REVIEW

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γ-Secretase Inhibitors for the Treatment of Alzheimer's Disease: The Current State

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SUMMARY

Aims: Drugs currently used for the treatment of Alzheimer's disease (AD) partially stabilize patients' symptoms without modifying disease progression. Brain accumulation of oligomeric species of β-amyloid (Aβ) peptides, the principal components of senile plaques, is believed to play a crucial role in the development of AD. Based on this hypothesis, huge efforts are being spent to identify drugs able to interfere with proteases regulating Aβ formation from amyloid precursor protein (APP). This article briefly reviews the profile of γ-secretase inhibitors, compounds that inhibit γ-secretase, the pivotal enzyme that generates Aβ, and that have reached the clinic. **Discussion:** Several classes of potent γ-secretase inhibitors have been designed and synthesized. Preclinical studies have indicated that these compounds are able to lower brain Aβ concentrations and, in some cases, reduce Aβ plaque deposition in transgenic mouse models of AD. The most developmentally advanced of these compounds is semagacestat, presently in Phase III clinical trials. In animals, semagacestat reduced Aβ levels in the plasma, cerebrospinal fluid (CSF), and the brain. However, studies have not reported on its cognitive effects. Studies in both healthy volunteers and patients with AD have demonstrated a dose-dependent inhibition of plasma Aβ levels, and a recent study in healthy subjects demonstrated a robust, dose-dependent inhibition of newly generated Aβ in the CSF after single oral doses. **Conclusions:** Unfortunately, γ-secretase inhibitors may cause intestinal goblet cell hyperplasia, thymus atrophy, decrease in lymphocytes, and alterations in hair color, effects associated with the inhibition of the cleavage of Notch, a protein involved in cell development and differentiation. Nevertheless, at least other two promising γ-secretase inhibitors are being tested clinically. This class of drugs represents a major hope to slow the rate of decline of AD.

Introduction

Alzheimer's disease (AD), an age-related progressive neurodegenerative disorder characterized by relatively slow chronic but progressive impairment in cognition, behavior, and functionality, is the most common form of dementia. Accurate AD epidemiological data have been recently released for the United States [1]. These 2009 figures suggested that 5.3 million Americans have AD and >15 million patients with AD worldwide. The prevalence is approximately 13% in people over the age of 65 years, and increases to >40% for those over 85 years of age. It is expected that the number of patients with AD in the United States will increase to 7.4 million

by 2020, with 400,000 new cases diagnosed each year caused largely by a significant increase in the aging population. The number of people suffering from AD, that currently affects more than 26 million people worldwide with an expected increase to more than 106 million by 2050 [2], is rising quickly because there are no effective treatments for the disorder available. At present, only cholinesterase inhibitors (ChEIs) and the N-methyl-D-aspartic acid (NMDA)-receptor antagonist memantine have received Food and Drug Administration (FDA) approval for the symptomatic treatment of AD [3,4]. Evidence from controlled clinical trials suggests that ChEIs in particular can stabilize patients' symptoms for periods of time ranging between 1 and 3 years but without modifying progression of the disease [3]. Despite many theoretical considerations suggesting that ChEIs or memantine may have a disease-modifying effect, only symptomatic effects of these compounds have been proven [3]. Individual ChEIs have additional pharmacological effects besides the inhibition of acetylcholinesterase [5,6]. However, a clinical benefit of these additional effects has not been convincingly shown [7]. A recent large *meta*-analysis based on 59 unique studies showed that both ChEIs and memantine had consistent effects in the domains of cognition and global assessment, but summary estimates showed small effect sizes [8]. Epidemiological studies mainly with cross-sectional data have suggested that nonsteroidal anti-inflammatory drugs (NSAIDs), estrogens, HMG-CoA reductase inhibitors (statins), and tocopherol (vitamin E) may be beneficial to reduce the incidence of AD [9]. However, bias of case selection and several other sources of error are inherent in epidemiological studies and subsequent clinical trials have often been disappointing.

