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**BACE1 AS A THERAPEUTIC TARGET IN ALZHEIMER'S DISEASE:
RATIONALE AND CURRENT STATUS**

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Abstract

Alzheimer's disease (AD) is a neurodegenerative disease of the central nervous system, which causes dementia in a large percentage of the aged population and for which there are only symptomatic treatments. Disease-modifying therapies that are currently being pursued are based on the amyloid cascade theory. This states that accumulation of amyloid β ($A\beta$) in the brain triggers a cascade of cellular events leading to neurodegeneration. $A\beta$, which is the major constituent of amyloid plaques, is a peptidic fragment derived from proteolytic processing of the amyloid precursor protein (APP), by sequential cleavages that involve β -site APP cleaving enzyme 1 (BACE1) and γ -secretase. Targeting BACE1 is a rational approach as its cleavage of APP is the rate-limiting step in $A\beta$ production and this enzyme is elevated in the brain of patients with AD. Furthermore, knocking out the *BACE1* gene in mice showed little apparent consequences. Ten years of intensive research have lead to the design of efficacious BACE1 inhibitors with favorable pharmacological properties. Several drug candidates have shown promising results in animal models, as they reduce amyloid plaque pathology in the brain and rescue cognitive deficits. Phase I clinical trials indicate that these drugs are well tolerated and the results from further trials in AD patients are now awaited eagerly. Yet, recent novel information on BACE1 biology and the discovery that BACE1 cleaves a selection of substrates involved in myelination, retinal homeostasis, brain circuitry, and synaptic function, alert us to potential side-effects of BACE1 inhibitors that will require further evaluation to provide a safe therapy for AD.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disease of the central nervous system which accounts for 70-80% cases of dementia and which affects an increasing proportion of the aged population [1]. AD prevalence has steadily risen over the last century to an estimated 25 to 30 million sufferers worldwide, and is set to double by 2030 and more than triple by 2050 [2]. These figures demonstrate the immediate imperative for developing effective AD therapies. This review focuses on recent progress in the development of inhibitors that target the β -site Amyloid precursor protein Cleaving Enzyme 1 (BACE1) and discusses promises and potential hurdles associated with this therapeutic approach.

2. The Amyloid Cascade Theory

Post-mortem histological studies have characterised the presence of senile plaques and intraneuronal tau neurofibrillary tangles as the pathological hallmarks of AD [3]. The identification of the amyloid β peptide ($A\beta$) as the key constituent of the senile plaques was an important step towards the understanding of AD pathology [4] and fostered a wealth of research into the pathological mechanisms of AD, particularly focusing on the role of $A\beta$ in AD pathogenesis. This led to the amyloid cascade hypothesis, which states that the cause of AD results from an imbalance between $A\beta$ production and clearance, with the consequent deposition of $A\beta$ plaques causing neuronal dysfunction and death [5, 6]. Extensive neurodegeneration is considered responsible for the progressive dementia which results in a gradual and severe loss of cognitive function and eventually death [7]. The amyloid cascade hypothesis has been revised over the years [8, 9], and it is proposed that $A\beta$ injury may be two-fold. Firstly, the direct toxicity of $A\beta$ oligomers, not the amyloid plaques, would cause injury to the neurons, in particular neuronal synapses. Secondly, this initial insult would trigger downstream effects, which potentially include: neural inflammation, oxidative stress, altered metal ion homeostasis and metal toxicity, as well as altered kinase and phosphatase activity, leading to tau hyperphosphorylation, microtubule dissociation, and the formation of tau neurofibrillary tangles [10]. Although the amyloid cascade hypothesis has been challenged [11, 12], this remains the most widely accepted hypothesis in AD research and it is supported by compelling genetic evidence that has linked mutations in the amyloid precursor protein and in the presenilins (which are the catalytic subunits of γ -secretase) to aggressive forms of familial AD, with accelerated production of $A\beta$ aggregating species [13]. Disappointing results of clinical trials of anti-amyloid therapies may have cast some doubt on

this approach to AD treatment but it should be highlighted that such clinical trials were conducted in patients with diagnostically established AD, thus at a stage of advanced neurodegeneration. PET imaging demonstrates A β accumulation in the brain of patients at the prodromal stage of the disease [14], well before the first signs of cognitive impairment and 20-30 years before AD clinical onset. Therefore, disease-modifying drugs should be given as an early therapeutic intervention, so as to limit neuronal loss and preserve the brain function.

