaway D, Xia W *et al.* Mutant presenilins of Alzheimer's disease increase production of 42-residue amyloid β-protein in both transfected cells and transgenic mice. *Nat. Med.* 3(1), 67–72 (1997).

46 Tomita T, Maruyama K, Saido TC *et al.* The presenilin 2 mutation (N141I) linked to familial Alzheimer disease (Volga German families) increases the secretion of amyloid β protein ending at the 42nd (or 43rd) residue. *Proc. Natl Acad. Sci. USA* 94(5), 2025–2030 (1997).

47 Zhang Z, Nadeau P, Song W *et al.* Presenilins are required for γ-secretase cleavage of β-APP and transmembrane cleavage of Notch-1. *Nat. Cell. Biol.* 2(7), 463–465 (2000).

48 Armogida M, Petit A, Vincent B, Scarzello S, da Costa CA, Checler F. Endogenous β-amyloid production in presenilin deficient embryonic mouse fibroblasts. *Nature Cell Biol.* 3(11), 1030–1033 (2001).

49 Wilson CA, Doms RW, Zheng H, Lee VM. Presenilins are not required for Aβ42 production in the early secretory pathway. *Nature Neurosci.* 5(9), 849–855 (2002).

50 Lai MT, Crouthamel MC, DiMuzio J *et al.* A presenilin-independent aspartyl protease prefers the γ-42 site cleavage. *J. Neurochem.* 96(1), 118–125 (2006).

51 Yagishita S, Futai E, Ishiura S. *In vitro* reconstitution of γ-secretase activity using yeast microsomes. *Biochem. Biophys. Res. Commun.* 377(1), 141–145 (2008).

52 Sevalle J, Ayral E, Hernandez JF, Martinez J, Checler F. Pharmacological evidences for DFK167-sensitive presenilin-independent γ-secretase-like activity. *J. Neurochem.* 110(1), 275–283 (2009).

53 Dovey HF, John V, Anderson JP *et al.* Functional γ-secretase inhibitors reduce β-amyloid peptide levels in brain. *J. Neurochem.* 76(1), 173–181 (2001).

54 Kreft AF, Martone R, Porte A. Recent advances in the identification of γ-secretase inhibitors to clinically test the Aβ oligomer hypothesis of Alzheimer's disease. *J. Med. Chem.* 52(20), 6169–6188 (2009).

55 Capell A, Grünberg J, Pesold B *et al.* The proteolytic fragments of the Alzheimer's disease-associated presenilin-1 form heterodimers and occur as a 100–150-kDa molecular mass complex. *J. Biol. Chem.* 273(6), 3205–3211 (1998).

56 Yu G, Chen F, Levesque G *et al.* The presenilin 1 protein is a component of a high molecular weight intracellular complex that contains β-catenin. *J. Biol. Chem.* 273(26), 16470–16475 (1998).

57 Yu G, Nishimura M, Arawaka S *et al.* Nicastrin modulates presenilin-mediated Notch/GLP-1 signal transduction and βAPP processing. *Nature* 407(6800), 48–54 (2000).

58 Edbauer D, Winkler E, Haass C, Steiner H. Presenilin and nicastrin regulate each other and determine amyloid β-peptide production via complex formation. *Proc. Natl Acad. Sci. USA* 99(13), 8666–8671 (2002).

59 Chavez-Gutierrez L, Tolia A, Maes E, Li T, Wong PC, de Strooper B. Glu (332) in the Nicastrin ectodomain is essential for γ-secretase complex maturation but not for its activity. *J. Biol. Chem.* 283(29), 20096–20105 (2008).

60 Shah S, Lee SF, Tabuchi K *et al.* Nicastrin functions as a γ-secretase-substrate receptor. *Cell* 122(3), 435–447 (2005).

61 Crystal AS, Morais VA, Pierson TC *et al.* Membrane topology of γ-secretase component PEN-2. *J. Biol. Chem.* 278(22), 20117–20123 (2003).

62 Francis R, McGrath G, Zhang J *et al.* Aph-1 and PEN-2 are required for Notch pathway signaling, γ-secretase cleavage of βAPP, and presenilin protein accumulation. *Dev. Cell* 3(1), 85–97 (2002).

63 Niimura M, Isoo N, Takasugi N *et al.* Aph-1 contributes to the stabilization and trafficking of the γ-secretase complex through mechanisms involving intermolecular and intramolecular interactions. *J. Biol. Chem.* 280(13), 12967–12975 (2005).