Drugs Targeting β-Amyloid

In the last decade, advances in understanding the neurobiology of AD have translated into an increase in clinical trials assessing various potential AD treatments [3,4,9]. In particular, most pharmaceutical research has been directed against the production and the accumulation of β-amyloid (Aβ) with the aim of slowing the deterioration rate of patients [10]. In fact, AD involves aberrant protein processing and is characterized by the presence of both intraneuronal protein clusters composed of paired helical filaments of hyperphosphorylated tau protein (neurofibrillary tangles [NFTs]), and extracellular protein aggregates (senile plaques [SPs]). Therefore, Alzheimer's classic pathological description of AD as a "two hallmarks disorder" [11] was confirmed by subsequent observations [12]. The exact relationship between these two neuropathological hallmarks remains unclear and how they may cause neuronal death is still an area of intense research effort [13]. However, these neuropathological hallmarks of AD strongly influenced recent therapeutic approaches [14]. SPs consist of a proteinaceous core composed of 5 to 10 nm amyloid fibrils surrounded by dystrophic neurites, astrocytic processes, and microglial cells. The Aβ peptide consists of 38 to 42 amino acids generated by the cleavage of amyloid precursor protein (APP), a type-1 transmembrane protein. The SPs are the result of misprocessing of the APP by β- and γ-secretases to form a toxic Aβ peptide of 40 to 42 amino acids that aggregates and initiates a pathogenic self-perpetuating cascade ultimately leading to neuronal loss and dementia [15]. The main form of Aβ contains 40 amino acids (Aβ40), but the carboxy-terminal-extended species, made up of 42 residues (Aβ42), is also produced. This longer form is more prone to aggregate into fibrils and Aβ42 makes up the major component of SPs. Extracellular and perhaps also intracellular Aβ exert neurotoxic effects [16]. Extracellular Aβ peptides cluster in a β-sheet structure to form SPs. According to the "amyloid cascade hypothesis" [17,18], the development of SPs is thought to precede and precipitate the formation of NFTs as a result of the cellular changes invoked, and the oligomeric forms of Aβ peptide are the main cause of neuronal death in AD [19]. APP may be metabolically processed according to two pathways. In the so-called nonamyloidogenic pathway, the α-secretase enzyme cleaves APP within the Aβ sequence and releases its transmembrane fragment soluble amyloid precursor protein α-cleaved (sAPPα) that appears to exert neuroprotective activity. In the amyloidogenic pathway, the β-secretase enzyme releases APP plus a 12-kDa protein fragment (C99), which in turn is cleaved by the γ-secretase enzyme giving way to Aβ. Accumulation of toxic, aggregated forms of Aβ seem crucial in the pathogenesis of familial forms of AD [19]. In fact, many studies showed a weak correlation between Aβ deposits and cognitive status [20]. Some other reports showed that cognitively healthy elderly people could have a substantial amyloid burden [21].

The manipulation of the enzymes responsible for the generation of Aβ (α, β, and γ-secretase) has been intensively pursued and several compounds have reached clinical testing. The last metabolic step in the generation of Aβ involves the enzymatic intramembranous cleavage of APP by a high-molecular weight complex called γ-secretase. This article briefly reviews the profile of γ-secretase inhibitors that have reached the clinic and discusses the clinical issues surrounding this new class of anti-AD compounds. Therefore, the review did not include active and passive immunization [22], drugs that target the proteases involved in both the nonamyloidogenic and the amyloidogenic

pathways of the APP metabolism (α-secretase activators and β-secretase inhibitors) [3], Aβ aggregation inhibitors [4], and drugs similar to tarenflurbil (R-flurbiprofen, FlurizanTM), a compound that recently failed in a large Phase III study, because it is not a γ-secretase inhibitor but just indirectly modulates its activity (shifting Aβ42 to Aβ38 production) by binding to APP (γ-secretase modulators) [9]. We reviewed studies from the English literature published before March 2010. We searched through the PubMed database of NCBI (available at http://www.ncbi.nlm.nih.gov) by author and the following keywords: drugs targeting β-amyloid; γ-secretase inhibitors; dementia syndromes, and AD.

