Future Therapeutics in Alzheimer's Disease: Development Status of BACE Inhibitors

Short Title (Running Head): Development Status of BACE Inhibitors

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Abstract

Alzheimer's disease (AD) is the primary cause of dementia in the elderly. It remains incurable and poses a huge socio-economic challenge for developed countries with an aging population. AD manifests by progressive decline in cognitive functions and alterations in behaviour, which are the result of the extensive degeneration of brain neurons. AD pathogenic mechanism involves the accumulation of amyloid beta peptide (A β), an aggregating protein fragment that self-associates to form neurotoxic fibrils that trigger a cascade of cellular events leading to neuronal injury and death. Researchers from academia and pharmaceutical industry have pursued a rational approach to AD drug discovery and targeted the amyloid cascade. Schemes have been devised to prevent the overproduction and accumulation of A β in the brain. The extensive efforts of the past twenty years have been translated into bringing new drugs to advanced clinical trials. The most progressed mechanism-based therapies to date consist of immunological interventions to clear A β oligomers, and pharmacological drugs to inhibit the secretase enzymes that produce A β , namely β -site amyloid precursor-cleaving enzyme (BACE) and γ -secretase. After giving an update on the development and current status of new AD therapeutics, this review will focus on BACE inhibitors, and particularly discuss the prospects of verubecestat (MK-8931) that has reached Phase 3 clinical trials.

Key Points

- Disease-modifying therapies for Alzheimer's disease are under development.
- BACE inhibitors represent the most promising novel therapeutics for Alzheimer's disease treatment
- At least four BACE inhibitors are currently in Phase 2/3 clinical trials, among which verubecestat is the most advanced
- Verubecestat can effectively decrease the levels of A β in the cerebrospinal fluid of healthy volunteers and of patients with mild to moderate Alzheimer's disease
- Verubecestat is being tested in large cohorts of patients with mild cognitive impairment and with mild Alzheimer's disease
- Because of the numerous BACE substrates, the potential adverse effects of BACE inhibitors will have to be monitored carefully

1. Introduction

Age-related diseases are becoming prevalent among the developed countries of which populations are getting older. Alzheimer's disease, in particular, poses a heavy socio-economic burden upon our societies [1]. It is currently estimated that one in twenty people aged over sixty will present with some form of dementia, and this figure can reach one in four among those aged over eight-five. Seventy percent of dementia cases are due to Alzheimer's disease, a progressive degenerative disease of the brain that develops over several decades of life. It manifests as a decline of cognitive function and alterations in behaviour that result in the patients' loss of autonomy and their need for permanent care and assistance. Although there is presently no cure for AD, and current therapies can only provide temporary relief from some of its symptoms, novel and promising mechanism-based therapies that aim to modify the course of the disease are undergoing clinical trials. These new therapeutic approaches are reviewed here, with particular focus on the BACE inhibitor verubecestat.

2. The Pathophysiology of Alzheimer's Disease

The definite diagnosis of Alzheimer disease (AD) is established at autopsy by the presence of pathological hallmarks in the brain of patients deceased with dementia [2, 3]. AD hallmarks consist of extracellular amyloid plaques, which accumulate in the brain parenchyma, and neurofibrillary tangles (NFT), which form within neuron bodies. Amyloid plaque deposition is the salient characteristic of AD, whereas NFT are also observed in other neurodegenerative diseases. Brain examination also indicates a major loss of grey matter that reflects extensive neuronal death. Plaques and tangles are principally localized in the hippocampus and frontal cortex, which are the brain regions associated with memory acquisition and retrieval. The essential component of amyloid plaques is a protein fragment termed A β , that self-associates to form oligomers and aggregates [4]. Although the pathophysiology of AD is not fully understood, extensive research has demonstrated the toxicity of A β oligomers and their direct injury to neurons, including inhibition of long-term potentiation (LTP), synaptic damage, and apoptotic cell death [5, 6]. The NFT are composed of hyperphosphorylated *tau* protein that self-associates as paired helical filaments [7]. *Tau* hyperphosphorylation causes its dissociation from the neurons' microtubules, with consequent loss of axon stability and cellular integrity, thus reduced viability. It is clear that the harmful combination of A β oligomers and hyperphosphorylated *tau* cause synaptic dysfunction and neuronal death in AD [8, 9]. The relationship between A β and *tau* is intricate and remains incompletely resolved. So far, experimental evidence supports that 1) A β fibrils promote *tau* phosphorylation; 2) *tau* phosphorylation mediates A β inhibition of long-term potentiation (LTP). In addition, it was recently shown that functional *tau* facilitates A β clearance and suggested that *tau* dysfunction could trigger A β oligomerisation and toxicity [10]. However, it is worth pointing that *tau* is also associated with neurodegenerative diseases such as frontotemporal dementia, Pick's disease, and other tauopathies that occur in the absence of A β pathology and which are caused by rare mutations in the *tau* gene, MAPT [11, 12]. Therefore, A β remains the primary pathological culprit in AD, and we will focus this review on the amyloidocentric therapeutic approaches that converge into preventing A β accumulation.

3. The Amyloid Cascade as the Basis for Rational Therapies in AD

3.1. Secretase processing of the Amyloid Precursor Protein

A β is a peptide fragment derived from cleavage processing of the amyloid precursor protein (APP), a membrane receptor involved in cell adhesion, synaptic plasticity and metal homeostasis [13-15]. APP is ubiquitously

expressed, and alternative splicing of the gene produces two major variants: APP₆₉₅, which is mostly expressed in neurons of the central nervous system (CNS), and APP₇₅₁, that is principally expressed in the periphery. APP undergoes proteolytic processing by two alternative cellular pathways that can either generate, or preclude formation of A β [16, 17] (refer to Figure 1). When following the amyloidogenic pathway, APP undergoes cleavage in its juxtamembrane region by γ -secretase, a proteolytic enzyme identified as γ -site APP cleaving enzyme (BACE, or BACE1 for distinction from its homologue BACE2). Cleavage of APP by BACE1 generates the soluble γ -cleaved APP fragment, sAPP γ , and a corresponding 99 amino acid, membrane-tethered C-terminal fragment (C99) that is the direct precursor to A β . Subsequent processing of C99 by β -secretase releases A β fragments of variable length that are secreted in the extracellular space, and concomitantly liberates the APP-intracellular domain (AICD) to allow its nuclear translocation together with transcription-active binding partners [18] [19]. γ -Secretase is a membrane-embedded catalytic complex composed of four subunits, among which presenilin represents the enzymatic entity [20]. Its mechanism involves sequential cuts that produce heterogeneous products [21]. Neuronal cells secrete A β peptides of 40 (A₄₀) and 42/43 (A₄₂/A₄₃) amino acids, in a ratio of approximately 95/5, with the longer peptides being more aggregating and cytotoxic. In the alternative non-amyloidogenic pathway, which is the default pathway for most cells apart from neurons, APP is cleaved by α -secretase, an ADAM metalloprotease (either ADAM10 or ADAM17) that sheds the large extracellular domain, sAPP α , following either a constitutive or a regulated pathway [22, 23]. Processing of the corresponding C-terminal fragment of 83 amino acids (C83) by α -secretase generates small fragments termed p3, as well as AICD [17]. The regulated cellular trafficking of APP controls its co-localisation with either secretase, and is a major determinant to A β production.

3.2. Evidence for the causative role of the amyloid cascade in AD

The main risk factors associated with AD, which are aging, oxidative stress, metabolic diseases, and inflammation, are all known to increase BACE expression and activity, and to favour the processing of APP through the amyloidogenic route [24-26]. A β is considered to be a normal product of cellular metabolism which, when produced in limited amounts, is degraded through proteolytic mechanisms that involve the extracellular enzymes, insulin-degrading enzyme (IDE), neprilysin (NEP) and matrix-metalloproteases (MMP) [27]. However, when the levels of A β increase, in response to BACE1 elevation or in reason of defective clearance, it can exceed a threshold concentration beyond which it self-associates, undergoes a change in conformation, and forms insoluble oligomers that are resistant to degradation [28-30].

The causative role of the amyloid cascade in AD pathogenesis is further exemplified by the rare, familial-inherited forms of AD (FAD), which develop at an earlier age and follow an aggressive course (for reviews, see [31, 32]). FAD patients carry autosomal dominant missense mutations either in the APP gene, which modify the sites of processing by the secretases, or in the presenilin genes, which impact the structure and activity of these catalytic subunits of γ -secretase. Both APP and presenilin FAD mutations alter the processing of APP to promote amyloidogenesis. For instance, the Swedish double point mutation in APP increases cleavage by γ -secretase, hence the rate of A β production [33]. Most of the other APP mutations alter the site of γ -secretase cleavage, leading to formation of longer and more aggregating A β peptides that are not only more toxic, but also harder to clear [34]. Another APP mutation (V715M) decreases A₄₀ production and increases formation of N-terminally truncated, aggregating, A₄₂ peptides [35]. The large majority of AD-causative mutations occur in the presenilins, and these also enhance the production of long A β variants [36]. Conversely, and in further support of the amyloid hypothesis, a very rare APP mutation that was identified in Icelandic families and which favours APP processing through the non-amyloidogenic pathway seems to protect its carriers from developing AD in their old age [37].

