

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,900

Open access books available

124,000

International authors and editors

140M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



---

# Dropping the BACE: Beta Secretase (BACE1) as an Alzheimer's Disease Intervention Target

---

Justin Read and Cenk Suphioglu

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/53603>

---

## 1. Introduction

The  $\beta$ -site amyloid precursor protein cleaving enzyme 1 (BACE1) is an important regulator for the production of amyloid plaques, a characteristic of the Alzheimer's disease (AD) brain. The proteolytic cleavage of the amyloid precursor protein (APP), by BACE1, produces an insoluble amyloid- $\beta$  ( $A\beta$ ) fragment which has the ability to aggregate and migrate onto the dendrites and cell body of neuronal cells, initiating chronic immune responses of inflammation and microglia activation.

The cleavage of  $A\beta$  fragments trigger a feedback system that complements production numbers. This increases  $A\beta$  loading to such an extent that it exceeds the defences required for natural elimination. The fragments aggregate, developing into an insoluble plaque that has the ability to effect normal functioning by causing dysfunction. Without early identification and effective inhibition of this pathogenic pathway, the disease is anticipated to become more widespread with an aging population, in which AD is most prevalent.

Attempts to inhibit BACE1 have been relatively fruitless with most therapeutic trials being aborted in the early stages. A number of obstacles can be detrimental to the ability of an inhibitor like solubility, bioavailability, potency and effectiveness. In addition to the complexity, there are also a number of substrates cleaved by BACE1 which are important in other pathways like voltage gated sodium channels and axon myelination [1, 2]. This can create adverse reactions beyond the reduction of plaques.

Inhibitors of BACE1 have, so far, looked at active-site mimics that diverge from small and large molecules to short peptidic structures and expression modulators. Other approaches look to utilize technology with modelling software to determine the BACE1 3D structure and formulate an effective inhibitor via domain analysis. The underlying issue is translat-

ing an analytically based, site-directed mimic into a potential inhibitor with pharmaceutical integrity. This process is generally thwarted by the blood brain barrier (BBB), a specialized endothelium that separates systemic blood flow from the central nervous system (CNS). The aim is to fabricate an inhibitor that can pass through the BBB whilst maintaining structure and function.

The importance of BACE1 and the influence it has on AD, has been investigated thoroughly since it was first identified in 1999. The enzyme is an intricate part of A $\beta$  cleavage and plaque formation instigating that inhibition could be the mechanism for relief in AD. In this chapter, we will summarize the structure and function of BACE1 and identify past attempts at its inhibition. The identification of important features regarding the protein-enzyme cleavage of APP will aid the understanding of the process and help theorize future perspectives of research.

## 2. Neurodegenerative disorders

Neurodegenerative disorders cover a wide range of brain conditions relating to the damage or death of neuronal cells [3]. Clinical characterisation suggests a regression in structure and function of the brain and central nervous system which is usually the final stage of a preceding period of neuronal dysfunction [4]. Specifically, dementia is of particular significance because of the devastating influence it bears on an ageing population and as the life expectancy of the general public increases, disease rates are predicted to escalate accordingly.

The burden of disease in relation to AD, the most predominant form of dementia, was calculated to be 26.6 million cases worldwide in 2006 [5]. This figure implicated 34% of the population over the age of 65 and 45% over the age of 85. The World Health Organization revised this figure in 2010 to incorporate dementia as a whole because of the difficulty to diagnose the varieties of neurodegenerative disorders [6]. The worldwide incidence was tallied at approximately 35.6 million with an estimated 7.7 million new cases annually instigating a new case arising every 4 seconds. The significance of this in regards to AD is that it covers 60-70% of dementia cases [6]. These figures could be inflated further because most cases go undiagnosed due to the requirement of post-mortem autopsy for confirmation.