64 Serneels L, Van Biervliet J, Craessaerts K *et al.* γ-secretase heterogeneity in the Aph1 subunit: relevance for Alzheimer's disease. *Science* 324(5927), 639–642 (2009).

65 Shirotani K, Edbauer D, Prokop S, Haass C, Steiner H. Identification of distinct γ-secretase complexes with different APH-1 variants. *J. Biol. Chem.* 279(40), 41340–41345 (2004).

66 Hébert SS, Serneels L, Dejaegere T *et al.* Coordinated and widespread expression of γ-secretase *in vivo*: evidence for size and molecular heterogeneity. *Neurobiol. Dis.* 17(2), 260–272 (2004).

67 Ma G, Li T, Price DL, Wong PC. APH-1a is the principal mammalian APH-1 isoform present in γ-secretase complexes during embryonic development. *J. Neurosci.* 25(1), 192–198 (2005).

68 Serneels L, Dejaegere T, Craessaerts K *et al.* Differential contribution of the three Aph1 genes to γ-secretase activity *in vivo*. *Proc. Natl Acad. Sci. USA* 102(5), 1719–1724 (2005).

69 Lai MT, Chen E, Crouthamel MC *et al.* Presenilin-1 and presenilin-2 exhibit distinct yet overlapping γ-secretase activities. *J. Biol. Chem.* 278(25), 22475–22481 (2003).

70 Dejaegere T, Serneels L, Schafer MK *et al.* Deficiency of Aph1B/C-γ-secretase disturbs Nrg1 cleavage and sensorimotor gating that can be reversed with antipsychotic treatment. *Proc. Natl Acad. Sci. USA* 105(28), 9775–9780 (2008).

71 Shirotani K, Tomioka M, Kremmer E, Haass C, Steiner H. Pathological activity of familial Alzheimer's disease-associated mutant presenilin can be executed by six different γ-secretase complexes. *Neurobiol. Dis.* 27(1), 102–107 (2007).

72 Gu Y, Chen F, Sanjo N *et al.* APH-1 interacts with mature and immature forms of presenilins and nicastrin and may play a role in maturation of presenilin.nicastrin complexes. *J. Biol. Chem.* 278(9), 7374–7380 (2003).

73 Prokop S, Shirotani K, Edbauer D, Haass C, Steiner H. Requirement of PEN-2 for stabilization of the presenilin N-/C-terminal fragment heterodimer within the γ-secretase complex. *J. Biol. Chem.* 279(22), 23255–23261 (2004).

74 Kim SH, Sisodia SS. A sequence within the first transmembrane domain of PEN-2 is critical for PEN-2-mediated endoproteolysis of presenilin 1. *J. Biol. Chem.* 280(51), 1992–2001 (2005).

75 Sato T, Diehl TS, Narayanan S *et al.* Active γ-secretase complexes contain only one of each component. *J. Biol. Chem.* 282(47), 33985–33993 (2007).

76 Lazarov VK, Fraering PC, Ye W, Wolfe MS, Selkoe DJ, Li H. Electron microscopic structure of purified, active γ-secretase reveals an aqueous intramembrane chamber and two pores. *Proc. Natl Acad. Sci. USA* 103(18), 6889–6894 (2006).

77 Ogura T, Mio K, Hayashi I *et al.* Three-dimensional structure of the γ-secretase complex. *Biochem. Biophys. Res. Commun.* 343(2), 525–534 (2006).

78 Osenkowski P, Li H, Ye W *et al.* Cryoelectron microscopy structure of purified γ-secretase at 12 Å resolution. *J. Mol. Biol.* 385(2), 642–652 (2009).

79 De Strooper B, Annaert W, Cupers P *et al.* A presenilin-1-dependent γ-secretase-like protease mediates release of Notch intracellular domain. *Nature* 398(6727), 518–522 (1999).

80 Sestan N, Artavanis-Tsakonas S, Rakic P. Contact-dependent inhibition of cortical neurite growth mediated by Notch signaling. *Science* 286(5440), 741–746 (1999).

81 Marambaud P, Wen PH, Dutt A *et al.* A CBP binding transcriptional repressor produced by the PS1/ε-cleavage of N-cadherin is inhibited by PS1 FAD mutations. *Cell* 114(5), 635–645 (2003).