3. A β Production

A β is produced through the metabolism of the Amyloid Precursor Protein (APP), a type 1 integral membrane receptor [15], which is involved in the regulation of neuronal activity, synaptic function, and neurogenesis, as well as metal homeostasis [16-19]. A β results from the dual cleavage of APP via the amyloidogenic pathway [20]. APP is first cleaved by β -secretase, to form a large, soluble extracellular fragment (sAPP β) and a small membrane bound amyloidogenic carboxyl terminal fragment of ninety-nine amino acids (C99). The C99 fragment is further processed by γ -secretase to release the APP intracellular domain (AICD) in the cytosol, and A β peptides on the luminal/extracellular space [21]. Soluble A β is thought to undergo further conformational changes, primarily obtaining a β -sheet conformation which is more prone to aggregation [22]. Alternatively, APP can be processed by a non-amyloidogenic pathway, where it is cleaved by α -secretase to produce the soluble extracellular fragment α (sAPP α) and a non-amyloidogenic C-terminal fragment (C83), which is subsequently cleaved by γ -secretase to produce a short p3 peptide and the AICD [20]. As the non-amyloidogenic pathway does not produce A β , a preference for the amyloidogenic over the non-amyloidogenic APP processing pathway predisposes AD pathology, as illustrated by the recent discovery of a mutation in the APP gene, in Finnish families, which impairs β -secretase cleavage, lessens A β production, and appears to protect its carriers against developing old age dementia [23, 24].

4. BACE1 Structure and Biology

BACE1 was identified in 1999 as the enzyme responsible for performing the β -secretase function, which is defined as the first cleavage step in the production of A β from APP (reviewed in [25]). BACE1 is a 501 amino acid, membrane-bound proteolytic enzyme, which represents the prototype of a new subclass of aspartyl proteases, with distinctive structural features and a unique arrangement of disulfide bonds [26-31]. Its crystal structure in complex

with an inhibitor has revealed an extended catalytic site that confers it with substrate specificity and has provided the bases for developing inhibitors [32]. Another distinct feature of BACE1 is its anchoring in cellular membranes through a C-terminal single transmembrane domain, which restricts its subcellular distribution, and dictates its orientation to facilitate an interaction with membrane-associated substrates, such as APP [26] [33]. [21, 34, 35]. BACE1 is synthesized in the endoplasmic reticulum as the proBACE1 precursor, and undergoes extensive post translational modifications, which take place while trafficking through the Golgi. These involve removal of the prosequence by furin, and complete glycosylation [31, 35-38]. The mature BACE1 is sorted and packaged by clathrin adaptor proteins for export to the endosome, which is an acidic cellular compartment with optimal pH for BACE1 enzymatic activity [35, 39]. BACE1 can also undergo phosphorylation at C-terminal serine 498, which mediates its retrieval from the plasma membrane to early endosomes under the control of the Golgi-localized, γ -ear-containing, Arf (ADP-ribosylation factor), GGA adaptor proteins [40-42]. Palmitoylation of cysteines 478, 482 and 485 facilitates its segregation to lipid raft membrane domains [38]. Ubiquitination of lysine 501 is a signal for BACE1 proteosomal degradation and for its recognition by the GGA3 adaptor protein for transport to lysosomal degradation [43] [44, 45]. BACE1 is cycled between the trans-Golgi network, cell surface, and early endosomes, and it is during this cycling that it recruits its APP substrate. Aberrant and continued recycling of BACE1 without degradation due to alterations of sorting proteins that control its cellular turnover, has been linked to increased A β production and AD [46, 47]. BACE1 activity can be enhanced by homodimerisation [48, 49] and the cellular mechanisms that prompt BACE1 dimer formation remain to be identified.

5. BACE1 in Alzheimer's Disease

The finding that a double mutation in APP, which segregates with early-onset AD in a Swedish family, increases BACE1 access to the β -secretase cleavage site and boosts A β production, together with the fact that transgenic mice overexpressing BACE1 have increased brain A β levels support that BACE1 activity plays a significant role in AD pathogenesis [50]. Post-mortem studies have shown that BACE1 protein levels and activity are upregulated in AD brain cortical regions [46, 51-55]. BACE1 activity can be correlated with A β deposition in the brain, suggesting an elevation in BACE1 activity is significant in the pathological production of A β [55]. BACE1 increase in AD brain has been attributed to changes in post-transcriptional and post-translational mechanisms [46, 52, 56]. BACE1 activity is also

elevated in the cerebrospinal fluid of AD patients [57-61], but its levels may vary at different stages of the disease progression, and this would explain why some researchers have also observed a decrease in BACE1 activity in AD CSF [62]. BACE1 is increased in patients with mild cognitive impairment (MCI), which is the prodromal stage of the illness, and high levels of BACE1 in CSF may help predict conversion from MCI to AD [58]. It also been reported that increased BACE1 activity in MCI patients correlates with change in hippocampal volume and with APOE ϵ 4 genotype [60].