The ripple effect of dementia extends far from those affected and into patient support networks. The worldwide costs are estimated to be US\$604 billion annually and as the number of affected increases this number is expected to follow suit [6]. The chronic onset of the disease indicates an eventual requirement for long term care. The financial burdens relating to carers, as the disease evolves and symptoms increase with severity, the eventual requirement for long term formal care is inevitable [7]. AD patients specifically require increased supervision after diagnosis because of the increased risk of developing associated diseases like cardiovascular disease, diabetes, and bone weakening [8].

AD is largely defined by chronic symptoms of progressive neuronal and synaptic apoptosis in the cerebral cortex and subcortical regions of the brain [4]. The direct consequence is cel-

lular atrophy, which includes degeneration in the temporal and parietal lobes, parts of the frontal cortex and cingulate gyrus [9, 10]. Progression of brain atrophy to well-defined brain structures results in symptoms of delusions, hallucinations, agitation, depression, anxiety, elation, apathy, disinhibition, irritability, aberrant motor behaviour and sleep disorders [11]. As the chronic nature of the disease progresses, the number of symptoms increases, becoming severely debilitating. Eventually, death results after long-term stress and reduction of brain structure and function [9].

Currently there are a variety of different intervention strategies being investigated to remedy these debilitating pathologies and focus to reduce the cause of neuronal cell death. Upwards of 100 irregular protein changes occur in a brain with AD, including hyperexpression, fragmentation and phosphorylation [12]. A number of these irregular protein modifications could be a result of degenerating neurons causing further damage to associated structures of the central nervous system [12]. The overall progression of AD is however, not restricted to the dysfunction of one mechanism. The number of different symptoms result from a conglomerate of sporadic cellular events that, as a consequence, stimulate the disease. The trigger or initial changes are not entirely clear and do not occur simultaneously which adds to the ambiguity of the disease.

Histopathological review of AD patients highlighted amyloid plaques and neurofibrillary tangles (NFT) as the two significant characteristics of cerebral regression identifying them as targets for the prevention of neuronal cell death [4, 13]. Investigation into the onset of NFTs implicate the tau protein as the leading cause [14]. The tau protein becomes hyperphosphorylated and releases from the intracellular microtubules decreasing structure and function and causing axons to become dishevelled and dedifferentiated. These subsequent microtubules bind together to become an insoluble fibre decreasing the ability of neurons to transmit action potentials along the axon and neurotransmitters across the synapse [15]. Alleviation from this facet of the disease is yet to be elucidated.

Amyloid plaque formation is characterized by an accumulation of amyloid- $\beta$  ( $A\beta$ ) sub-units on the cell membrane of neurons that, as a result, cause a decrease in cellular function [16]. The progressive damage of amyloid plaques cause neuronal apoptosis by activating the complement cascade and stimulating the membrane attack complex [17]. In developed cases of AD, where there is a high concentration of  $A\beta$ , significant damage to the physical structure of the brain can be a result of the immune response. The activation of the complement cascade involves microglia and astrocytes, which have both protective and destructive attributes. The eventual outcome is apoptosis because of the overwhelming immune response, which cannot be hindered by anti-inflammatory drugs [18]. The aggregations of  $A\beta$  deposits are neurotoxic, insoluble and become vastly distributed around the brain as they increase in numbers [19]. AD needs to be proactively prevented before amyloid plaque formation gains the ability to manipulate regular brain function and cause irreversible brain damage.

Closer investigation of the AD brain will encounter a plethora of imperative neuronal cell functions failures like synaptic failure [20], depletion of neurotransmitters [21], mitochondrial dysfunction [22], decreased cholesterol metabolism [23], reduction of neurotrophin [24] and axonal transport deficiencies [25] which can be classified as associated effects of the dis-

ease. These associated deficiencies become apparent with the onset of plaque formation, which precedes tau deconstruction [26]. The repercussions of tau mutations are a characteristic of frontotemporal dementia (FTD) and Parkinson's like defects rather than AD, which makes plaque formation the focus of this chapter [27].