82 Ni CY, Murphy MP, Golde TE, Carpenter G. γ-secretase cleavage and nuclear localization of ErbB-4 receptor tyrosine kinase. *Science* 294(5549), 2179–2181 (2001).

83 Müller T, Meyer HE, Egensperger R, Marcus K. The amyloid precursor protein intracellular domain (AICD) as modulator of gene expression, apoptosis, and cytoskeletal dynamics-relevance for Alzheimer's disease. *Prog. Neurobiol.* 85(4), 393–406 (2008).

84 Wong GT, Manfra D, Poulet FM *et al.* Chronic treatment with the γ-secretase inhibitor LY-411,575 inhibits β-amyloid peptide production and alters lymphopoiesis and intestinal cell differentiation. *J. Biol. Chem.* 279(13), 2876–2882 (2004).

85 Milano J, McKay J, Dagenais C *et al.* Modulation of Notch processing by γ-secretase inhibitors causes intestinal goblet cell metaplasia and induction of genes known to specify gut secretory lineage differentiation. *Toxicol. Sci.* 82(1), 341–358 (2004).

86 Olson RE, Albright CF. Recent progress in the medicinal chemistry of γ-secretase inhibitors. *Curr. Top Med. Chem.* 8(1), 17–33 (2008).

87 Li YM, Xu M, Lai MT *et al.* Photoactivated γ-secretase inhibitors directed to the active site covalently label presenilin 1. *Nature* 405(6787), 689–694 (2000).

88 Esler WP, Kimberly WT, Ostaszewski BL *et al.* Transition-state analogue inhibitors of γ-secretase bind directly to presenilin-1. *Nat. Cell Biol.* 2(7), 428–434 (2000).

89 Lewis HD, Pérez Revuelta BI, Nadin A *et al.* Catalytic site directed γ-secretase complex inhibitors do not discriminate pharmacologically between Notch S3 and β-APP cleavages. *Biochemistry* 42(24), 7580–7586 (2003).

90 Imbimbo BP, Speroni F. β-amyloid therapeutic strategies for Alzheimer's disease. In: *Burger's Medicinal Chemistry and Drug Discovery (6th Edition).* Abraham DJ (Ed.). John Wiley & Sons Inc, Hoboken, NJ, USA, 1–115 (2005).

91 Comery TA, Martone RL, Aschmies S *et al.* Acute γ-secretase inhibition improves contextual fear conditioning in the Tg2576 mouse model of Alzheimer's disease. *J. Neurosci.* 25(39), 8898–8902 (2005).

▪ **This is the only published on the cognitive effects of single and prolonged administration of γ-secretase inhibitors in animal models of Alzheimer's disease, with single oral doses of *N-S*-phenylglycine t-butyl ester (DAPT) (100 mg/kg) reversed the contextual fear-conditioning deficit of Tg2576 mice only when administered before training.**

92 Imbimbo BP. Alzheimer's disease: γ-secretase inhibitors. *Drug Disc. Today-Ther. Strategies* 5(3), 169–175 (2008).

93 Best JD, Jay MT, Otu F *et al.* Quantitative measurement of changes in amyloid-β(40) in the rat brain and cerebrospinal fluid following treatment with the γ-secretase inhibitor LY-411575 [*N*2-[(2*S*)-2-(3,5-difluorophenyl)-2-hydroxyethanoyl]-*N*1-[(7*S*)-5-methyl-6-oxo-6,7-dihydro-5*H*-dibenzo[b,d]azepin-7-yl]-L-alaninamide]. *J. Pharmacol. Exp. Ther.* 313(2), 902–908 (2005).

94 Abramowski D, Wiederhold KH, Furrer U *et al.* Dynamics of Aβ turnover and deposition in different β-amyloid precursor protein transgenic mouse models following γ-secretase inhibition. *J. Pharmacol. Exp. Ther.* 327(2), 411–424 (2008).

95 Garcia-Alloza M, Subramanian M, Thyssen D *et al.* Existing plaques and neuritic abnormalities in APP:PS1 mice are not affected by administration of the γ-secretase inhibitor LY-411575. *Mol. Neurodegener.* 4, 19 (2009).