Therapeutic Potential of γ-Secretase Inhibitors

γ-Secretase is an unconventional aspartyl protease that resides and cleaves its substrates within the lipid bilayer. 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 [23]. AD is believed to be caused by a progressive cerebral accumulation of Aβ, and the γ-secretase activity, which consists of both presenilin-dependent and presenilin-independent activities [24–26], determines the length of Aβ and therefore controls the ratio Aβ42/Aβ40 [27]. In fact, at least four subunit proteins form γ-secretase: presenilin (PS), nicastrin, anterior pharynx-defective-1 (Aph-1), and presenilin enhancer-2 (Pen-2) [28] (Figure 1). Presenilins are of exceptional pathophysiological importance because more than 175 autosomal dominant point mutations are known in these proteins, and most of which cause aggressive early-onset AD elevating the Aβ42/Aβ40 ratio and interfering with the processing of APP and other γ-secretase substrates [29,30]. The catalytic component of γ-secretase complex is PS, with two aspartate residues forming the active site. The inhibition of the catalytic unit of the γ-secretase enzymatic complex appears a logical strategy to counteract Aβ accumulation in patients with AD [31,32]. γ-Secretase processes a wide range of type-I integral membrane proteins, some of them with critical cellular functions. However, despite the fact that more than 70 type-I integral membrane proteins are known to be cleaved by γ-secretase [33], the physiological function of these proteolytic events is poorly understood. 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 (notch intracellular domain [NICD]) which is essential for many cell differentiation events and neurite outgrowth [34,35]. Proteolysis of N-cadherin leads to degradation of the transcriptional factor CBP (cAMP response element-binding protein), and cleavage of ErB4 inhibits astrocyte differentiation by interacting with repressors of astrocyte gene expression [36,37]. Cleavage of APP generates an APP intracellular domain (AICD), although its role in signal transduction remains controversial [38]. 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 inhibitors. 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 2001, the first *in vivo* testing of a γ-secretase inhibitor was reported, the dipeptidic compound DAPT (N-[N-(3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester) developed by Elan and Eli Lilly [27]. Later data demonstrated that DAPT reversed contextual

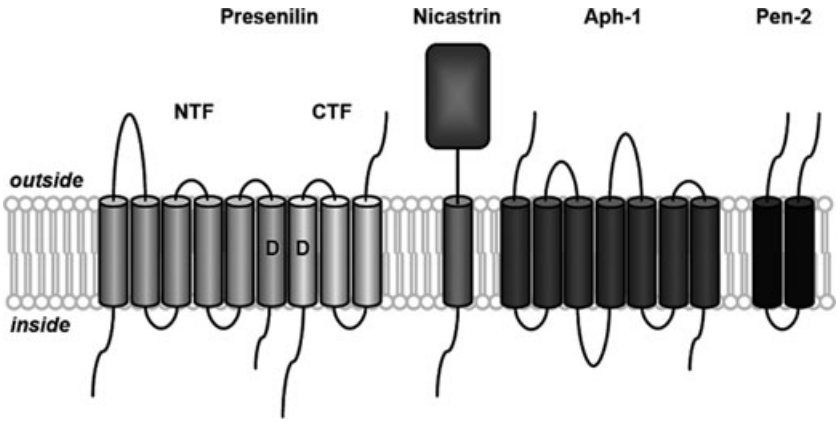

Figure 1 Schematic representation of the γ-secretase complex. γ-Secretase is composed of four different integral membrane proteins: presenilin (PS), nicastrin, anterior pharynx-defective-1 (Aph-1), and presenilin enhancer-2 (Pen-2). Presenilin undergoes endoproteolysis into an N-terminal fragment (NTF) and a C-terminal fragment (CTF) that remain associated. Two conserved aspartates (D) within adjacent transmembrane domains are essential for both presenilin endoproteolysis and γ-secretase activity.

memory deficit in a transgenic mouse model of AD [39]. Several other nonpeptidic, orally-available, γ-secretase inhibitors have been reported to lower brain Aβ concentrations in both transgenic and nontransgenic animals [40]. The first γ-secretase inhibitor to reach clinical development appears to be BMS-299897, a sulfonamide derivative synthesized at Bristol-Myers Squibb and the former SIBIA Neurosciences [40]. 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 [40]. Benzodiazepine analog LY-411575 and benzolactam semagacestat (LY-450139), developed at Eli Lilly, are highly potent γ-secretase inhibitors that have been tested extensively *in vivo* [41,42]. At least five other γ-secretase inhibitors reached the clinic, and most of the information on these other compounds is derived from conference communications: MK-0752 [43], BMS-708163 [44], PF-3084014 (abandoned) [45], and GSI-953 (begacestat) [46], and E2012 [40] (Table 1). Some γ-secretase inhibitors appear to spare Notch cleavage *in vitro* and are relatively well tolerated in man. Three of these compounds affect Aβ levels in the cerebrospinal fluid (CSF) of humans, which is a potential biomarker of the disease. These compounds are Merck & Co Inc's (Whitehouse Station, NJ, USA) MK-0752, Bristol-Myers Squibb Co's (New York City, NY, USA) BMS-708163, and Eli Lilly & Co's (Indianapolis, IN, USA) semagacestat [40]. The best documented and most advanced of these compounds is semagacestat [47].

Semagacestat

Semagacestat is 3-fold more potent in inhibiting APP cleavage than Notch cleavage [42]. In experimental animals, the effects of semagacestat on Aβ levels in brain, CSF, and plasma were well characterized in transgenic mice [48], nontransgenic mice [49], guinea pigs [50], and dogs [51]. However, the drug failed to show a statistically significant effect on brain plaque deposition in chronic studies in transgenic mice expressing mutated human APPV717F (PDAPP mice) [52]. More importantly, no data are available on the cognitive or behavioral effects of the drug in animal models of AD.