### 3. Amyloid plaque formation

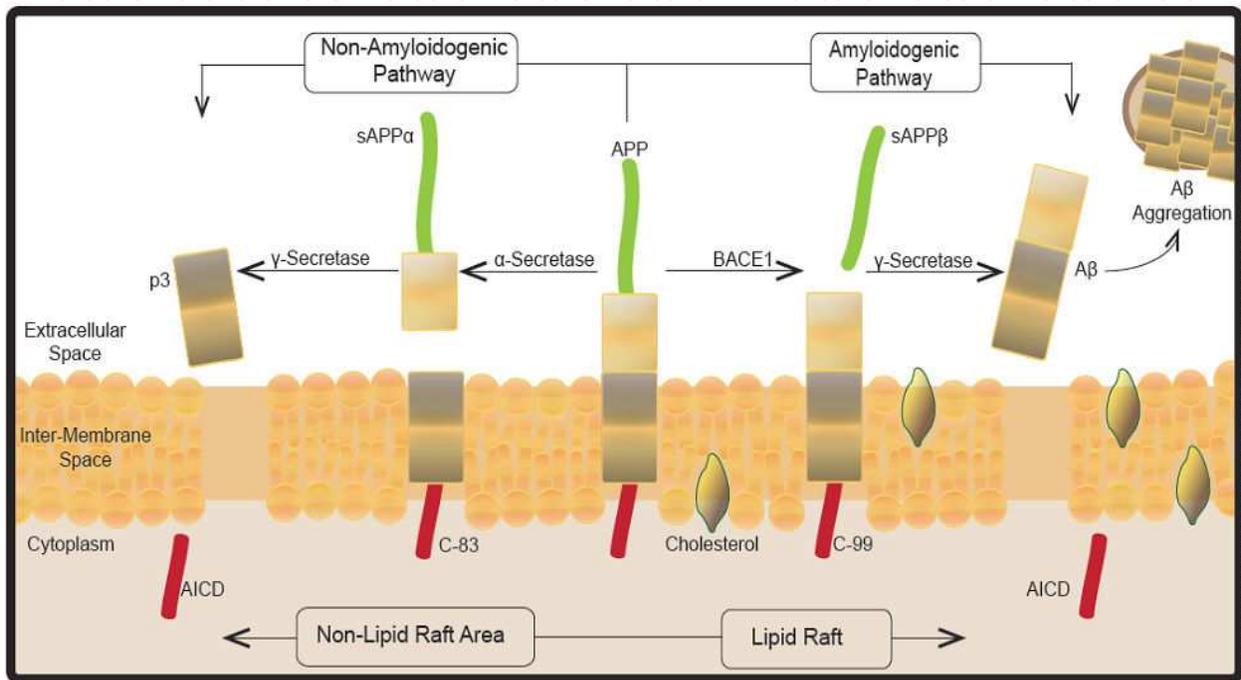
Amyloid plaques were originally purified in the early 80s and examined to contain peptides of approximately 40 amino acids that aggregated as oligomers, later to be generically named amyloid-beta ( $A\beta$ ) [28]. Gene cloning and cDNA analysis of these monomers lead to the realization that the origins of this peptide remained part of a larger precursor protein [29]. This protein was later identified as the 695 amino acid, membrane bound cell receptor, amyloid precursor protein (APP), which contained the  $A\beta$  sequence in the extracellular domain [30]. It is the proteolytic cleavage, by that of multiple secretases, which releases the  $A\beta$  product.

Proteolytic processing of APP occurs by one of two pathways, the amyloidogenic (pathogenic) or the non-amyloidogenic (non-pathogenic). Transport to the membrane via endosomes is chaperoned by the intracellular adaptor protein, sorting nexin 17 (SNX17), where it becomes available for processing by the secretases [31]. The determinant of amyloidogenesis depends on the initial proteolytic cleavage in the extracellular space by either  $\alpha$ - or  $\beta$ -secretase to create a soluble or insoluble fragment [19]. Cleavage of APP by the  $\alpha$ -secretase, a protein investigated as part of the disintegrin and metalloprotease (ADAM) family, cleaves APP at the  $\alpha$ -site releasing a soluble fragment sAPP $\alpha$  into the extracellular space [32]. This leaves the C-terminal fragment of 83 amino acid residues (C-83), which is abruptly cleaved by  $\gamma$ -secretase in the intramembrane space. This creates two subsequent fragments of the APP intracellular domain (AICD), released in the cytosol, and p3 which is released into the extracellular space (Figure 1).