Furthermore, other genetic factors in AD can be linked to the amyloid cascade [38]. Such an example is the association of late onset AD with rare mutations in the ADAM10 gene that attenuate γ -secretase function, and thereby augment APP processing by γ -secretase [39]. Polymorphisms in other genes that confer susceptibility for late-onset AD may be also be connected to the amyloid cascade, considering that some of these genes control the trafficking of APP (SORL1, CD2AP) or BACE (BIN1) and APP endocytosis (PICALM), which may alter the colocalisation of APP and BACE; some of the other genes are involved in lipid metabolism (APOE, ABCA7, CLU, PLD3), a cellular factor that affects cellular membrane composition, thus endocytosis and γ -secretase activity; some genes (PRNP, ZCWPW1) mediate homeostasis of metals that are implicated in A β aggregation and in the generation of reactive oxygen radicals that promote BACE1 expression and A β production; another group of AD susceptibility genes are linked to inflammation (TREM2, EPHA1, CLU, CR1), which also triggers A β production, and which mediates the clearance of A β and *tau* aggregates [40-42] (also refer to ref [43])

In summary, there is a large amount of evidence to support the accumulation of A β in selected brain regions, through increased production and aggregation, and/or defective clearance of the amyloid peptide, to underlie the causative mechanism in AD, and to lay the bases for a rational therapeutic intervention.

4. Progress and Challenges of Amyloidcentric Clinical Trials to Date

4.1 Immunotherapy Strategies to Clear A β Deposits and Oligomers

This is in principle the most straightforward approach, however it has encountered considerable difficulties. Immunotherapy has been experimented over the past twenty years, first with the aim of clearing amyloid plaques that were initially believed to cause neurodegeneration, and more recently with the purpose of removing toxic A β oligomers. The immunotherapy approach consists in either challenging the organism with A β , or A β fragments to elicit an immune response that produces antibodies selective for A β (this is called active immunization), or in dispensing specific anti-A β monoclonal antibodies exogenously prepared in the laboratory (this is called passive immunization). Immunotherapy has been so far successful in cancer therapy, with active immunotherapy being used in the development of preventive cancer vaccines, and monoclonal antibodies becoming widely used in cancer treatment. The development of AD immunotherapies has been more challenging than expected and has faced major problems with safety and efficacy.

4.1.1. Active immunisation: Initial experiments, in which the whole A $_{42}$ peptide was injected in an AD transgenic mouse model, supported that this intervention would be safe and effective, achieving protection against amyloid deposition in young mice, and a decrease in brain amyloid burden in older mice which also showed moderate improvement in spatial memory tasks [44-46]. Unfortunately, such promising results did not translate successfully when trialled in humans.

AN 1792: The Elan/Wyeth group reported that the Phase 1 administration of AN 1792 (A $_{42}$ plus QS21 adjuvant) to patients with mild to moderate AD was well tolerated, but that only 53% of patients showed evidence of an immune response. The following Phase 2 trial was terminated because 6% of those given the A $_{42}$ -containing formulation developed meningoencephalitis due to a neuroinflammatory reaction [45]. *Post-mortem* histological examination proved that the immunisation had been successful at reducing A β amyloid plaque in the brain, had improved neurite dystrophy in the hippocampus, and had moderately reduced tau pathology [47]. However, no significant cognitive improvement was reported, probably because the patients selected for the study had already reached a too far advanced stage of the illness, with extensive neuronal loss [48]. A likely reason for the adverse effects of AN 1792 was the toxicity of administered A $_{42}$ peptide that caused an inflammatory reaction.

Substituting A₄₂ for smaller fragments was then tested and established that the N-terminal region of A₄₂, which binds to B-cells, induced a humoral immunity response, whereas the mid and C-terminal regions of A₄₂ were predominantly presented to T-cells and induced an inflammatory response. Further research in A₄₂ vaccine development then moved onto using N-terminal A₄₂ fragments and short peptides that are unlikely to activate T-cells. Novartis, Janssen-Pfizer, Affiris and AC Immune have begun clinical trials with peptides corresponding to the A₄₂ N-terminal region [49].

CAD106: Researchers of the Karolinska Institute (Sweden), with funding support from Novartis, reported data from a Phase I trial on the safety and tolerability of CAD106 (an antigen derived from A₄₂ 1-6), which could produce an antibody response in a cohort of patients (aged 50-80)[50]. Phase 2a studies in patients with mild AD further confirmed that CAD106 vaccination was generally well-tolerated and elicited an immune response in ~64% of patients, with little side-effects, except for one case of cerebral haemorrhage [51]. A Phase 2/3 trial in people “at-risk” of developing AD - which means they carry one or two copies of the apolipoprotein E gene 4 allele (APOE 4)- has just started and will go on for the next five years as part of the Alzheimer Prevention Initiative [52]

ACC-001: After reporting favourable data on the safety and tolerability of ACC-001/PF-05236806 in a Phase 1 trial, Janssen/Pfizer have discontinued their Phase 2 trials in mild to moderate AD participants after the patients showed no improvement in cognitive function and suffered some adverse reactions [53] (<https://trialbulletin.com/lib/entry/ct-00955409>).

AD02 and AD04: Researchers at AFFiRiS have screened peptide libraries to identify novel “affitopes” that elicit the production of antibodies that bind exclusively to A₄₂ N-terminus, are selective for A₄₂ aggregates, and do not bind to APP or other fragments. Preclinical trials of selected AD01 and AD02 antigens have been encouraging since immunized AD mice showed a reduction in amyloid burden and brain neuropathology, and a significant improvement in cognitive tasks [54]. A Phase 2 study of AD02 in patients with early AD has shown a stabilization of their cognitive function after 18 months of treatment. Surprisingly, 47% of patients -mostly at the disease early stage- who were treated for 18 months with the AD04 control antigen, saw their hippocampal volume stabilize, and this was correlated with a stabilisation of cognitive function. Although the mechanism of AD04 remains to be elucidated, this study supports that a disease-modifying therapy capable of halting hippocampal degeneration could stop cognitive decline [55](

Overall, the potential problems associated with A₄₂ active immunisation are the lack of a response in some aged patients who have a defective immune system, and the production of self-antibodies against APP and its soluble fragment sAPP, the functions of which are not completely understood. If the second problem can be resolved, A₄₂ active immunisation might be of value as prevention in asymptomatic patients.

4.1.2. Passive immunisation: Some encouraging preclinical data were first obtained in AD mouse models, which received injections of antibodies produced against A₄₂. Some antibodies crossed successfully the blood brain barrier, but only those that targeted an epitope within the N-terminal region of A₄₂ were able to bind to amyloid plaques and induce their clearance by activating microglia and inducing phagocytosis [56]. The efficacy of other exogenous A₄₂ antibodies that did not reach the CNS was attributed to an alternative mechanism by which they acted as a peripheral sink that depletes A₄₂ from the blood circulation, and thereby increases its transfer from brain to plasma [57]. Monoclonal mAb266 proved to be effective at ameliorating cognitive impairments in AD mice without decreasing brain amyloid plaque burden, suggesting it worked by redistributing the soluble toxic A₄₂ oligomers from the CNS into the blood and CSF [58]. A dozen of clinical trials of passive immunisation treatment have been registered, which are summarized below [49].

Bapineuzumab: Janssen/Pfizer antibody *Bapineuzumab* (also called 3D6) is a humanized monoclonal antibody targeting A_β 1-5, which was initially developed by Elan/Wyeth to target amyloid plaques. It has unfortunately failed to meet its clinical endpoints in human trials. In a phase 2 multicentre study, a group of patients with mild to moderate AD who were treated for 18 months showed no cognitive improvement, although the study remained inconclusive because of its lack of statistical power. Vasogenic oedema (water accumulation in the brain) was observed in 10% of treated patients and was more frequent in APOE 4 carriers and in subjects being given the higher doses of antibody, possibly due to the removal of vascular amyloid [59]. PET imaging of A indicated that *Bapineuzumab* removed cortical fibrillar amyloid from the brain of treated patients [60] and CSF analysis showed a decrease of *tau* and *phospho tau*, two key signature markers in AD [61]. Two Phase 3 trials in patients with mild to moderate AD, one study with over one thousand asymptomatic APOE 4 carriers and the other with one thousand non APOE 4 carriers, showed no clinical improvement in either group, in spite of changes in CSF biomarkers in the cohort of APOE 4 carriers [62]. 0.9% of patients who received the antibody treatment developed neoplasia, with a fatal outcome. Brain image analysis showed that the drug had hit its target and decreased fibrillary amyloid, and thus it might be beneficial as prevention in asymptomatic patients [63]. A study at Novartis with two models of AD transgenic mice has shown that mAb3D6, a murine equivalent of bapineuzumab, can clear amyloid pathology, but cannot rescue cognitive deficits, and even aggravates neuronal dysfunction [64]. The mechanism is unclear, but electrophysiology indicates neuronal hyperactivity and unusual synchrony, which may be caused by cross-reactivity with APP and other APP-derived fragments.