In a Phase I study, semagacestat was administered to healthy men and women, aged 45 years and above, for up to 14 days at doses of 5, 20, 40, and 50 mg once daily [53]. Two subjects in the 50-mg-dose group developed possibly

Table 1 γ-Secretase inhibitors in clinical development for the treatment of Alzheimer's disease (AD)

Compound	Mechanism of action	Side effects	Development status	Company	References
Semagacestat (LY-450139)	Decreases newly synthesized Aβ in CSF of AD patients	No significant effects on brain plaque burden in transgenic mice. Lack of data on behavioral effects in animal models of AD. Gastro-intestinal and skin side effects in AD patients	Phase III	Eli-Lilly	[42]
MK-0752	Decreases Aβ40 levels in CSF of healthy volunteers	Inhibits Notch cleavage. Significant gastro-intestinal toxicity in humans	Abandoned[a]	Merck	[43]
E2012	Notch sparing	Lenticular opacity in rats	Phase I	Eisai	[40]
BMS-708163	Notch sparing Decreases Aβ levels in CSF of healthy volunteers	Lack of data on brain plaque deposition in transgenic mice. Lack of data on behavioral effects in animal models of AD	Phase II	Bristol Myers Squibb	[44]
PF-3084014	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. Lack of data on behavioral effects in animal models of AD	Abandoned[a]	Pfizer	[45]
GSI-953 (begacestat)	Improves memory in a transgenic mouse model of AD.	Does not decrease Aβ40 levels in CSF of AD patients	Phase II	Wyeth	[46]

[a]In development as anticancer agent.
Aβ, β-amyloid; CSF, cerebrospinal fluid.

drug-related adverse events and discontinued treatment (significant increases in serum amylase and lipase with moderate abdominal pain and diarrhea positive for occult blood). The 50-mg dose caused a maximal 40% reduction in total plasma Aβ that returned to baseline within 8 h. However, after returning to baseline, plasma Aβ levels increased to about 300% of baseline values at 15 h before slowly 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 in this study [53]. A second Phase I study evaluated the safety and tolerability and biomarker responses to single oral doses of 60, 100, or 140 mg in 31 healthy male and female volunteers (40 years and older) [54]. No clinically significant adverse events or laboratory changes were observed in this study. A dose-dependent decrease in plasma Aβ40 levels was demonstrated with maximum inhibition (–73%) at 6 h after the administration of the 140-mg dose. Again, a rebound effect on plasma Aβ40 levels was observed at 8–12 h after administration and lasted for at least 24 h. CSF concentrations of Aβ were unchanged 4 h after drug administration [54].

Semagacestat has been evaluated also in AD patients in Phase II studies. In a randomized, placebo-controlled trial, 70 patients received the drug for 6 weeks (30 mg once a day for 1 week followed by 40 mg once a day for 5 weeks) [55]. Six patients taking the drug reported diarrhea and a 76-year-old man on semagacestat had gastrointestinal bleeding associated to a Barrett esophagus. Approximately 4 months after discontinuing treatment, this older 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. Plasma Aβ40 concentrations of patients taking the compound decreased significantly by 38% compared to baseline. Aβ40 concentrations in CSF did not decrease significantly in this study. In another Phase II study, 51 individuals with mild-to-moderate AD were randomized to receive placebo (n = 15) or semagacestat [100 mg (n = 22) or 140 mg (n = 14)] for 14 weeks, with 43 patients completing the treatment phase [56]. Patients on semagacestat received 60 mg/day for 2 weeks, then 100 mg/day for 6 weeks, and then either 100 or 140 mg/day for 6 additional weeks. There were 7 cases with skin rashes and 3 reports of hair color change in the drug treatment groups. There were 3 adverse event-related discontinuations, including 1 transient bowel obstruction. Compared to placebo, Aβ40 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 in this study. No differences were seen in cognitive or functional measures between placebo- and semagacestat-treated patients [56]. No data were reported on the effects of semagacestat on Aβ42 levels. More recently, the results of a study on the effects of semagacestat on Aβ synthesis and clearance in CSF of AD patients were reported [57]. CSF was collected hourly for 36 h with a lumbar catheter. Semagacestat significantly decreased newly generated Aβ in a dose-dependent fashion, with inhibition of Aβ generation of 47%, 52%, and 84% over the first 12-h period with doses of 100, 140, and 280 mg, respectively. However, ELISA determinations revealed that there was a late rebound of CSF Aβ42 levels compared to placebo in the 20–36-h period especially after the highest dose of 280 mg [57].