96 Gitter BD, Czilli DL, Dieckman DK, Bender MH, Nissen JS. Stereoselective inhibition of amyloid β peptide secretion by LY450139, a novel functional γ secretase inhibitor. *Neurobiol. Aging* 25(Suppl. 2), Abstr. P4–339 (2004).

97 Boggs LN, Fuson SF, Gitter BD *et al. In vivo* characterization of LY450139, a novel, stereoselective, functional γ-secretase inhibitor. *Neurobiol. Aging* 25(Suppl. 2), Abstr. P1–419 (2004).

98 May PC, Yang Z, Li W, Hyslop PA, Siemers E, Boggs LN. Multi-compartmental pharmacodynamic assessment of the functional γ-secretase inhibitor LY450139 in PDAPP transgenic mice and non-transgenic mice. *Neuobiol. Aging* 25(Suppl. 25), Abstr. O3–06–07 (2004).

99 Lanz TA, Karmilowicz MJ, Wood KM *et al.* Concentration-dependent modulation of Aβ *in vivo* and *in vitro* using the γ-secretase inhibitor, LY-450139. *J. Pharmacol. Exp. Ther.* 319(2), 924–933 (2006).

100 Cocke PJ, Boggs LN, Ness DK, May PC. Comparison of cisternal and lumbar cerebrospinal fluid amyloid β concentrations following oral administration of LY450139 dihydrate to Beagle dogs. *Alzheimers Dement.* 4(Suppl. 2), Abstr. P1–017 (2008).

101 Ness DK, Boggs LN, Hepburn DL *et al.* Reduced β-amyloid burden, increased C-99 concentrations and evaluation of neuropathology in the brains of PDAPP mice given LY450139 dihydrate daily by gavage for 5 months. *Neurobiol. Aging* 25(Suppl. 2), Abstr. P2–053 (2004).

102 Imbimbo BP, Peretto I. Semagacestat, a γ-secretase inhibitor for the potential treatment of Alzheimer's disease. *Curr. Opin. Investig. Drugs* 10(7), 721–730 (2009).

103 Bittner T, Fuhrmann M, Burgold S *et al.* γ-secretase inhibition reduces spine density *in vivo* via an amyloid precursor protein-dependent pathway. *J. Neurosci.* 29(33), 10405–10409 (2009).

104 Siemers ER, Dean RA, Friedrich S *et al.* Safety, tolerability, and effects on plasma and cerebrospinal fluid amyloid-β after inhibition of γ-secretase. *Clin. Neuropharmacol.* 30(6), 317–325 (2007).

▪ **First Phase I, third-party unblinded, single ascending dose trial that examined 31 healthy male and female volunteers (≥40 years) receiving single oral doses of semagacestat (LY-450139) 60, 100 or 140 mg.**

105 Siemers E, Skinner M, Dean RA *et al.* Safety, tolerability, and changes in amyloid β concentrations after administration of a γ-secretase inhibitor in volunteers. *Clin. Neuropharmacol.* 28(3), 126–132 (2005).

▪ **Second Phase I study, in which semagacestat (LY-450139) was administered to healthy men and women (≥45 years) for up to 14 days at doses of 5, 20, 40 and 50 mg once-daily.**

106 Siemers ER, Quinn JF, Kaye J *et al.* Effects of a γ-secretase inhibitor in a randomized study of patients with Alzheimer disease. *Neurology* 66(4), 602–604 (2006).

▪▪ **First randomized, placebo-controlled Phase II trial, with 70 Alzheimer's' disease patients receiving semagacestat (LY-450139) for 6 weeks (30 mg once-daily for 1 week followed by 40 mg once-daily for 5 weeks).**

107 Fleisher AS, Raman R, Siemers ER *et al.* Phase 2 safety trial targeting amyloid β production with a γ-secretase inhibitor in Alzheimer disease. *Arch. Neurol.* 65(8), 1031–1038 (2008).

▪▪ **Main Phase II trial of semagacestat (LY-450139) on 51 Alzheimer's disease patients treated for 14 weeks.**

108 Bateman RJ, Siemers ER, Mawuenyega KG *et al.* A γ-secretase inhibitor decreases amyloid-β production in the central nervous system. *Ann. Neurol.* 66(1), 48–54 (2009).