Solanezumab: Monoclonal antibody *Solanezumab* (LY2062430 from Eli Lilly has provided more promising results and remains in clinical trials. It is directed to the mid region of A_β and targets the soluble monomer. Early clinical trials have shown that it is usually well tolerated, although most treated patients complained from headaches. A 12-weeks Phase 2 study in patients with AD has shown an increase of A_β₄₂ in the CSF, suggesting that this antibody depletes peripheral A_β and displaces A_β associated with amyloid plaques [65]. Two 80-weeks Phase 3 trials (EXPEDITION1 and EXPEDITION2) in mild to moderate AD have failed to meet their cognitive and functional endpoints [66]. A first partial analysis of the data suggested a greater cognitive decline in the patients given the drug compared to those treated with the placebo, leading to interrupting the trials. Also 0.9 % of the patients who received *Solanezumab* experienced side effects, such as brain oedema and microhemorrhages [66]. Follow-up analysis of the clinical outcomes has now established some slight, but significant benefits of *Solanezumab* treatment in patients with mild cognitive impairment, and found that these benefits increase over time [67]. Lilly has thus extended to 3.5 years its EXPEDITION3 trial that involves about 2000 patients with mild AD [68]. Further trials also include asymptomatic “at-risk” patients as part of the Anti-Amyloid Treatment for Asymptomatic Alzheimer’s Disease (A4) and the Dominantly Inherited Alzheimer Network (DIAN) initiatives.

Cremazumab: This humanized monoclonal IgG4 antibody, developed by AC Immune/ Genentech/ Roche, targets aggregated A_β, including oligomeric and fibrillar species and amyloid plaques and has been selected for not inducing microglia activation [69]. After successful Phase 1 trials, a Phase 2 multidose trial in mild to moderate AD patients for 68 weeks has failed to meet its clinical endpoints, but has shown a trend for a modest cognitive improvement in the patients with mild AD who received the highest dose of the drug. Two other Phase 2 studies remain ongoing, and *Cremazumab* is also being tested in preventive trials (Alzheimer Prevention Initiative) in people ‘at risk’ (APOEε4 homozygotes) and in carriers of the E280A PS1 FAD mutation in a Colombian kindred [49].

Gantenerumab: Roche/Genentech has conducted trials of *Gantenerumab* (RO4909832), an IgG1 against A β N-terminus that specifically targets A β fibrils. Results showed efficacy at reducing amyloid burden in the brain, but also serious adverse effects such as inflammation or vasogenic edema in some patients [70]. At the end of 2014, Roche has announced the termination a Phase 3 trial of *Gantenerumab* in patients with prodromal AD (SCarlet RoAD trial), although biological changes indicated that the drug had hit its target and was able to decrease brain amyloid in a number of patients [71].

Aducanumab: Biogen is conducting trials of *Aducanumab* (BIIB037), a human IgG1 antibody that was identified by screening natural human antibodies from healthy aged donors, and which targets a conformational epitope of A β . Data from a Phase 1b study in patients with prodromal and mild AD (PRIME) showed that injections of this antibody could reduce amyloid burden at 26 weeks, and even further at 54 weeks, with a slower rate of cognitive decline compared to patients receiving a placebo (Data presented at AD/PD 2015 [72]). Phase 3 trials are being carried out in 150 centres worldwide, and are recruiting patients with prodromal and mild AD.

In summary, after many disappointments, mostly due to the selection of patients at a far too-advanced stage of illness for clinical trials, passive immunisation is starting to offer better promise, and results of preventive treatments are awaited with interest. Janssen/Pfizer (AAB-003), Lilly (N3pG-A) and Astra-Zeneca (MEDI814) have also begun Phase 1 trials of their anti-A β in patients with mild to moderate AD. However, the cost of the treatment and the frequency of reported serious adverse effects might limit the clinical applications of passive A β immunotherapy in AD.

4.2. Targeting γ -Secretase to Prevent or Modulate A β Formation

Targeting the secretases has been considered as a primary choice of intervention because proteolytic enzymes can be inhibited by small molecules that can translate into cost-effective orally-available drugs. Successes of the antiretroviral medications targeting the HIV protease, and of the inhibitors of circulating enzymes such as renin and kallikrein have demonstrated the feasibility of this therapeutic approach.

4.2.1. Gamma-Secretase Inhibitors (GSI): GSI were first developed without knowledge of the identity of the target enzyme. Biochemical evidence supported that the enzyme responsible for the γ -secretase cleavage of APP was an aspartyl protease [73, 74], spurring the design of transition-state analogues of this class of enzymes based on the sequence of the APP cleavage site. Hydroxyisosteres, such as L-685,458 [75], difluoroketones, such as DFK-167 [76], hydroxyether ureas, such as WPE-III31C [77], and pepstatin derivatives that contain a statinoic acid isostere [78] were shown to inhibit γ -secretase activity at low or sub micromolar concentrations, *in vitro* and in cellular assays. Demonstration that these inhibitors interacted with key aspartate residues within transmembrane domains of the presenilins established the function of these proteins as novel intramembranous aspartyl proteases, and provided more substantial bases for rational drug design [79-82]. High-throughput screen of non-targeted drug libraries to identify compounds capable of inhibiting A β production from cellular models provided other classes of GSI. Following this approach, ELAN discovered a hit dipeptide derivative that was further refined into the lead compound DAPT [83], and Dupont (now Bristol Myers Squibb) identified the benzodiazepine derivative, Compound E [82]. Both of these compounds work through an allosteric mechanism and do not directly engage with the γ -secretase active site aspartates. Because such compounds were discovered through cellular screen, they had already passed the test of membrane permeability and had the advantage of a better bioavailability over the substrate analogues. DAPT was the first GSI reported to be orally active and to lower brain A β levels in an AD mouse model [83].

Parallel studies with neuronal cultures derived from PS1 knockout mouse embryos revealed that γ -secretase activity was required to mediate Notch signalling during brain development, through the release of the Notch intracellular domain (NICD) that signals gene transcription [84]. Notch signaling controls cell-cell interactions, cell differentiation, and cell-fate decision in the brain and in many body tissues, not only during development but also throughout life [85]. Therefore, the future of γ -secretase inhibitor development in AD therapy appeared to be precarious, although it was argued that Notch signalling might not play an essential role in aging adults. However, serious side-effects of the DAPT-derived LY-411,575 were observed in mice treated with the compound for 15 days, as the mice showed decreased maturation of peripheral B cells and altered intestinal tissue morphology caused by inhibition of Notch signaling [86]. From then on, research focussed on finding “Notch-sparing” GSIs.

Semagacestat: Researchers at Lilly produced the carboxamide-containing GSI, LY-350139 (*Semagacestat*) with marginal selectivity for APP against Notch. Acute dosing and short treatments with this compound lowered A β in the plasma, CSF and brain of animal models, and in the plasma and CSF of humans [87, 88]. Unfortunately, a Phase 2 trial in patients with mild or moderate AD did not lower A β levels in CSF, but exposed some side-effects of the drug consisting of skin lesions and intestinal toxicity [89]. A Phase 3 trial in AD patients that was initially planned to last for 21 months was abruptly terminated because cognitive decline worsened in the group treated with *Semagacestat* compared to the group given a placebo. In addition, serious adverse reactions were observed, including gastrointestinal bleeding, skin cancers, and decreased resistance to infection due to immunosuppression, all of which were incumbent to defective peripheral Notch processing.

Avagacestat: Bristol Myers Squibb developed BMS-708163 (*Avagacestat*), a carboxamide-substituted sulfonamide with nearly 200-fold selectivity for APP over Notch, and which proved to be effective at lowering A β in brain, plasma and CSF of rats and dogs [90] and in the plasma and CSF of humans [91]. Phase 1 safety trials in different age groups, including patients with mild cognitive impairment (MCI), indicated that the drug was safe and capable of decreasing A β levels in plasma [92, 93]. A 24-week Phase 2 trial in patients with mild to moderate AD showed that the highest doses of *Avagacestat* worsened the patients’ cognitive decline, and caused a range of adverse secondary effects [94]. Trials in AD were thus terminated. A multicentre Phase 2 trial conducted in patients with MCI and who tested positive for CSF AD biomarkers, showed no benefit of the drug as the patients receiving *Avagacestat* progressed to dementia at the same rate as those given a placebo, with no significant effect on brain atrophy, PET amyloid imaging, or CSF biomarkers [95]. Gastrointestinal, cutaneous, and reversible renal adverse effects (probably related to Notch inhibition) were more frequently observed in patients treated with the drug.