At present, two ongoing Phase III clinical trials were listed on the NIH's clinical trials registry. In March 2008, the first trial, called Interrupting Alzheimer's Dementia by EvaluatiNg Treatment of AmyloId PaThologY (IDENTITY) trial, was a Phase III, randomized, double-blind, placebo-controlled, parallel-assignment, multicenter clinical trial (clinicaltrials.gov identifier: NCT00594568; H6L-MC-LFAN) in patients with mild-to-moderate AD (expected n = 1500). Patients would be treated with semagacestat (100 or 140 mg p.o., q.d.) 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 incorporates 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 endpoints include Aβ levels in plasma and CSF and other brain biomarkers determined by neuroimaging [58]. Eli Lilly estimated the trial would be completed by May 2011.

The second trial, called IDENTITY-2, was also a Phase III, randomized, double-blind, placebo-controlled, parallel-assignment, multicenter clinical trial (clinicaltrials.gov identifier: NCT00762411; H6L-MC-LFBC) in patients with mild-to-moderate AD (expected n = 1100). Patients would be treated with semagacestat (60 mg p.o., q.d., titrated to 140 mg p.o., q.d.) 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 incorporates 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 endpoints 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 [59]. Eli Lilly estimated the trial would be completed by March 2012. Finally, in December 2009, Eli Lilly launched an open-label extension called Identity XT (ClinicalTrials.gov Identifier: NCT01035138) for AD patients who completed one of two semagacestat Phase III double-blind studies, IDENTITY, or IDENTITY 2 (H6L-MC-LFAN or H6L-MC-LFBC), with an estimated enrollment of 1700 patients and an estimated study completion by January 2014 [60].

MK-0752

Merck is developing a γ-secretase inhibitor (MK-0752) that does not distinguish between APP and Notch. A Phase I study evaluated the safety, tolerability, pharmacokinetics, and pharmacodynamics of single oral doses (from 110 to 1000 mg) of MK-0752 in 27 healthy young men [43]. 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 about 20 h. Doses of 500 mg significantly inhibited for 12 h Aβ40 concentrations in CSF with a peak effect of –35%. After 1000 mg, CSF Aβ40 inhibition was sustained over 24 h. Plasma Aβ40 concentrations also showed a dose-dependent decrease but were followed by a later rebound over baseline levels.

Although MK-0752 was initially developed as a treatment for AD, there is increasing interest in the applicability of this drug to the treatment of cancer. Indeed, MK-0752 has been shown to inhibit γ-secretase-mediated cleavage of Notch with an IC_{50} of 55 nM. Increasing evidence implicates the Notch pathway in normal T-cell lymphopoiesis and the pathogenesis of several human malignancies. Prolonged activation of the Notch signal transduction pathway occurs in more than 50% of patients with T-cell acute lymphoblastic leukemias and is important in the pathogenesis of the disease. Preclinical studies indicate that pharmacologic inhibition of γ-secretase activity suppresses T-cell acute lymphoblastic leukemias cell growth and induces apoptosis by preventing cleavage of Notch, thus preventing prolonged activation in downstream pathways. Studies with MK-0752 in pediatric and adult patients with T-cell acute lymphoblastic leukemias and acute myeloid leukemia reported that drug was associated with gastrointestinal toxicity and fatigue without substantive clinical activity [60,61]. An intermittent dosing schedule appears to reduce toxicity while demonstrating adequate target inhibition, warranting further evaluation [61,62].

BMS-708163

BMS-708163 is a potent, Notch-sparing, γ-secretase inhibitor in development at Bristol-Myers Squibb. *In vitro*, the drug shows a 193-fold selectivity versus Notch cleavage [44]. Studies in rats and dogs have shown that BMS-708163 is able to decrease brain and CSF Aβ40 levels without causing Notch-related gastrointestinal and lymphoid toxicity [44]. 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/day after multiple doses for 28 days [44]. 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 $\approx$ 40 h). Single oral doses of BMS-708163 decrease dose dependently Aβ40 levels in CSF with a peak inhibition of about 55% after 400 mg [44]. A multicenter, randomized, double-blind, placebo-controlled, Phase II trial of the safety, tolerability, and pharmacodynamic and pharmacokinetic effects of oral BMS-708163 (25 to 125 mg q.d. for 12 weeks) in 200 patients with mild-to-moderate AD began in February 2009. At that time, the trial was estimated to complete in September 2010 (clinicaltrials.gov identifier: NCT00810147).