The event that triggers BACE1 cleavage of APP is yet to be elucidated. Growing experimental evidence from cellular and animal experiments supports that BACE1 is involved in stress response, as increased BACE1 expression was shown to correlate with oxidative stress and aging [63-67], which are key factors in AD onset. The tight control of BACE1 cellular trafficking appears to be the key determinant of its substrate accessibility [43]. Our latest results show that exposing neuronal cultures to non-toxic levels of oxidative stress can prompt the redistribution of BACE1 and enhance its co-localization with APP, hence increase the production of APP amyloidogenic fragments [68].

6. Pharmacological BACE1 inhibition

Due to its critical role in AD etiology, BACE1 has attracted much attention for its potential as a therapeutic target [69, 70]. Initially spurred on by the fact that *BACE1* knockout mice showed complete A β suppression and no obvious disease phenotype [71] and by the reports that ablation of the *BACE1* gene in AD transgenic mouse models could rescue memory deficits [72, 73], many companies have embarked into BACE1 inhibitor discovery programs and produced compounds with high *in vitro* potency. BACE1 inhibitors were originally designed from substrate analogues, with a non-hydrolysable bond at the BACE1 cleavage site, such as an hydroxyethylene or an hydroxyethylamine isostere [34]. These have provided high affinity inhibitors, which are effective at low nanomolar concentrations *in vitro*, but which lack blood-brain-barrier permeability due to their extended size that is tailored to occupy the large BACE1 catalytic site. Recent research has yielded potent non-peptidic BACE1 inhibitors through structure-guided drug design and refinement, based on data from co-crySTALLISATION of inhibitors with BACE1 and helped identify new inhibitor cores through *in silico* screening of chemical libraries (reviewed in [74]). Some of the major challenges researchers have encountered in the developement of BACE1 inhibitors have been i) a lack of selectivity for BACE1 versus other aspartyl proteases such as cathepsin D and BACE2 (with

the first being involved in general protein catabolism, and the latter possibly being involved in A β degradation); ii) insufficient blood brain barrier permeability, mostly due to a large molecular size and/or efflux by P-glycoprotein, which impairs gastrointestinal absorption as well as capillary delivery to the brain [75-77]; iii) interference with potassium channel subunit, human Ether-à-go-go-Related Gene (hERG), leading to potential cardiac toxicity [78]. These are common problems associated with CNS drug discovery, and thousands of compounds had to be trialled for selecting the best drug candidates.

CTS-21166, which was developed from an hydroxyethylene peptide isostere by Ghosh and Tang's team, with Comentis/Astellas, was among the first BACE1 inhibitors reported to be effective *in vivo*. This compound was shown to reduce A β levels in plasma of laboratory animals [79], and successfully reached Phase I clinical trials, where it demonstrated good tolerance and further showed its ability to reduce plasma A β [80]. The same research group also described GRL-8234, an hydroxyethylamine isostere peptidomimetic with high potency against BACE1 activity *in vitro* (K_i =1.8 nM) and in cellular systems (ED_{50} =0.4-1 nM), with good selectivity versus BACE2 (39-fold) and cathepsin D (23-fold), and with demonstrated brain penetration and suitable pharmacological properties [81]. A sustained 12-hour reduction in A β_{40} levels was observed in the plasma and brain interstitial fluid of Tg2576 AD mice given the drug by intravenous injection. Chronic administration for six months at a dose of 33.4 μ g/g/day through implanted osmotic pumps showed good tolerance and a reduction of plasma A β_{40} and A β_{42} by 60-80%. Remarkably, cognitive performance tests demonstrated that upon prolonged treatment (5-7.5 months) the inhibitor rescued cognitive decline in the animals that received the drug from the age of 12 months, and this was accompanied by a reduction of brain amyloid plaque load. This study demonstrates that BACE1 inhibition can reduce amyloid deposition in the brain and rescue memory deficits in an animal model, and supports the promise of BACE1 inhibitors for AD therapy.