Begacestat: The thiophene sulfonamide derivative, GSI-953 (*Begacestat*), which has been developed by Wyeth, has encountered similar problems. After performing favorably in preclinical studies where AD transgenic mice showed reduced A β levels in the brain, plasma, and CSF, and improved behaviour in contextual fear-conditioning tests, the drug entered clinical trials. However, Phase I results showed no significant effect on CSF A β levels and led to discontinuing human trials because the initial reduction in plasma level of A β s followed with an increase [96]. Determining the right dosage of the drug may be critical because some GSI show a biphasic curve and increase A β formation at low concentrations [97].

Taken together, trials of GSI in humans have shown no benefit on cognitive function and have demonstrated a range of adverse effects caused by inhibition of Notch signalling.

4.2.2. Gamma-Secretase Modulators (GSM): The evidence of the toxicity of drugs that block the activity of γ -secretase called for a new approach to targeting γ -secretase. Novel classes of molecules termed GSM have been

developed that can modulate the enzyme's activity, alter its kinetics through an allosteric mechanism, and enhance the production of shorter, less aggregating A_β peptides, whilst not compromising the release of the Notch NICD fragment or the cleavage of other γ -secretase substrates. γ -Secretase is a multimolecular complex that behaves like a proteolytic machinery and digests receptors' transmembrane domains by a series of cuts. A first endoproteolytic cleavage on the cytosolic side of the membrane facilitates the release of the transcriptionnally-active cytosolic domains of receptors [20]. Then, serial cleavages by a carboxyl-peptidase-like activity progress along one side of the membrane domain α -helix, beginning on its C-terminal end and cutting at every third amino-acid residue [21]. Its cleavage of APP creates A_β fragments of 49, 46, 43 and 40 amino acids, with only the shorter ones being released from the membrane. FAD mutations in APP impair the first cleavage and cause it to shift, resulting in the production of A_β fragments of 48, 45, 42 amino acids. The FAD presenilin mutations also modify γ -secretase by decreasing the rate of the enzyme's kinetics, and cause processing termination at A₄₃ or A₄₂ [98]. In contrast, some modulators can accelerate the carboxypeptidase reaction, leading to the formation of shorter A_β peptides, A₃₈ and A₃₇ [99].

NSAID and Tarenflurbil: The first class of molecules that were discovered to modulate the γ -secretase cleavage of APP *in vitro* is the non-steroidal anti-inflammatory drugs (NSAID). Ibuprofen, indomethacin, and sulindac sulphide were found to inhibit production of A₄₂ in cellular and transgenic mouse models, with no effect on Notch processing [100]. *Tarenflurbil*, a flurbiprofen enantiomer that does not affect cyclo-oxygenase I (COX-1) activity, was tested for 6-months in patients with mild AD, but provided no benefit on cognitive decline or daily living activities, probably due to its very low binding affinity [101].

CHF5074: The Tarenflurbil-analogue produced by CHIESI, *CHF5074* has shown promise so far. It can decrease brain amyloid plaque burden and A_β levels, and also rescue cognitive deficits in AD transgenic mice. Phase 1 clinical trials showed it to be safe and well-tolerated upon a single administration, but a Phase 2 study has revealed some adverse effects upon 40-week treatment in the patients who received high doses of the compound, with rare cases of worsened cognitive decline and, more commonly gastro-intestinal upset. Some patients, who tolerated the drug and reached 64-weeks of treatment, showed a significant improvement, with a lesser cognitive decline compared to those given a placebo. This progress was particularly evident in APOEε4 carriers. CSF analysis indicated a decrease in the neuroinflammation markers, TNF- α and sCD40 after 12 weeks of dosing, supporting the multi-target mechanism of the drug, and *CHF5074* has since been considered to be primarily a microglial modulator rather than a GSM (Alzheimer's Association International Conference (AAIC) 2013. Oral Presentation: O3-06-5. Presented July 14, 2013). The drug has now been licensed to CereSpir Inc. and has progressed into Phase 3.

The search for GSM remains active, and the new information recently obtained about the structure of γ -secretase will assist in designing specific GSM [102, 103]. Novel proteins found to interact with C99, or presenilin, and which can modify the processing of APP by γ -secretase, such as TMP 21 [104], the transient receptor potential canonical, TRPC 6 [105], and the γ -secretase activating protein (GSAP) [106] may also open new therapeutic avenues, although the effect of the latest on γ -secretase activity and A_β production remains controversial [107]. However, targeting γ -secretase remains a difficult task because the concept of intramembrane proteolysis is rather novel, and the mechanism of γ -secretase that combines endoproteolytic and carboxypeptidase activities is poorly understood. In addition, the enzymatic activity is contained within a complex comprised of at least four subunits, and the expression of subunit homologues, alternative splice variants, and post-translational modifications can potentially generate a diversity of proteolytic complexes with

different kinetics and substrate preferences [108]. Therefore, further basic research in the biology of γ -secretase is needed to advance the design of specific inhibitors and modulators.

4.3. BACE Inhibition as a Strategy to Prevent and Control A β Formation

Considering the challenges encountered with γ -secretase inhibition, most of the field has shifted focus to β -secretase inhibitors. BACE1 was identified over fifteen years ago as the gene responsible for β -secretase activity [109]. Ablation of the BACE1 gene in mice did not cause any major defect in embryonic or early-life development, and the mice reached adulthood with no anatomical abnormalities or overt phenotype [110, 111]. This encouraged the rapid development of BACE1 inhibitors. Reports that BACE1 levels were increased in the brains of AD patients further prompted intensive research into the therapeutic application of BACE1 inhibitors [112-115]. BACE1 is the founder of a new subclass of membrane-tethered aspartyl-proteases, with sequence homology to BACE2, renin, and cathepsin D. The three-dimensional structure of BACE1 catalytic domain was determined to facilitate the design of inhibitors [116]. It revealed an extended catalytic site that posed a challenge for designing high-affinity inhibitors that would be small enough to penetrate through cellular membranes and to cross the blood brain barrier. In addition, the close homology of BACE1 with BACE2 further has complicated the design of specific BACE1 inhibitors.

Indeed, BACE2 shares 45% sequence identity and 75% homology with BACE1, including a close structural similarity in the active site region [117-120]. However, the two enzymes differ by their cleavage specificity. BACE2 was ruled out as a β -secretase candidate because it cleaves APP at an alternative site, close to the cleavage site of γ -secretase.[117, 121, 122]. The patterns of expression of BACE1 and BACE2 also differ considerably, with BACE1 being principally expressed in the brain where little or no BACE2 is detectable [123, 124]. The *BACE2* knockout mice display an increased mass of pancreatic β -cell, and alterations in coat colour that are consistent with hypopigmentation. Physiological and biochemical examinations of these mice have revealed that BACE2 controls the proliferation and function of β -cells in the pancreas by cleaving the type I transmembrane protein TMEM27 [125]. The *BACE2* knockout mice show increased insulin levels and improved glucose metabolism, which support further investigations to determine if BACE2 inhibition could be beneficial to patients with type 2 diabetes. BACE2 also plays a role in the regulation of melanosomes by cleaving the premelanosome protein PMEL [126], thus BACE2 inhibition may increase photosensitivity in treated patients. Other substrates for BACE2 have also been uncovered by proteomic analysis [127]. Most inhibitors under current development inhibit both BACE1 and BACE2, therefore the potential secondary effects of BACE2 inhibition will have to be taken into consideration.

Hundreds of compounds that potently inhibit BACE1 *in vitro* have been described, and we have reviewed them previously [128, 129]. Here, we will only summarize key information and the latest progress in the development of BACE1 inhibitors.

4.3.1. Peptidomimetics: The initial approach to designing BACE1 inhibitors was to synthesize transition-state analogues based on the APP sequence, where the peptide bound at the cleavage site is replaced with a non-cleavable isostere such hydroxyethylene, hydroxyethylamine, or statine/norstatine groups, and then improve their pharmacological properties by chemical substitution and structure refinement, according to a strategy that has been successful in producing efficacious inhibitors for other aspartyl proteases [130]. This approach produced large and polar molecules, with a high binding affinity for BACE1, but unfavourable pharmacological properties and poor brain penetration. Another hurdle has been that some of the substrate and peptidomimetics that were able to pass across cellular membranes and to reach their target faced the problem of efflux by the P-

glycoproteins (PgP) that control the blood-brain barrier. These compounds had to be administered together with a PgP inhibitor to demonstrate their efficacy in reducing A β formation and amyloid deposition in the brain in animal studies [116, 131-133]. Thus these were unlikely to be applied in the clinic.