▪ **Innovative study evaluating the effects of semagacestat (LY-450139; 100, 140 and 280 mg) on β-amyloid synthesis and clearance in cerebrospinal fluid of 20 healthy volunteers with a relatively new technique, based on the intravenous administration of a stable isotope-labeled aminoacid (13C6-leucine).**

109 Siemers ER. Update on the semagacestat program. Presented at: *The 3rd Conference – Clinical Trials on Alzheimer's Disease.* Toulouse, France, 3–5 November 3–5 2010.

110 Ross JS, Imbimbo BP. Are γ-secretase inhibitors detrimental for Alzheimer's disease patients? *J. Alzheimers Dis.* 22(2), 401–404 (2010).

111 Lanz TA, Wood KM, Richter KE *et al.* Pharmacodynamics and pharmacokinetics of the γ-secretase inhibitor PF-3084014. *J. Pharmacol. Exp. Ther.* 334(1), 269–277 (2010).

112 Brodney, M. Discovery of PF-3084014: A novel γ-secretase inhibitor for the treatment of Alzheimer's disease. Presented at: *The 238th ACS National Meeting.* Washington, DC, USA, 16–20 August 2009.

113 Qui R, Willavize S, Fullerion T, Gastonguay MR. Modeling and simulation of plasma Aβ in humans after multiple oral doses of Pf-3084014, a potent γ-secretase inhibitor. *Alzheimers Dement.* 5(Suppl. 1), Abstr. P1–261 (2009).

114 Martone RL, Zhou H, Atchison K *et al.* Begacestat (GSI-953): a novel, selective thiophene sulfonamide inhibitor of amyloid precursor protein γ-secretase for the treatment of Alzheimer's disease. *J. Pharmacol. Exp. Ther.* 331(2), 598–608 (2009).

115 Frick G, Raje S, Wan H *et al.* GSI-953, a potent and selective γ-secretase inhibitor – modulation of β amyloid peptides and plasma and cerebrospinal fluid pharmacokinetic/pharmacodynamic relationships in humans. *Alzheimers Dement.* 4(Suppl. 2), Abstr. P4–366 (2008).

116 Wan H, Bard J, Martone R *et al.* GSI-953, a potent and selective γ-secretase inhibitor, modulates Aβ peptides in mice and humans: translating the PK/PD biomarker relationships in different biological compartments between rodent and human. *Alzheimers Dement.* 4(Suppl. 2), Abstr. P2–101 (2008).

117 Gillman KW, Starrett JE, Parker MF *et al.* Discovery and evaluation of BMS-708163, a potent, selective and orally bioavailable γ-secretase inhibitor. *ACS Med. Chem. Lett.* 1(3), 120–124 (2010).

118 Gillman KW, Starrett JE, Parker MF *et al.* BMS-708163, a potent and selective γ-secretase inhibitor, decreases CSF Aβ at safe and tolerable doses in animals and humans. Presented at: *The 11th International Conference on Alzheimer's Disease (ICAD).* Chicago, IL, USA, 26–31 July 2008.

119 Tong G, Castaneda L, Wang JS *et al.* A study to evaluate the effects of single oral doses of BMS-708163 in the cerebrospinal fluid of healthy young men. *Alzheimers Dement.* 6(Suppl. 1), Abstr. O3–07–07 (2010).

120 Wang JS, Castaneda L, Sverdlov A *et al.* A placebo-controlled, ascending, multiple-dose study to evaluate the safety, pharmacokinetics (P) and pharmacodynamics (PD) of BMS-708163 in healthy young and elderly subjects. *Alzheimers Dement.* 6(Suppl. 1), Abstr. P3–303 (2010).

121 Kambhampaty A. Alzheimer's disease snapshot. *BioPharm Insight*. Ha K (Ed.). 28 February (2011).

122 Morimoto B. Drug development for neurodegenerative diseases – second annual marcus evans conference. Advances in drug development for NDD and expediting discovery through novel compounds and sound clinical trials. *IDrugs* 13(7), 440–443 (2010).

123 Rosen LB, Stone JA, Plump A *et al.* The γ-secretase inhibitor MK-0752 acutely and significantly reduces CSF Aβ40 concentrations in humans. *Alzheimers Dement.* 2(Suppl. 1), Abstr. O4–03–02 (2006).

124 Deangelo DJ, Stone RM, Silverman LB *et al.* A phase I clinical trial of the Notch inhibitor MK-0752 in patients with T-cell acute lymphoblastic leukemia/lymphoma (T-ALL) and other leukemias. *J. Clin. Oncol.* 24(Suppl. 18), 6585 (2006).