PF-3084014

PF-3084014 is a 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 IC50 of 6.2 nM [45]. In a whole-cell assay, PF-3084014 displays an IC50 of 1.3 nM. In fetal thymus organ culture assay, PF-3084014 appears to be a weak inhibitor of Notch signaling with an IC50 of 1915 nM. The APP to Notch selectivity ratio is 1.473. 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 to 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. Aβ40 was most potently inhibited in all compartments. Aβ42 showed approximately 20% less reduction than Aβ40 in all compartments. In a Phase I study in healthy volunteers, single doses of 1–120 mg were safe and well tolerated, and the maximum tolerated dose was not identified. The plasma half-life of the drug was approximately 19 h [63]. The analysis of the pharmacokinetic and pharmacodynamic data derived from another Phase I study employing multiple doses has recently led to the decision to end development of the compound for AD [64]. In this study, plasma drug concentrations and plasma Aβ levels were collected from 18 healthy volunteers that received 40 or 90 mg once daily for 14 days. Pharmacokinetic/ pharmacodynamic modeling of these data yielded the finding that exposure levels needed to reach the prespecified inhibition of plasma Aβ40 levels were 2- to 3-fold higher than exposure limits based on animal toxicology data. As a result, much higher doses than those previously used would be needed to attain a high probability of pharmacological activity in man. PF-3084014 has recently entered clinical studies as an anticancer agent. A Phase I study in patients with advanced solid tumors and leukemia, with an estimated enrollment of 60, is currently recruiting patients. The open-label, dose-escalation study is designed to evaluate safety (ClinicalTrials.gov Identifier: NCT00878189).

GSI-953 (Begacestat)

GSI-953 is a potent γ-secretase inhibitor in development at Wyeth (Madison, NJ, USA). This compound inhibits Aβ production with low nM potency in both cellular ($A\beta_{1-42}$ IC_{50} = 15 nM) and cell-free (IC_{50} = 8 nM) assays [65]. Cellular assays of Notch cleavage reveal that this compound is 15-fold selective for the inhibition of APP cleavage [65]. In Tg2576 mice, high doses of GSI-953 significantly reduced $A\beta_{1-40}$ levels in brain, CSF, and plasma [65]. At lower doses, GSI-953 significantly reduced $A\beta_{1-40}$ only in brain and plasma but not in CSF. Importantly, this compound has been reported to reverse contextual memory deficits in Tg2576 transgenic mice [65].

The pharmacokinetic, pharmacodynamic, and tolerability profile of GSI-953 after single oral administration has been recently described in young subjects and in AD patients [46]. GSI-953 was well tolerated and no dose-limiting adverse events were observed. The drug 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 10-fold lower than plasma in both young and AD subjects. For both young healthy subjects and AD patients, a biphasic pattern of plasma Aβ40 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 Aβ40 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 Aβ42 than Aβ40. Maximum reductions in plasma Aβ42 were only 7% in AD subjects and 17% in young subjects. Subsequent increases in Aβ42 above baseline were similar to those of Aβ40 in magnitude and duration. No significant effects of the drug on CSF Aβ40 levels were observed in either AD patients or young volunteers.

E2012

E2012 is a novel γ-secretase inhibitor claimed to lower Aβ by modulating γ-secretase without inferring with Notch processing [40]. It was developed by Eisai in a partnership with Torrey-Pines Therapeutics (La Jolla, CA, USA). In mid-2006, the compound entered Phase I clinical development. In February 2007, the Phase I study was put on hold because lenticular opacity was observed in a high-dose group of a 13-week safety preclinical study in rats. At the time when the study was suspended, no medical concerns were reported. Lenticular opacity was not observed in a later 13-week safety study in monkeys [40]. An additional 13-week multiple dosing study in rats did not reveal ocular toxicity and the clinical hold on E2012 was lifted in April 2008. The drug is now headed back into a Phase 1 clinical trial [40].

Efficacy of γ–Secretase Inhibitors

The hypothesis that Aβ is the key pathologic factor affecting the disease process has been strongly questioned. The most recent challenge derives from a recently published article: although immunization with preaggregated Aβ42 (AN-1792; formerly in development by Elan Corp plc and Wyeth) resulted in almost complete clearance of SPs from the brain of patients with AD, plaque removal did not prevent progressive neurodegeneration [66]. Aβ may have a physiological role in modulating synaptic plasticity [67] and hippocampal neurogenesis [68]. Aβ deposition could simply represent a host response to an upstream pathophysiologic process [20] or serving a protective function [69] likely as an antioxidant/metal chelator [70,71]. Nevertheless, pharmaceutical companies have

devoted huge efforts and finances to the development of agents that block γ-secretase with several compounds being actively studied in humans, including AD patients [40]. Semagacestat is the most advanced γ-secretase inhibitor in clinical development with two Phase III trials ongoing [47].