CTS-21166: The hydroxytethylene isostere substrate analogue CTS-21166/ASP1702 that was developed by Ghosh and colleagues, proved to potently inhibit BACE1 *in vitro* with a $K_i = 1$ nM, and was among the first BACE1 inhibitors to be successfully trialled in AD transgenic mice [134]. Intravenous injections in Tg2576 mice decreased A β levels by 70% in plasma and by 55% in the brain (Table 1). Further preclinical trials demonstrated brain penetration in non-transgenic animals, including mice, rats, dogs and monkeys, and showed that single and multiple doses of the compound given to rats could lower A β in the brain and CSF by up to 50% [135]. A Phase 1 clinical trial, in which CTS 21166 was given intravenously to healthy male volunteers at doses up to 225 mg, proved that the drug could decrease A β levels in plasma in a dose-dependent manner, with an effect lasting for up to 72 h. A second Phase 1 study, where participants received an oral liquid solution of the compound, consolidated these results. These Phase 1 trials proved that CTS 21166 was well-tolerated and attested for the first time that a BACE1 inhibitor could lower A β production in human, although this was only demonstrated in plasma [130]. There is no other trial of this drug currently registered in the USA. The group of Ghosh has also produced GRL-8234, a hydroxyethylamine peptidomimetic with a high-affinity for BACE1, good selectivity, and blood brain barrier permeability, and showed it could lower A β levels in the brain of AD transgenic mice [136]. Chronic administration of GRL-8234 for 6 months produced exciting results as, not only this could decrease brain amyloid burden in treated animals, but it could also rescue cognitive deficits. This provided the first proof-of-concept that BACE1 inhibition could ameliorate cognitive performance in an AD model, and has fostered the further development of BACE1 inhibitors.

4.3.2. Small molecules and fragment-based BACE1 inhibitors:

As an alternative approach to substrate analogues, pharmaceutical companies have screened libraries of small molecules, either by virtual simulation -where the chemical entities are tested for docking into a model of the BACE1 catalytic site-, or by using high-throughput *in vitro* assays to identify new warheads capable of altering BACE1 enzymatic activity. The hits obtained from these screens provided lead compounds that were further refined to improve potency, specificity, and to demarcate each company's intellectual property space (for a review see [137]). This approach had the advantage of providing small molecules, with better bioavailability than the peptidomimetics, but also had the disadvantage of producing inhibitors that might block only part of the BACE active site, thus lack specificity for BACE1 relative to BACE2 or to the ubiquitous lysosomal protease cathepsin D. These disadvantages were the key factors in discontinuing the clinical trials of some BACE1 inhibitors.

LY2811376: Eli Lilly and Co designed the cyclic isothiourea derivative LY2811376 that provided very encouraging results in animal studies. Testing in AD mice and beagle dogs showed dose-dependent reductions in plasma and CSF of A β peptides and of the APP-derived products produced by BACE1 cleavage, C99, and sAPP [138]. Results from a Phase I clinical trial in healthy volunteers indicated that LY2811376 could lower CSF A β by up to 55%, a finding that was for the first time observed in humans and that provided the evidence that the drug had engaged with its target. However, the clinical trials of LY2811376 were terminated as a preventative measure when retinal toxicity was observed in parallel toxicological studies in animals. Rats chronically treated for three months with LY2811376 developed an accumulation of lipofuscin that led to cellular degeneration in the retinal epithelium, and to a lesser extent, in the brain neurons and glia [138]. Lipofuscin consists of

autofluorescent granule pigments that build up with aging in the lysosomes. Because a similar phenomenon was also observed in the BACE1 knockout mice, it was concluded that *LY2811376* toxicity was caused by the lack of specificity of the drug that also inhibits cathepsin D.

LY2886721: Lilly next-generation small molecule BACE inhibitor *LY2886721* appeared to be more potent and more specific for BACE1 in preclinical testing [139]. Four Phase 1 studies were carried out in healthy volunteers and one in AD patients to test the safety and efficacy of ascending doses of the drug (NCT01133405, NCT01227252, NCT01367262, NCT01534273, NCT01775904, NCT01807026). These indicated that the drug was well-tolerated and capable of decreasing levels of A₄₀, A₄₂, and sAPP in CSF by up to 74%. The Phase 2 study NCT01561430 was set up to trial the drug in MCI patients, but this had to be discontinued because of liver toxicity that was attributed to an off-target effect of the drug [140].

AZD3839: Astra-Zeneca reported promising preclinical data of the efficacy and *in vivo* performance of the aminoisoindole derivative, *AZD3839* [141]. *In vitro* studies established the selectivity of the compound to be three-order of magnitude greater for BACE1 compared to cathepsin D, and 14-fold versus BACE2. Oral dosing of *AZD3839* in AD transgenic mice, in guinea pigs, and in monkeys demonstrated dose-dependent decreases in the levels of A in brain, CSF, and plasma. The safety and pharmacological properties of the drug were tested in a Phase I trial in 54 healthy volunteers (NCT01348737)[142]. A dose-dependent decrease was observed in the levels of plasma A₄₀ and A₄₂, which reached a maximum effect of 55% in those given the highest dose of 300 mg. These data are consistent with findings in animal studies. However, a concern was raised regarding the safety of the drug because one patient experienced a moderate adverse event, three reported events of presyncope, and others reported milder effects, including dizziness and headaches. Cardiac electrophysiology examination indicated that the drug caused arrhythmia, with a dose-dependent prolongation of the QT interval [143]. This long QT syndrome adverse effect is attributed to the inhibition of potassium ion channel activity. With the consideration that doses of *AZD383* that do not disrupt the heart electrical activity can only marginally decrease plasma A β levels, the clinical trials of *AZD3839* were terminated.

BI-1147560/VTP37948: Boehringer-Ingelheim has collaborated with Vitae Pharmaceuticals to test VTP37948 in Phase 1 trials. The drug had given hopes from preclinical testing. Initial Phase 1 trials in healthy volunteers gave promising results, with a single dose treatment decreasing A β levels in CSF by 80% [144]. (Further Phase I trials that involved 10-day multiple dosing in healthy young and aged volunteers in Germany and in Belgium (NCT02254161) were placed on hold in the first quarter of 2015 in reason of skin reactions in some of the study participants. Another Phase 1 and Phase 2/3 trials have now been terminated.

NB-360 Novartis has also several BACE inhibitors in their pipeline. The cyclic sulfoxide hydroxyethylamine derivative, NB-04 proved to be a potent BACE inhibitor *in vitro*, and capable of reducing A levels in the brain of AD mice and in the CSF of beagle dogs [145]. It could not be advanced into clinical trials because of P-gP efflux issues. Based on these results and on the failures of other BACE inhibitors, Novartis have produced NB-360, a new-generation BACE inhibitor with an amino-1, 4-oxazine core that provided enhanced pharmacological properties, and resulted in improved potency, selectivity, and brain penetration [146]. NB-360 pharmacokinetics is also favourable, as it does not bind to plasma proteins and it is slowly cleared from the blood, as demonstrated in several animal models, including dogs. Preclinical safety and toxicity studies are ongoing.

5. BACE Inhibitors in Clinical Development

5.1. Verubecestat

The joint efforts and expertise of the chemists and biologists at Merck/Schering-Plough have led to the discovery of the potent BACE inhibitor SCH-900931/MK-8931, recently renamed **verubecestat**. The chemists used a fragment-based discovery and a fragment combination approach to produce a series of iminothiadiazine dioxide derivatives capable of binding to the BACE1 active site. The compounds were validated as BACE1 inhibitors in an APP substrate cleavage assay, and *verubecestat* was further selected based on its ability to reduce A β levels in the CSF of AD transgenic mice. It is a potent inhibitor of both BACE1 and BACE2, with a K_i for BACE1 equal to 1.75 nM, and a K_i for BACE2 equal to 0.37 nM (Patent US8729071 B2, compound 25). Chronic oral administration of *verubecestat* to APP transgenic TgCRND8 mice prior to amyloid plaque deposition resulted in a considerable reduction of A β accumulation in the brain compared to mice treated with vehicle only [147]. The patent covering the compound also states that A β levels dropped by 49% in the brain cortex of rats at 3 h after receiving a dose of 10 mg/kg of the drug. Preclinical characterization of the drug has recently been reported, including its dose-dependent reduction in levels of A β in plasma, CSF and brain of rats and cynomolgus monkeys [148]. An oral dose of 10 mg/kg caused a drop by 80% of CSF A β_{40} at 12 h, which lasted for up to 24 h. A 72% decrease of A β was observed in the brain cortex at 4 h after dosing. The potential toxicity of the drug has also been thoroughly investigated and it is predicted to be low because *verubecestat* does not activate cytochrome P450, a major inducer of liver toxicity, and it is a weak inhibitor of hERG, which controls the electrical activity of the heart.