125 Krop IE, Kosh M, Fearen I *et al.* Phase I pharmacokinetic (PK), and pharmacodynamic (PD) trial of the novel oral Notch inhibitor MK-0752 in patients (pts) with advanced breast cancer (BC) and other solid tumors. *J. Clin. Oncol.* 24(Suppl. 18), 10574 (2006).

126 Brigham E, Quinn K, Kwong G *et al.* Pharmacokinetic and pharmacodynamic investigation of ELND006, a novel APP-selective γ-secretase inhibitor, on amyloid-β concentrations in the brain, CSF and plasma of multiple nonclinical species following oral administration. *Alzheimers Dement.* 6(Suppl. 1), Abstr. P3–320 (2010).

127 Brigham E, Quinn K, Motter R *et al.* Effects of single and multiple dose oral administration of ELND006, a novel APP-selective γ-secretase inhibitor, on amyloid-β concentrations in the brain and CSF of cynomolgus monkeys. *Alzheimers Dement.* 6(Suppl. 1), Abstr. P3–322 (2010).

128 Schroeter A, Brigham E, Motter R *et al.* APP-selective γ-secretase inhibitor ELND006 effects on brain parenchymal and vascular amyloid-β in the PDAPP mouse model of Alzheimer's disease. *Alzheimers Dement.* 6(Suppl. 1), Abstr. P3–321 (2010).

129 Minati L, Edginton T, Bruzzone MG, Giaccone G. Current concepts in Alzheimer's disease: a multidisciplinary review. *Am. J. Alzheimers Dis. Other Demen.* 24(2), 95–121 (2009).

130 Maillard I, Adler SH, Pear WS. Notch and the immune system. *Immunity* 19(6), 781–791 (2003).

131 Stanger BZ, Datar R, Murtaugh LC, Melton DA. Direct regulation of intestinal fate by Notch. *Proc. Natl Acad. Sci. USA.* 102(35), 12443–12448 (2005).

132 Panelos J, Massi D. Emerging role of Notch signaling in epidermal differentiation and skin cancer. *Cancer Biol Ther.* 8(21), 1986–1993 (2009).

133 Searfoss GH, Jordan WH, Calligaro DO *et al.* Adipsin, a biomarker of gastrointestinal toxicity mediated by a functional γ-secretase inhibitor. *J. Biol. Chem.* 278(46), 46107–46116 (2003).

134 Lathia JD, Mattson MP, Cheng A. Notch: from neural development to neurological disorders. *J. Neurochem.* 107(6), 1471–1481 (2008).

135 Costa RM, Honjo T, Silva AJ. Learning and memory deficits in Notch mutant mice. *Curr. Biol.* 13(15), 1348–1354 (2003).

136 Wang Y, Chan SL, Miele L *et al.* Involvement of Notch signaling in hippocampal synaptic plasticity. *Proc. Natl Acad. Sci. USA* 101(25), 9458–9462 (2004).

137 Xia X, Qian S, Soriano S *et al.* Loss of presenilin 1 is associated with enhanced β-catenin signaling and skin tumorigenesis. *Proc. Natl Acad. Sci. USA* 98(19), 10863–10868 (2001).

138 He G, Luo W, Li P *et al.* γ-secretase activating protein is a therapeutic target for Alzheimer's disease. *Nature* 467(7311), 95–98 (2010).

139 Chen F, Hasegawa H, Schmitt-Ulms G *et al.* TMP21 is a presenilin complex component that modulates γ-secretase but not ε-secretase activity. *Nature* 440(7088), 1208–1212 (2006).

140 Thathiah A, Spittaels K, Hoffmann M *et al.* The orphan G protein-coupled receptor 3 modulates amyloidβ peptide generation in neurons. *Science* 323(5916), 946–951 (2009).

141 Checler F, Dunys J, Pardossi-Piquard R, Alves da Costa C. p53 is regulated by and regulates members of the γ-secretase complex. *Neurodegener. Dis.* 7(1–3), 50–55 (2010).

142 Goodger ZV, Rajendran L, Trutzel A *et al.* Nuclear signaling by the APP intracellular domain occurs predominantly through the amyloidogenic processing pathway. *J. Cell Sci.* 122(Pt 20), 3703–3714 (2009).