May et al have reported in detail the encouraging preclinical trials of LY-2811376, a non-peptidic inhibitor that was designed by Lilly Research Laboratories from a fragment-based screening approach by combining hit structures to maximize occupancy of the BACE1 active site. This compound displayed a good oral bioavailability and an impressive *in vivo* efficacy [82]. Dosed to PD-APP mouse model at 10-100 mg/kg, it achieved a dose-dependent reduction of brain A β levels (by 47-68%), of C99 fragment (by 56-78%), and of sAPP β (by 33-43%). Trials in beagle dogs showed this could attenuate A β levels in the plasma and CSF,

by 85% and 70%, respectively. Clinical administration to healthy volunteers indicated its good tolerability and its sustained effect at reducing A β and sAPP β in the CSF, with concomitant increase in sAPP α [82]. These data provide a convincing proof-of-concept that BACE1 inhibition can be achieved in human. As the results of preclinical toxicity studies of LY-2811376 in rats showed lipofuscin accumulation and degenerescence in the retina [82], the clinical trials of this drug candidate had to be interrupted. This problem was attributed to an off-target effect of the drug that showed insufficient selectivity over cathepsin D. Lilly researchers have now begun Phase I trials of a new compound, LY-2886721, with expected safer properties.

Several other companies have also brought BACE1 inhibitors into clinical trials. Merck Research Laboratories have screened thousands of molecules, explored new scaffolds with success [83-87], and produced MK-8931 that is undergoing Phase II clinical trials and expected to enter Phase III later this year. Preclinical trials reported at conferences showed that this, or a related compound was effective when given orally to monkeys at a dose of 10 mg/kg, lowering A β levels in the brain and CSF by about 90%. Also, researchers at Astra-Zeneca have recently described AZD3839 as a clinical drug candidate [88]. This compound, which was designed from fragment-based screening and structure-assisted refinement, proves to be 14-fold selective for BACE1 against BACE2, and more than 1,000-fold over cathepsin D, and to have favourable pharmacological properties. Effective at lowering A β in the plasma, brain, and CSF of mouse, guinea pig, and primate models, it has now advanced to Phase I clinical trials. Amgen is also known to have begun clinical trials and reported that AC-3/AMG0683, when given orally to rats at 10 mg/kg can lower A β in the brain and CSF by 73% and 86%, respectively [89]. And Eisai is conducting clinical trials of its compound, Eisai 2609, which has passed successfully preclinical tests and is now in a Phase II study in subjects with mild cognitive impairment.

7. Potential adverse effects of BACE1 inhibition

BACE1 inhibition appears to be generally well tolerated in Phase I clinical trials, which involve short treatment in healthy volunteers, and the clinical therapeutic value of BACE1 inhibitors will soon be determined when Phase II and III have been completed. Whether BACE1 inhibition will really prevent A β accumulation may be questioned after the publication of a recent report showing that several BACE1 inhibitors administered to animal models produced the expected reductions of A β ₁₋₄₀ and A β ₁₋₄₂ in the CSF, but also enhanced

production of A β ₅₋₄₀, suggesting that an alternative processing pathway may take over that of BACE1 to process APP and produce potentially aggregating A β peptides [90]. Another strong concern is that BACE1 cleaves substrates, other than APP, which may be important for the maintenance of neurons and synaptic function in aging. The first major BACE1 substrate that was uncovered is neuregulin 1 (NRG1), which is the ligand for ErbB receptor tyrosine kinases. The NRG1 precursor isoforms, type I β 1a and type III β 1a require BACE1 cleavage processing to release their epidermal-growth factor domain and activate ErbB receptors [91-93]. BACE1 knockout mice show some defects in myelination of the central and peripheral nervous systems [91, 94] that reflect an impairment of NRG1/ErbB4 signaling. NRG1 type III plays an important role during embryonic development as it regulates myelin ensheathment, Schwann cell and oligodendrocyte differentiation, as well as neuronal migration [95]. There is also evidence for its role in the regulation of receptors for the neurotransmitters, γ -Aminobutyric acid (GABA) and N-methyl-D-aspartate (NMDA) [96, 97]. Notably, NRG1 is a susceptibility gene for schizophrenia and other psychotic disorders, thus tampering with its signaling may have adverse consequences. BACE1 was also shown to regulate the expression of the *Disrupted-in-Schizophrenia-1* gene, another schizophrenia susceptibility gene, which has been linked to the NRG1/ErbB/Akt cascade [98]. Consistently with these findings, Savonenko et al have reported that BACE1 knockout mice have phenotypic changes and behaviour deficits that are reminiscent of schizophrenia and can be rescued by antipsychotic drugs [99]. Although treatment of transgenic mice with BACE1 inhibitor Merck-3 had apparently no effect on NRG1 levels [100], the intricate expression, processing, and biological function of NRG1 in the brain [93] will require further investigation so as to foresee the potential side effects of inhibiting BACE1 processing of NRG1, particularly in aging. Monitoring psychotic symptoms in the patients who will receive BACE1 inhibitor treatment may be recommended. BACE1 also plays an important role in modulating membrane excitability, as it is involved in the processing of the β (β 1, β 2, β 3, and β 4) auxiliary subunits of the Na_v1 voltage-gated sodium channel (VGSC) [101-103], and of the KCNE1 and KCNE2 subunits of voltage-gated potassium channels [104]. Other BACE1 substrates include the amyloid precursor-like proteins, APLP-1 and APLP-2 [92, 105], the alpha2,6-sialyltransferase, which is involved in liver copper homeostasis [106], the cell-adhesion P-selectin glycoprotein ligand-1 [101], the low density lipoprotein receptor related protein (LRP), which is required for endocytosis and lipid homeostasis [107], and other substrates that are specifically expressed in the brain and are involved in cell adhesion