The Aβ reduction in plasma or CSF required to modify the rate of AD progression remains largely unknown [40]. A study in transgenic mice suggested that single doses of DAPT causing 25 to 30% reductions in soluble brain Aβ levels were associated with attenuation of cognitive deficit [39]. As outlined above, semagacestat produced significant and long-lasting inhibition of brain Aβ levels at relatively low doses in transgenic mice. However, in nontransgenic animals (mice, guinea pigs, and dogs), the effect of the drug appears to be much more complex with inhibitory or stimulatory effects depending on the dose and the time. In healthy human volunteers, semagacestat dose-dependently inhibited Aβ in CSF with approximately 80% inhibition of newly formed Aβ and by approximately 50% inhibition of total Aβ levels at high doses. However, some rebound effects have been observed, particularly on Aβ42 levels in CSF. The potential clinical consequences of the rebound in this neurotoxic Aβ species are not known.

The effects of chronic administration of γ-secretase on brain Aβ deposition have been documented in a few studies [32]. The first published study was with respect to MRK-560, a potent γ-secretase inhibitor (IC50 = 4.3 nM) investigated by Merck. In this study, MRK-560 (3 mg/kg/day p.o. for 3 months) decreased the number of Aβ deposits by 49% in Tg2576 mice. The mean size of the remaining SPs remained unchanged suggesting that MRK-560 did not alter deposit growth once initiated, which may suggest that an earlier intervention with γ-secretase inhibitors would be more effective on brain Aβ pathology [72]. Another recent study reported that LY-411575 (3 mg/kg/day p.o. for 3 months) reduced neocortical plaque area by 80% in transgenic mice (bearing the Swedish mutation). This group also reported on LY-411575 (3 mg/kg/day p.o. for 2 months) in different transgenic mice (bearing both Swedish and "London" [Val717Ile] mutations), which demonstrate more diffuse and AD-like plaque pathology. Aβ deposition was reduced by only 31% in the neocortex but by 79% in the caudate putamen, which has a lower Aβ load than the neocortex [73]. These observations suggest that γ-secretase inhibitors effectively reduce further Aβ formation in brains already containing SPs and that the efficacy is inversely correlated with the initial Aβ load. At present, only one study describing the effects of chronic semagacestat treatment had been reported (3, 10, or 30 mg/kg/day p.o. for 5 months in PDAPP mice) [74]. Quantitative analysis of Aβ immunohistochemistry data did not demonstrate significant changes in total plaque burden between treatment groups. However, the median plaque burden was reduced by 43 and 48% in the cortex and hippocampus, respectively, of treated animals compared with controls. Unfortunately, the study was not fully published but these data suggested that the inhibitory effects of semagacestat on plaque burden were not dramatic, although median inhibition provides more robust data [74].

Studies on the cognitive and behavioral effects of γ-secretase inhibitors in animal models of AD are very limited [41]. While single oral administration of DAPT or begacestat or the acute intrahippocampal administration of L-685458 (Merck's peptidic γ-secretase inhibitor) may improve contextual or spatial memory in rats [75] or in Tg2576 transgenic mice [39,65], data on the cognitive effects of prolonged administration of oral γ-secretase inhibitors is lacking. No data on the behavioral effects of semagacestat are available from either acute or chronic dosing studies. It is possible that any beneficial effects on learning and memory caused by the antagonism of brain Aβ production and deposition may be more than offset by other biochemical changes resulting from γ-secretase inhibition, such as the chronic accumulation of the neurotoxic C-terminal fragments of APP in neurons and the cerebral cortex [76]. This hypothesis is supported by the observation that conditional inactivation of PS-1 is accompanied by beneficial effects on cognition in 3-month-old transgenic mice but not in 6-month-old animals [77]. The lack of chronic behavioral data represents the major weakness of the preclinical documentation of semagacestat and may undermine the confidence on the cognitive efficacy potential of the drug in AD patients [47].