143 Müller T, Meyer HE, Egensperger R, Marcus K. The amyloid precursor protein intracellular domain (AICD) as modulator of gene expression, apoptosis, and cytoskeletal dynamics-relevance for Alzheimer's disease. *Prog. Neurobiol.* 85(4), 393–406 (2008).

144 Chang WP, Koelsch G, Wong S *et al. In vivo* inhibition of Aβ production by memapsin 2 (βsecretase) inhibitors. *J. Neurochem.* 89(6), 1409–1416 (2004).

145 Kim J, Onstead L, Randle S *et al.* Aβ40 inhibits amyloid deposition *in vivo*. *J. Neurosci.* 27(3), 627–633 (2007).

146 Glenner GG, Wong CW. Alzheimer's disease and Down's syndrome: sharing of a unique cerebrovascular amyloid fibril protein. *Biochem. Biophys. Res. Comm.* 122(3), 1131–1135 (1984).

147 Armstrong RA. The pathogenesis of Alzheimer's disease: a reevaluation of the 'amyloid cascade hypothesis'. *Int. J. Alzheimers Dis*. 2011, 630865 (2011).

148 Nochlin D, van Belle G, Bird TD, Sumi SM. Comparison of the severity of neuropathologic changes in familial and sporadic Alzheimer's disease. *Alzheimer Dis. Assoc. Disord*. 7(4), 212–222 (1993).

149 Iurescia S, Fioretti D, Mangialasche F, Rinaldi M. The pathological cross talk between apolipoprotein E and amyloid-β peptide in Alzheimer's disease: emerging gene-based therapeutic approaches. *J. Alzheimers Dis*. 21(1), 35–48 (2010).

150 Holmes C, Boche D, Wilkinson D *et al.* Long-term effects of Aβ42 immunisation in Alzheimer's disease: follow-up of a randomised, placebo-controlled phase I trial. *Lancet* 372(9634), 216–223 (2008).

■■ **This case series demonstrated that the burden of Aβ correlated inversely with antibody titer. Although this was not the first postmortem study of an AN1792-treated patient, it strongly suggested that Aβ immunization facilitated plaque clearance in humans; however, doing so had no effect on survival or progression of dementia.**

151 Castellani RJ, Lee HG, Zhu X, Perry G, Smith MA. Alzheimer disease pathology as a host response. *J. Neuropathol. Exp. Neurol.* 67(6), 523–531 (2008).

152 Castellani RJ, Lee HG, Siedlak SL *et al.* Reexamining Alzheimer's disease: evidence for a protective role for amyloid-β protein precursor and amyloid-β. *J. Alzheimers Dis.* 18(2), 447–452 (2009).

153 Selkoe DJ. Soluble oligomers of the amyloid β-protein impair synaptic plasticity and behavior. *Behav. Brain Res.* 192(1), 106–113 (2008).

154 Koffie RM, Meyer-Luehmann M, Hashimoto T *et al.* Oligomeric amyloid β associates with postsynaptic densities and correlates with excitatory synapse loss near senile plaques. *Proc. Natl Acad. Sci. USA* 106(10), 4012–4017 (2009).

155 Shankar GM, Bloodgood BL, Townsend M, Walsh DM, Selkoe DJ, Sabatini BL. Natural oligomers of the Alzheimer amyloid-β protein induce reversible synapse loss by modulating an NMDA-type glutamate receptor-dependent signaling pathway. *J. Neurosci.* 27(11), 2866–2875 (2007).

156 Lesnè S, Koh MT, Kotilinek L *et al.* A specific amyloid-β protein assembly in the brain impairs memory. *Nature* 440(7082), 352–357 (2006).

157 Tampellini D, Rahman N, Gallo EF *et al.* Synaptic activity reduces intraneuronal Aβ, promotes APP transport to synapses, and protects against Aβ related synaptic alterations. *J. Neurosci.* 29(31), 9704–9713 (2009).

158 Abramov E, Dolev I, Fogel H, Ciccotosto GD, Ruff E, Slutsky I. Amyloid-β as a positive endogenous regulator of release probability at hippocampal synapses. *Nature Neurosci.* 12(12), 1567–1576 (2009).

159 Puzzo D, Privitera L, Leznik E *et al.* Picomolar amyloid-β positively modulates synaptic plasticity and memory in hippocampus. *J. Neurosci.* 28(53), 14537–14545 (2008).