and inflammation [108-110]. Indeed, proteomic analyses have revealed among BACE1 substrates, the neural cell adhesion molecules L1 and “close homologue of L1” (CHL1), the Seizure protein 6 homologue (SEZ-6), and contactin-2, which are required for axonal guidance and formation of neuronal circuits during the brain development, and also for the maintenance and regeneration of sensory neurons later in life. Impaired cleavage processing of these substrates and defects in axonal guidance were demonstrated in mice with *BACE1* gene knockout and in animals treated with the BACE1 inhibitor LY2811376 [109, 111]. These findings are consistent with the reports of seizures in the *BACE1*^{-/-} mice [112, 113], as well as synaptic deficits [114, 115]. Retinal pathology is another potential side-effect of BACE1 inhibition that should be followed. Cai *et al* have recently reported retinal thinning, reduced retinal vascular density, and accumulation of lipofuscin age pigment in *BACE1*^{-/-} mice [116]. The authors also demonstrated that BACE1 suppression by siRNA or with a chemical inhibitor caused a dysregulation of retinal angiogenesis, due to the suppression of vascular endothelial growth factor receptor 1 processing [116]. The accumulation of lipofuscin was attributed to a decrease in lysosomal function.

In sum, an increasing number of potential side-effects of BACE1 inhibition will require careful attention. The ethical issues of giving patients BACE1 inhibitors which may have long term negative effects could be questioned, while considering that AD is an illness that can span ten years or more and which requires a lasting treatment. However, it is important to point out that the data showing toxicity of BACE1 inhibition result from the suppression of BACE1 activity in young animals [112, 113, 116]. The patients to be targeted with BACE1 inhibitors are elderly individuals with high BACE1 levels, thus it is essential to investigate further the consequences of elevated BACE1 on brain pathology, besides increased A β production, to balance the positive and negative outcomes of BACE1 inhibition. As inflammation, seizures, and hyperexcitability are some features of the AD brain, and these may be driven and exacerbated by BACE1 elevation, BACE1 inhibition might actually offer additional advantages to decreasing A β pathology. A recent study shows with an APP transgenic mouse model that high BACE1 levels and increased cleavage of sodium channel β 2 subunit associate with aberrant neuronal excitability and with cognitive deficits [117]. Therefore, we could assume that a safe therapeutic approach will require establishing the right dosage of BACE1 inhibitors that can restore BACE1 activity to near normal levels, and take care not to decrease it beyond levels that would compromise its normal function.

8. Alternative Approaches to Modulating BACE1 Activity

Besides designing molecules that target the BACE1 active site, other strategies have been explored to modulate BACE1 activity. Such an approach consists in interfering with BACE1 association with cellular membranes using chemical compounds that were identified through cellular screening as capable of modulating β -secretase cleavage of APP. The compound KMI-574 was proposed to modify BACE1 conformation and molecular associations, and thereby to dislodge the enzyme from lipid rafts, possibly to alter its trafficking, and prevent its interaction with APP [118]. Takeda compound, TAK-070 was also shown to function as a non-competitive inhibitor by presumably binding to the BACE1 transmembrane domain [119]. Oral administration of this compound showed a significant reduction, although modest, in levels of $A\beta_{40}$ and $A\beta_{42}$ in the brain of Tg2576 mice, with concomitant increase in sAPP α . Chronic treatment at a dose of 7 mg/kg/day for six months decreased amyloid plaque burden by ~60%. An amelioration in cognitive performance in memory behaviour tests was observed in mice given orally 3mg/kg/day, and none of the behavioural phenotypes that were observed by others in the BACE1 knockout animals. These data suggest that achieving a partial reduction in BACE1 activity is sufficient to decrease amyloid load in the brain and rescue cognitive deficits, without noticeable secondary effects.