Safety of γ-Secretase Inhibitors

Although γ-secretase has in many ways been an attractive target for AD therapeutics, γ-secretase inhibition may have safety drawbacks. The PS knockout in mice resulted in an early embryonal lethality with the phenotype presenting skeletal and CNS defects similar to those of a Notch knockout [78]. Indeed, γ-secretase inhibitors block proteolysis of Notch-1, a particular γ-secretase substrate, the Notch receptor by inhibiting cleavage at site 3 ("S3") [79]. Physiological cleavage of Notch leads to release of the NICD 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 inhibition of Notch cleavage has been associated with goblet cell hyperplasia in intestinal

epithelium and changes in the immune system with a decrease of lymphocytes in the spleen and thymus [80,81]. Unfortunately, semagacestat does not spare Notch cleavage *in vitro* and this may have pharmacological and clinical consequences [47]. In the first Phase II clinical trial, T-lymphocyte and eosinophil counts were found to be slightly but statistically increased in semagacestat-treated patients compared to placebo-treated subjects. A decrease in an early marker (CD69) of activated mature T cells and thymocytes was also found. However, this change was no longer present 90 days after treatment discontinuation [55]. Indeed, clinical trials in AD patients have identified a relatively high rate of gastrointestinal side effects (nausea, vomiting, and diarrhea) that could be Notch mediated. Much more data have to be collected from human studies to establish the separation between well-tolerated and toxic doses of γ-secretase inhibitors in chronic treatment of AD patients. Recently, hair color changes in animals have been linked to the inhibition of Notch processing [82]. Interestingly, alterations in hair color have also been observed in a clinical study with semagacestat [56]. The use of a Notch-related toxicity biomarker such as adipsin may be useful for the early detection of potential toxicity [83]. Compounds have been described and are being developed that specifically modulate the proteolytic activity of γ-secretase. These compounds, called γ-secretase modulators, shift the production of Aβ42 to Aβ38 and do not affect Notch cleavage, meaning they may be more attractive from a safety point of view than the traditional γ-secretase inhibitors [32].

Conclusions

γ-Secretase is a rationale target for the treatment of AD because it regulates the final step of the Aβ formation. One of the components of the γ-secretase, PS, is of exceptional biological importance since point mutations of this protein cause the most frequent and aggressive forms of familial AD. Although γ-secretase is a complicated and unusual proteolytic complex, several classes of potent inhibitors have been discovered. At least six compounds have entered clinical development. In order to rationally guide their clinical development, efforts are being pursued to characterize their pharmacodynamic profile, not only by measuring Aβ concentrations in plasma and CSF over time, but also by estimating the effects of these drugs on newly synthesized Aβ and its clearance with recently available isotopic techniques. Studies in both transgenic and nontransgenic animals have indicated that γ-secretase inhibitors, administered by the oral route, are able to lower brain Aβ concentrations. However, scanty data are available on the effects of these compounds on soluble Aβ oligomers, the neurotoxic molecular species believed to be responsible for neuronal death in AD. Studies on the effects of γ-secretase inhibitors on brain Aβ deposition after prolonged administration are also few. Behavioral and cognitive studies after chronic administration in animal models of AD have not been published. The major contribution to side effects of γ-secretase inhibitors is thought to originate from the interference with intestinal and lymphatic cell differentiation associated with the inhibition of Notch cleavage. Fortunately, some γ-secretase inhibitors appear to spare Notch cleavage *in vitro* and are well tolerated *in vivo*. One γ-secretase inhibitor (semagacestat) has been properly characterized from the pharmacodynamic point of view in initial Phase I and II studies and now is in Phase III clinical testing. We will know if semagacestat does work in the next couple of years.

It was forecasted that by 2014 there would be at least two new anti-AD drugs on the market. One could be Wyeth/Elan Corp's bapineuzumab (humanized mAb) and the other could be semagacestat [84], although the recent discontinuation of the highest dose in a Phase III trial of bapineuzumab [85] and the mixed clinical data we have reported here for semagacestat, may cast doubt on these predictions. Nevertheless, the approval of either one of these agents could have a considerable impact on the way AD is treated in the future as they target the underlying cause of the disease and may therefore be used in combination with other agents to stop or slow the disease progression. It is expected that the commercial market will at least double in size upon entry of any new agents and it is believed that a small molecule compound (like semagacestat) with disease-modifying properties could easily reach US$7 billion in sales in the United States alone [86]. However, semagacestat could be a relatively high development risk based on its poor selectivity on Notch cleavage, lack of cognitive, and behavioral data in preclinical animal models of AD and complex time- and dose-dependent pharmacodynamics with preclinical and clinical indications of late Aβ42 rebounds in the CSF. Nevertheless, it and other γ-secretase inhibitors represent a major hope to slow the rate of decline of AD and to modify the natural history of this devastating disease.

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Conflicts of Interest

No Disclosures to Report.

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