The drug has been tested in clinical trials for the past 4 years, and has now moved to Phase 3. Impressive data have been reported for several Phase 1 studies. A two-part Phase 1 trial that was conducted in 88 healthy volunteers, aged 18-45 proved that *verubecestat* was well tolerated and produced a profound and lasting decrease of A β in the CSF [149, 150]. In a randomized, double-blind, placebo-controlled, single ascending dose study, a dose-dependent decrease in levels of A β_{40} in the CSF was observed at 36 h after administration, with a 21% reduction in those given a 20 mg dose, 75% reduction in those given a 100 mg dose, and 92% reduction who received 550 mg of the drug. In a multiple ascending dose study, the participants were administered daily doses of the drug for 14 days. CSFA β_{40} levels, which were measured at the end of the study and compared to the baseline, were reduced by as much as 32% for the 10 mg dose, 91 % for the 150 mg dose, and 94% for the 250 mg dose. A Phase 1b clinical trial was also conducted to test the safety, tolerability and pharmacodynamics of the drug in AD participants (NCT01496170) [151]. Thirty patients, aged 50-85, with a history of cognitive decline and who were classified as mild to moderate AD, were administered either 12 mg, 40 mg, or 60 mg daily doses of *verubecestat* or a placebo for 7 days. Dose-dependent, sustained reductions in A β_{40} and A β_{42} , and sAPP β in CSF by up to near 90% were observed at 36 h after the last dose. The data were consistent between AD patients and healthy controls, suggesting that brain amyloid load does not impair the drug efficacy [152]. A study carried out in Japan corroborated the data obtained in the USA, and showed a sustained 88% reduction in baseline level of CSFA β_{40} at 24 h after oral administration of a 450 mg single dose of *verubecestat* [153].

The challenge remains now to demonstrate the cognitive benefits of the drug in MCI, mild AD, and “at-risk” patients. Merck has now registered two Phase 2/3 clinical trials. The EPOCH study began in 2012 with a Phase 2 trial in 400 patients with mild to moderate AD, who received a daily oral dose of 12 mg, 40 mg, or 60 mg of *verubecestat*, or a placebo, to assess the safety and efficacy of the drug over an 18 month treatment period (NCT01739348). There has been no report of adverse reactions so far, but the highest dose has been dropped to 40 mg in the extension of the study that is being continued for up to 5 years. The EPOCH Phase 3 has also

begun, which involves 1560 additional participants who are dosed daily with 12 or 40 mg of the drug. The primary outcomes of the EPOCH study will be the observation at 18 months of a change from baseline in psychometric tests that measure cognitive function (ADAS-Cog score) and performance in daily activities (ADCS-ADL score). If the drug proves to be beneficial, the trial will be extended to 5 years. Secondary outcomes will also be measured, which include the Clinical Dementia Rating Sum of Boxes (CDR-SB) score to obtain a precise rating of dementia stage, mini-mental score examination (MMSE), changes in hippocampal volume determined by MRI, changes in CSF tau, and changes in brain amyloid load determined by PET imaging. The final data will be collected mid 2017 and the study is expected to be completed by July 2019. Another Phase 3 study, called APECS, is still recruiting. This aims to test daily treatment with 12 mg or 40 mg of *verubecestat* in 1500 participants with prodromal AD, for an initial period of 2 years that could be extended to additional 5 years (NCT01953601). The participants should be aged 50-85, have a history of progressive memory decline, and test positive for AD PET biomarkers, but not meet the criteria for dementia. The outcomes of this study are the same as those of the EPOCH trial. APECS is due to be completed in March 2021, and since it has started in 2013, interim 24-month data may become available very soon.

In summary, *verubecestat* is the most advanced BACE1 inhibitor in clinical trials today. Its high potency and its selectivity for BACE1 against cathepsin D limit off-target effects. Preclinical trials, as well as Phase 1 and 2, have met their objectives. The design of Phase 2/3 trials, involving patients with moderate and mild cognitive impairment, supported by thorough clinical investigations and by state-of-the-art methodologies to monitor biomarkers of the disease progression, represents the best possible set up to achieve a positive outcome. Results of these trials will provide the ultimate test of the effectiveness of BACE1 inhibitors in AD therapy. The equal specificity of *verubecestat* for BACE1 and BACE2, and the diversity of BACE1 substrates remain potential sources of mechanism-based toxicity, especially during prolonged treatment.

5.2. Other promising BACE inhibitors

Merck has been leading the field of BACE inhibitors for the last five years, however three other companies are currently determined to catch up and are expediting the recruitment of participants for Phase 2 and Phase 3 trials of their proprietary compounds. The timeline for completion of these trials are compared to those of *verubecestat* in Figure 2. The three following drugs are the direct competitors of *verubecestat*.

AZD3293: Astra-Zeneca is now testing *AZD3293/LY-3314814* in human trials in collaboration with Lilly. This double-spiro amino imidazole derivative, which was designed from a fragment-based screening approach, is an orally available and brain penetrant BACE inhibitor with a high potency (*in vitro* IC₅₀ = 0.2 nM). It has a 25,000-fold selectivity for BACE against cathepsin D, but it inhibits BACE2 and BACE1 equally. It has been proved efficacious at inhibiting A₄₀ secretion in various neuronal systems. Experiments in animals have demonstrated dose dependent decreases of A₄₀, A₄₂, and sAPP levels in the brain and plasma of AD mice, and in the brain, plasma, and CSF of guinea pigs and dogs [154, 155].

Phase 1 safety and efficacy clinical trials began in late 2012, with 72 young healthy volunteers being administered 1 to 1,000 mg single doses of the drug (NCT01739647). Other Phase I trials were carried out to assess the safety and pharmacodynamics of the drug. Trials in the US (NCT01795339) and in Japan (NCT02005211), in which healthy participants and AD patients were given single (15, 50 or 150 mg) or multiple doses (15 mg or 50 mg) of the compound or placebo to evaluate tolerability and effects on plasma and CSF biomarkers indicated that the drug caused no serious adverse effect. A strong, dose-dependent reduction of CSF

A β levels was observed in both healthy volunteers and AD patients [156, 157]. In the study carried out in Japan with young and elderly volunteers, a single 50 mg dose of *AZD3293* could lower plasma levels of both A β_{40} and A β_{42} by ~75%. Daily dosing for 14 days resulted in similar >75% decreases of A β_{40} and A β_{42} in plasma and CSF [158]. A Phase 2/3 trial has begun in December 2014, which is due to end in May 2019 and to be fully completed by 2021 (NCT02245737). This is a 24-month multicentre, randomized, double-blind study in patients with early Alzheimer's disease (AMARANTH study) that is expected to involve 2202 participants over fifteen countries. The primary measured outcome will be a change from baseline in the Clinical Dementia Rating (CDR) score observed at 24 months. The patients are also being assessed for changes in psychometric tests that consist of cognitive score (ADAS-COG) and functional activities in daily living (ADCS-ADL), and for changes in brain volume identified by MRI. A series of AD biomarkers will be measured, including CSF levels of A β_{42} , A β_{40} , total Tau, and phospho-Tau, as well as brain PET imaging of amyloid accumulation and glucose metabolism.

E2609: This small oral BACE inhibitor developed by Eisai Co Ltd was reported to decrease A β levels in the plasma, brain, and CSF of animal models, including rats, guinea pigs, and rhesus monkeys [159]. Biogen is conducting the clinical development of the drug. Phases 1 trials have shown that the drug is well tolerated, has a prolonged half-life, and can effectively lower A β levels in plasma by up to 90% [160] and in CSF by 71% after 100 mg daily dosing for 14 days [161]. The drug was proved to engage with its target since it was also found to decrease BACE1 enzymatic activity in the CSF by up to 99%, in a dose-correlated manner, without changing the levels of BACE1 protein [162]. Eight Phases 1 clinical trials have been completed in Japan and in the USA, involving elderly healthy participants, and patients with MCI and moderate AD (among which NCT02207790, NCT01294540, NCT01511783, NCT01600859). A Phase 2 placebo-controlled, double-blind, randomized, proof-of-concept study is currently being conducted in the USA, that involves 700 patients, including MCI and mild to moderate AD. The primary outcome of the study is to establish the safety and tolerability of the drug given as a daily dose regimen, and to evaluate its cognitive benefits in MCI patients after 18 months of treatment, as defined from changes from baseline in the derived Alzheimer's Disease Composite Score (NCT02322021). Other data to be recorded are 1) changes in hippocampal atrophy at 12 and 18 month; 2) changes in CSF A β levels at 4 weeks and 18 months; 3) changes in Alzheimer's Disease Composite Score in patients with mild to moderate AD at 18 months. The study is due to be completed by January 2018.