160 Morley JE, Farr SA, Banks WA, Johnson SN, Yamada KA, Xu L. A physiological role for amyloid-β protein: enhancement of learning and memory. *J. Alzheimers Dis.* 19(2), 441–449 (2010).

161 Bennett DA, Schneider JA, Arvanitakis Z *et al.* Neuropathology of older persons without cognitive impairment from two communitybased studies. *Neurology* 66(12), 1837–1844 (2006).

162 Aizenstein HJ, Nebes RD, Saxton JA *et al.* Frequent amyloid deposition without significant cognitive impairment among the elderly. *Arch. Neurol.* 65(11), 1509–1517 (2008).

163 Struble RG, Ala T, Patrylo PR, Brewer GJ, Yan XX. Is brain amyloid production a cause or a result of dementia of the Alzheimer's type? *J. Alzheimers Dis.* 22(2), 393–399 (2010).

164 Vossel KA, Zhang K, Brodbeck J *et al.* Tau reduction prevents Aβ-induced defects in axonal transport. *Science* 330(6001), 198 (2010).

165 Duce JA, Tsatsanis A, Cater MA *et al.* Iron-export ferroxidase activity of β-amyloid precursor protein is inhibited by zinc in Alzheimer's disease. *Cell* 142(6), 857–867 (2010).

166 Dubois B, Feldman HH, Jacova C *et al.* Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS-ADRDA criteria. *Lancet Neurol.* 6(8), 734–746 (2007).

167 Dubois B, Feldman HH, Jacova C *et al.* Revising the definition of Alzheimer's disease: a new lexicon. *Lancet Neurol.* 9(11), 1118–1127 (2010).

168 McKhann GM, Knopman DS, Chertkow H *et al.* The diagnosis of dementia due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement.* 7(3), 263–269 (2011).

169 Albert MS, Dekosky ST, Dickson D *et al.* The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement.* 7(3), 270–279 (2011).

170 Sperling RA, Aisen PS, Beckett LA *et al.* Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement.* 7(3), 280–292 (2011).

171 Weiner MW, Aisen PS, Jack CR Jr *et al.* Alzheimer's Disease Neuroimaging Initiative. The Alzheimer's disease neuroimaging initiative: progress report and future plans. *Alzheimers Dement.* 6(3), 202–211 (2010).

172 John V, Beck JP, Bienkowski MJ *et al.* Human β-secretase (BACE) and BACE inhibitors. *J. Med. Chem.* 46(22), 4625–4630 (2003)

■ Websites

201 ClinicalTrial.gov. Effect of LY450139 on the long term progression of Alzheimer's disease www.clinicaltrials.gov/ct2/show/NCT00594568?term=LY450139&rank=3]

202 ClinicalTrial.gov. Effects of LY450139, on the progression of Alzheimer's disease as compared with placebo (IDENTITY-2) www.clinicaltrials.gov/ct2/show/NCT00762411?term=LY450139&rank=2]

203 ClinicalTrial.gov. A study of semagacestat for Alzheimer's patients (identity XT) www.clinicaltrials.gov/ct2/show/NCT01035138?term=LY450139&rank=5]

204 ClinicalTrial.gov. A trial in patients with advanced cancer and leukemia www.clinicaltrials.gov/ct2/show/NCT00878189?term=NCT00878189&rank=1]

205 ClinicalTrial.gov. A Phase II, multicenter, double-blind, placebo-controlled safety, tolerability study of BMS-708163 in patients with mild to moderate Alzheimer's disease www.clinicaltrials.gov/ct2/show/NCT00810147?term=NCT00810147&rank=1]

206 ClinicalTrial.gov. A multicenter, double-blind, placebo-controlled, safety and tolerability study of BMS-708163 in patients with prodromal Alzheimer's disease www.clinicaltrials.gov/ct2/show/NCT00810147?term=BMS-708163&rank=6]

207 ClinicalTrial.gov. Phase I/II study of MK-0752 followed by docetaxel in advanced or metastatic breast cancer www.clinicaltrials.gov/ct2/show/NCT00645333?term=NCT00645333&rank=1]

208 ClinicalTrial.gov. Study Of MK-0752 in combination with tamoxifen or letrozole to treat early-stage breast cancer www.clinicaltrials.gov/ct2/show/NCT00756717?term=NCT00756717&rank=1]