The use of monoclonal antibodies offers another approach to interfere with BACE1 activity. Zhou et al demonstrated the feasibility of this method by showing that monoclonal antibodies that target BACE1 surface loops can reduce $A\beta$ secretion from primary neuronal cultures [120]. Direct injection into the brain of APP transgenic mice significantly decreased levels of $A\beta_{40}$, $A\beta_{42}$, and C99 fragment. Researchers at Genentech also developed a BACE1 monoclonal antibody that targets a surface epitope of the enzyme, and proved its efficacy at inhibiting BACE1 activity and cleavage of APP in cellular systems and animal models, including mice and monkeys [121]. The advantages of this antibody are its excellent selectivity for BACE1 and its ready intracellular delivery, as it becomes endocytosed together with BACE1 after binding the protease at the surface of neurons. Repeated peritoneal injections in Tg2576 mice resulted in 70% decreases of $A\beta_{1-40}$ and $A\beta_{1-42}$ in the plasma, but reductions of brain $A\beta_{1-40}$ and $A\beta_{1-42}$ were more modest (~20%). A reduction of peripheral and CSF $A\beta_{1-40}$ was also demonstrated in primates. To improve brain penetrance, the Genentech researchers designed a bispecific antibody by combining a low affinity transferrin receptor antibody and a BACE1 antibody [122].

Methods of RNA interference are also being explored as a means to control BACE1 expression levels. BACE1 suppression by small interfering RNA (siRNA) was demonstrated to inhibit production of A β and C99 using primary neural cultures [123] and to ameliorate neuropathology and behaviour in transgenic mice [124]. Injecting lentiviral plasmids expressing BACE1 siRNA into the brain of APP transgenic mice not only decreased BACE1 expression, but also decreased levels of A β ₁₋₄₂ and APP C99 fragment, and rescued cognitive deficits in spatial memory tests. Improving the methodology for siRNA delivery to the brain is in progress [125, 126].

9. Conclusions

BACE1 initiates the amyloid cascade and is therefore a very attractive therapeutic target. A panoply of inhibitors and other tools are now available to modulate BACE1 activity. These have proved to be effective in preclinical trials, as these reduce brain amyloid deposition and rescue cognitive deficits in animal models. With recent progress at detecting biomarkers changes in the brain and CSF of patients at the prodromal stage of AD, we may expect that the early administration of BACE1 inhibitors will decrease A β formation and prevent A β -mediated neurodegeneration. However, the function of BACE1 remains to be fully elucidated, in particular the role it plays in the maintenance of neuronal circuits and synaptic activity, in the regulation of retinal homeostasis, and in lysosomal function, so as to anticipate and compensate potential side-effects of BACE1 inhibition.

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Figure Legend

Figure 1. Secretase Processing of the Amyloid Precursor Protein

The amyloid precursor protein (APP) is a type I membrane protein, with a large extracellular domain (ectodomain), a transmembrane domain, and a short cytoplasmic domain. The ectodomain can be secreted through two alternative pathways. In the amyloidogenic pathway, cleavage by β -site APP cleaving enzyme 1 (BACE1) releases the soluble APP N-terminal fragment (sAPP β), thereby producing a membrane-tethered C-terminal fragment of ninety-nine amino acids (C99), which is the direct precursor to A β . C99 is further processed by γ -secretase (γ -sec) to produce A β and release the APP intracellular domain (AICD) in the cytosol. A β self-aggregates to form toxic oligomers that trigger degeneration of neuronal cells. In the non-amyloidogenic pathway, APP is cleaved by α -secretase (α -sec), which produces the soluble sAPP α N-terminal fragment and a membrane-tethered, eighty-three amino acid-long C-terminal fragment (C83). α -Secretase cleavage occurs within the A β domain and precludes A β amyloid formation. C83 is further processed by γ -secretase to produce the 3kDa, non-amyloidogenic peptide, p3, and release AICD.