JNJ-54861911: Data recently reported by Janssen/Johnson & Johnson indicate that they are also at the forefront of the competition to bring a BACE inhibitor in the clinic. They have registered fourteen clinical trials for the compound *JNJ-54861911*. Phase 1 trials in a group of healthy participants aged 55 to 85 have been recently reported [163]. A single dose of the compound efficiently decreased A β levels in a dose-dependent manner, with a maximum effect being recorded in plasma at 3-4 h and in CSF at 6-8 h. Up to 95% reductions in CSF A β levels were observed after 14 days of daily dosing. sAPP β was also decreased, with a concomitant 2-3 fold increase in sAPP α . Janssen has just completed a study in Europe and is finishing a study in Japan, both of which involve participants with MCI who received 10 mg or 50 mg of the drug, or a placebo, daily for 4 weeks in order to investigate the outcomes of the treatment on the products of APP processing in plasma and CSF (NCT01978548 and NCT02360657). They are also carrying on a multicentre study to test the safety and tolerability of the drug in 100 patients with early AD who are receiving a daily dose of 10 or 50 mg of *JNJ-54861911* for 10 months (NCT02260674). The efficacy of the drug on the levels of A β and APP fragments in

plasma and CSF is also being analysed at 1 and 6 months. This study has already been extended for twelve months (NCT02406027). Janssen has also begun Phase 2/3 studies in Europe, USA, Canada, Mexico, and in Australia, with focus being placed on prevention and early intervention. A 54-month study has started with patients aged 60-85, classified as at risk of developing AD because either they have a family history for dementia (first degree relative), or they are APOE ϵ 4 allele carriers, or they have tested positive for A β accumulation in the brain by CSF analysis or PET imaging (NCT02569398). The participants are given a daily oral dose of *JNJ-54861911* (either 5 mg or 25 mg) or a placebo. The primary outcome measurement will be the finding of a change from baseline in cognitive assessment tests at the end of the study. A battery of psychometric and psychological tests will be performed, and the patients will be carefully monitored for adverse reactions. Changes in AD imaging biomarkers will be recorded.

5.3. Potential problems associated with BACE inhibition

Although the first reports on BACE1 knockout mice stated that these animals had no overt phenotypes, further examination has revealed subtle abnormalities in behaviour, physiology, histochemistry, and electrophysiology. An increased rate of perinatal mortality, a smaller adult size, an hyperactive behaviour, and some alterations in the inactivation of voltage-gated sodium channels were recorded [164]. Furthermore, the evidence of hypomyelination in the central and peripheral nervous systems of these mice revealed that the membrane-bound isoforms of neuregulin 1 Nrg1 were substrates of BACE1 [165, 166]. BACE1 is required to activate the Nrg1/erbB cascade that regulates the thickness of myelin sheath during neuronal development early in life. BACE1 expression is also needed in both neurons and Schwann cells to mediate remyelination upon injury [167]. Considering that Nrg1 is an established susceptibility gene for schizophrenia and other psychotic disorders, the behaviour of the BACE1^{-/-} mice was also thoroughly examined, and this revealed ‘schizophrenia-like’ phenotypes associated with defective Nrg1-erbB4 signalling [168]. BACE1 cleavage is also activates the function of Nrg1 in muscle-spindle formation, which underlies proper motor coordination [169]. In addition, BACE1 is also implicated in the regulation of voltage-gated sodium channels by cleaving their regulatory subunits [170]. Proteomics analyses have demonstrated that three dozens of neuronal type I integral proteins are misprocessed in the absence of BACE1 expression [127, 171, 172]. BACE1 substrates include cell adhesion molecules and receptors required for axonal growth and guidance, regulatory molecules involved in synaptic plasticity and in the activity of sodium and potassium ion channels, as well as proteins that contribute to inflammation and repair processes. Therefore, it can be expected that blocking BACE1 activity will give rise to an array of mechanism-based side effects. As an example, BACE inhibitors were shown to cause retinal toxicity in animal models by a mechanism that entails misprocessing of the vascular endothelial growth factor receptor 1 and defective lysosomal function [173]. Also, because the active sites of BACE1 and BACE2 are structurally very similar, most BACE inhibitors inhibit equally both proteases. BACE2 is principally expressed in the periphery, and plays an important role in the pancreas where it controls the proliferation and activity of β -cells [127]. It is also required for the processing of the melanocyte protein PMEL that controls skin pigmentation [174]. Therefore, potential adverse reactions will have to be cautiously monitored in patients receiving BACE inhibitors, and ideally the drugs should be dosed to restrict A β production to non-toxic levels, while sustaining some degree of BACE activity to carry on the cleavage of BACE1 and BACE2 various substrates. Newly discovered cleavages of APP upstream from the BACE1 cleavage site, which are mediated by matrix metalloproteinases (MMP) and that influence subsequent processing by α - and γ -secretases will also have to be

taken into consideration [175, 176]. The biological importance of the so-called “eta-secretase” (η -secretase; MMP-7), which acts in tandem with either BACE1 or γ -secretase to generate fragments that modify long-term potentiation is under scrutiny and may well complicate the applications of BACE inhibitors [175]. The membrane-type 5-matrix metalloproteinase (MT5-MMP) that was shown to promote the amyloidogenic processing of APP in an AD mouse model might offer an alternative therapeutic target [176].

5.4. Rationale for the continued use of BACE inhibitors in AD therapy

Because the clinical trials of novel therapies based on the amyloid cascade have so far provided a succession of disappointing and negative results, the question of the validity of this approach has again been raised. However, the amyloid hypothesis cannot be denied from the results of unsuitable drug trials. Each failed trial has taught us important lessons to move forward. First, we now know that the choice of the right cohort of participants is primordial and that an intervention in patients with advanced AD is unfortunately unlikely to succeed. The recent progress in the development of diagnostic tools has helped to better understand the disease progression and it has highlighted that A β begins to accumulate in the brain years before the occurrence of AD symptoms [177-180]. Therefore, a therapeutic intervention should ideally be initiated in asymptomatic people who report memory complaints and who test positive for AD imaging and CSF biomarkers, and in those who carry a genetic risk for developing AD, such as the APOE 4 allele. Also, safety trials should be conducted over several months to detect potential adverse effects of the drug. And it is evident that an orally-available drug will be easier to dose and administer than an antibody. Based on these considerations, BACE inhibitors remain strong candidates in future AD therapy.

Little study has been done to assess the pathology associated with BACE1 elevation, which happens in a large proportion of patients with sporadic AD, and also particularly in patients with MCI [181]. Considering the number and variety of BACE substrates, adverse consequences of high BACE1 activity in the brain, apart from the overproduction of A β , should therefore be expected. A study has shown that overexpression of BACE1 in transgenic mice caused increased processing of voltage gated sodium channel α subunits, which resulted in decreased cell surface expression of the sodium channels, hence impaired propagation of action potentials and epileptic phenotypes [170, 182]. Other potential effects of BACE1 elevation in AD merit further investigation. Indeed, it was shown that the direct C-terminal product of APP cleavage by BACE1, C99, accumulates in the brain earlier than A β and significantly contributes to neurodegeneration and cognitive alterations in transgenic mice [183]. In addition, A β toxicity itself induces BACE1 expression, as evidenced from its accumulation in dystrophic neurites surrounding amyloid plaques [112, 184]. Therefore, BACE1 inhibition will provide a mean to break this vicious cycle of BACE1 elevation and A β toxicity, and could also prevent the deleterious effects of BACE1 excess activity in the cleavage of its other substrates.

The risks associated with BACE1 inhibition have been essentially assessed from the observation of BACE1 knockout mice, or from wild type animals treated with levels of inhibitors that completely get rid of BACE1 activity. For BACE1-targeted therapy to be safe, it will be important to monitor the levels of BACE1 activity and to determine its normal range in the CSF and/or blood cells so that BACE inhibitors could be used to restore these levels, but not for completely removing BACE1 activity. It may also be beneficial to use a combined therapy in patients with mild to moderate AD, with a BACE inhibitor being administered in conjunction with an antibody targeting A β , or Tau. Indeed, recent studies in mice have shown that passive Tau immunotherapy can successfully reduce Tau pathology and ameliorate cognitive deficits in mouse models [185] [186] and some Tau antibodies are now being tested in Phase 1 clinical trials. Inhibitors of glycogen-synthase kinase-3 (GSK-3 β),

the major kinase responsible for Tau hyper-phosphorylation, have also emerged as promising drug candidates in AD [187, 188]. GSK-3 has been proposed as the possible link between A and Tau [189]. It was also shown to repress BACE1 expression.[190]. Recently, dual inhibitors that consist of an arm targeting BACE1 and another arm that targets GSK-3 have been described that possess favourable pharmacological properties and can cross the blood brain barrier [191, 192]. These attest that the search for new therapeutics in AD is still progressing.

6. Conclusions

Despite the failure of early BACE inhibitors that lacked potency and specificity, a new generation of BACE inhibitors stand as strong candidates in AD therapy. BACE1 inhibition represents a rational and promising approach to break the pathological cycle of amyloid toxicity. The design of clinical trials in AD has made considerable progress and is now supported by diagnostic tools that permit to select patients at the prodromal stage of AD, who are those the most likely to benefit from this treatment. Verubecestat is the most advanced among the new BACE inhibitors, and some interim results of its Phase 3 trials may soon become available. Several other compounds are also entering Phase2/3 trials. It may be expected that by the next decade, BACE inhibitors will be used in the clinic for AD therapy. However, research must continue to improve understanding of the biology of BACE1, as a prerequisite to evaluate and prevent, or compensate the potential adverse effects of BACE1 inhibition.

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Figure Captions

Figure 1. Therapies targeting the amyloid cascade

The scheme depicts the amyloidogenic processing of the amyloid precursor protein, APP, and the targets of therapies in clinical trials. First, α -site APP cleaving enzyme 1 (BACE1) cleaves APP to secrete sAPP and produce the C-terminal membrane-tethered fragment C99. The BACE inhibitors target this reaction. Next, cleavage of C99 by γ -secretase produces A β peptides and releases the APP intracellular domain (AICD). Gamma-Secretase Inhibitors (GSI) and Gamma-Secretase Modulators (GSM) are designed to interfere with this cleavage. A β can self-associate to form toxic oligomers. Immunotherapy targets either A β monomers or oligomers.

Figure 2. Timeline of BACE Inhibitors in clinical trials

Table 1. Comparison of Verubecestat and other BACE Inhibitors in Clinical Trials

Compound (name, class, company)	Potency and Specificity (<i>in vitro</i> and cell assays)	Animal trials	Human Trials	
			Effects on AD biomarkers	Safety
AZD3839 (amino isoindole) Astra Zeneca	$K_i=26$ nM (iv) ^a <i>Selectivity:</i> 14 x vs BACE2 1,000 x vs Cath D $IC_{50}=17$ nM (c) ^b ↓ A ₄₀ , sAPP (neurons)	<ul style="list-style-type: none"> Tg mice: ↓ A in brain and plasma Guinea pigs: ↓ A in brain, CSF and plasma 	<ul style="list-style-type: none"> Ph1 in HC up to 55% ↓ plasma A₄₀ and A₄₂ Discontinued 	Moderate adverse effects (Heart toxicity)
AZD3293 /LY3304814 (double-spiro amino imidazole) Astra Zeneca/Lilly	$IC_{50}=0.2$ nM $K_i=0.4$ nM ↓ A secretion from mouse, guinea pigs neurons, human SH-SY5Y ($IC_{50}=610$ pM, 310 pM, and 80 pM) <i>Selectivity:</i> none vs BACE2 25,000 x vs Cath D	<ul style="list-style-type: none"> Tg mice: ↓ A₄₀, A₄₂, sAPP in brain and plasma Guinea pigs: ↓ A₄₀, A₄₂, sAPP in brain, plasma and CSF Beagle dogs ↓ A₄₀ in brain, plasma and CSF (2-weeks daily dosage) 	<ul style="list-style-type: none"> Ph1 in HC (young and older): <i>Single dose:</i> ↓ plasma A₄₀ and A₄₂ by 70-76% t 50 mg; by 60-80% at 15 mg <i>Multiple doses</i> (14 days): a ↓ CSF A₄₀ and A₄₂ by 75% at 50 mg daily; 50% at 15 mg daily; ↓ CSF sAPP, ↑ CSF sAPP Ongoing Ph1 in AD Ongoing Ph 2/3 (AMARANTH: >2200 MCI and prodromal AD) 	Well tolerated
CTS 2116 (hydroxyethylene) Comentis	$K_i=1.1$ nM (iv) $IC_{50}=39$ nM (c)	<ul style="list-style-type: none"> Tg2576 mice: ↓ plasma A (70%) and brain A (55%) Rats: ↓ CSF A (~50%) 	<ul style="list-style-type: none"> Ph1 in HC: ↓ A in plasma 	Well tolerated
E2609 (small molecule) Eisai/Biogen	BACE $IC_{50}=7$ nM (c)	<ul style="list-style-type: none"> Rats: ↓ CSF A (~80%), brain A (55%) Guinea pigs ↓ CSF A (85%), brain A (97%) Monkeys ↓ plasma A₄₀ and A₄₂ ($ED_{50}=1.3$ nM) ↓ CSF A₄₀ and A₄₂ (90%) 	<ul style="list-style-type: none"> Ph1 in HC: ↓ plasma A₄₀ (92% at 800 mg/day / 6 days) ↓ CSF A (80% at 200 mg/day; 46% at 25 mg/day; 6 days) Ph1b in HC: ↓ plasma A₄₀ and A₄₂ (50-90% at 25-200 mg/day; 14 days) ↓ CSF A (85% at 200mg/day; 14 days); ↓ CSF sAPP ; ↑ CSF sAPP Ph1c in AD Ongoing Ph2 	Safe and Well-tolerated
JNJ 54861911 Janssen/Shionogi			<ul style="list-style-type: none"> Ph1 in HC: <i>Single dose:</i> ↓ plasma A₄₀ (lasting 36 h) ↓ CSF A (90%), ↓ CSF sAPP ; ↑ CSF sAPP <i>Multiple doses</i> (14 days) ↓ CSF A₃₇, A₃₈, A₄₀ (~95%), A₄₂ Ongoing Ph1b and Ph2/3 in AD/MCI/at risk (10 and 50 mg) 	
LY2811376 Eli Lilly and Co	$IC_{50}=239$ nM (iv); 300 nM (c) Lacks selectivity	<ul style="list-style-type: none"> TgPDAPP mice: ↓ brain A (68%); C99 (78%); sAPP (63%) Beagle dogs ↓ plasma A (85%); brain A (70%) 	<ul style="list-style-type: none"> Ph1 in HC: ↓ CSF A by 90%; ↓ CSF sAPP ; ↑ CSF sAPP ; Discontinued 	

LY2886721 Eli Lilly and Co	IC_{50} = 20 nM (iv); 19 nM (c); 11 nM (neurons) <i>Selectivity:</i> >15,000 x vs Cath D; 2x more active vs BACE2	<ul style="list-style-type: none"> TgPDAPP mice: ↓ brain A (~65%), C99 (~55%), sAPP (~50%) Beagle dogs ↓ CSF A (70%); ↓ plasma A (80%) 	<ul style="list-style-type: none"> Ph1 in HC and AD: <i>Single dose:</i> ↓ CSF A₄₀ (74% at 70 mg; 40% at 35 mg), A₄₂, sAPP ; ↑ CSF sAPP ↓ plasma A₄₀ (83%) and A₄₂ (74%) <i>Multiple doses</i> (70 mg; 14 days): ~70% ↓ CSF A₄₀, A₄₂, and sAPP ; ~55-70% ↑ CSF sAPP ~80% ↓ plasma A₄₂ Ph2 in MCI: Discontinued 	
Verubecestat (iminothiadiazine dioxide) Merck/Schering	K_i (BACE1) = 1.75 nM K_i (BACE2) = 0.37 nM IC_{50} (BACE1) = 2 nM (c) <i>Selectivity:</i> >45,000 x vs Cath D >45,000 x vs Cath E >15,000 x vs renin	<ul style="list-style-type: none"> TgCRND8 Mice ↓ brain A plaque Rats ↓ brain A by 49%, 3h after oral dose (10 mg/kg) CSF ED₅₀ = 5 mg/kg Monkeys ↓ CSF A by 80 % at 12 h after 10 mg./kg dose; by 60% at 8 h after 3 mg/kg dose ↓ brain cortex A by 72% at 4h after 10mg/kg dose 	<ul style="list-style-type: none"> Ph1 in HC (18-45 yo) <i>single dose:</i> ↓CSF A (92% at 550 mg; 75% at 100 mg; 21% at 20 mg)) <i>multiple doses</i> (14 days): ↓CSF A (94% at 250 mg daily; 32% at 10 mg daily) Ph1b, mild-mod AD <i>multiple doses</i> (7 days) ↓CSF A₄₀ (57%), A₄₂, (47%), sAPP (37%), at 12 mg daily, Ongoing Ph2/3 EPOCH ~2000 mid-mod AD patients, 78 w treatment APECS: ~1500 MCI, 104 w treatment 	Well tolerated after 3 month daily dosing

^a (iv) *in vitro* assay against purified BACE1; ^b (c) cellular assay in permanent cell lines.

Abbreviations: A , amyloid ; AD, Alzheimer's disease; APP, amyloid precursor protein; BACE, -site-APP cleaving enzyme; Cath D, cathepsin D; CSF, cerebrospinal fluid; HC, healthy control; IC_{50} , concentration inhibiting enzyme activity by 50%; K_i , inhibitory constant; MCI, mild cognitive impairment; Ph, clinical trial phase; sAPP , soluble/secreted APP fragment produced by -secretase cleavage; sAPP , soluble/secreted APP fragment produced by BACE1; Tg, transgenic. NCT numbers refer to the study codes in the ClinicalTrials.gov database.