The amyloidogenic pathway also utilises APP as a target. This pathway is much like that of the non-amyloidogenic pathway but instead  $\alpha$ -secretase is substituted for  $\beta$ -secretase or beta site APP converting enzyme 1 (BACE1). This enzyme also cleaves APP in the extracellular space at the  $\beta$ -site, which is 18 amino acids towards the N-terminal, releasing a much shorter soluble APP- $\beta$  (sAPP $\beta$ ) fragment [33, 34]. The remaining C-terminal fragment of 99 amino acid residues (C-99) remains membrane bound until cleaved by  $\gamma$ -secretase. This releases two fragments of AICD and  $A\beta$  (Figure 1).

The non-amyloidogenic pathway has the ability to nullify the amyloidogenic pathway by simply having  $\alpha$ -secretase cleave APP prior to that of BACE1. Since the  $\alpha$ -site of APP is situated between that of  $\beta$ - and  $\gamma$ -, the shorter p3 fragment is produced instead of  $A\beta$ . The question over competition for the cleavage of APP is still debated but there is evidence suggesting that  $\alpha$ -secretase nullification does not increase BACE1 activity *in vitro* [35]. The subsequent fragments of sAPP $\alpha$  and AICD from the non-amyloidogenic pathway suggest a purpose from the subsequent cleavage of APP. The sAPP $\alpha$  has shown to have neurotrophic

and neuroprotective properties as well as promoting neurite expansion, synapse production and cell adhesion, while AICD has a role in p53 expression and caspase 3 activation, both associated with cell death, and in maintaining cellular actin dynamics [36-38]. This indicates that the non-amyloidogenic cleavage of APP is imperative to the maintenance of neuronal growth and function.



**Figure 1.** The cleavage of APP, depending whether by BACE1 or  $\alpha$ -secretase, results in the production of an amyloid plaque. The non-amyloidogenic pathway (non-pathogenic) begins with  $\alpha$ -secretase, which releases sAPP $\alpha$  externally from the endosome or cell membrane. The resulting C83 fragment is cleaved by  $\gamma$ -secretase in the intermembrane space releasing both the AICD and p3 fragments. The release of p3 into the extracellular space is not associated with plaque formation. Alternatively, the amyloidogenic (pathogenic) pathway begins with BACE1, instead of  $\alpha$ -secretase, and cleaves APP in a lipid raft region of the membrane. The sAPP $\beta$  released is considerably shorter than sAPP $\alpha$  and is still released externally. The  $\gamma$ -secretase cleaves APP, the same as in the non-amyloidogenic pathway, but releases the 38-42 amino acid A $\beta$ , and AICD. The externally released fragment, A $\beta$ , has the capability to form an amyloid plaque by aggregation.

The underlying difference between the two processes is the release of the A $\beta$  and p3 fragments. The p3 is a shorter bi-product of the overall APP cleavage that has no known function. However, the p3 fragment does not have the same ability to form stable oligomeric intermediates like that of A $\beta$ , which poses no threat to synaptic function instigating that it is not the cause of amyloid plaques and is the reason for being the non-amyloidogenic pathway [39, 40]. This suggests that the longer A $\beta$  peptide is the main neurotoxic fragment established from the APP fragment. The cleaved product becomes a peptide of 40 amino acid residues but this can be varied between 38 and 42 depending on where the  $\gamma$ -secretase cleaves APP.

### 3.1. Amyloid- $\beta$

The  $\gamma$ -secretase complex is composed of four proteins: either one of presenilin 1 (PS1) or presenilin 2 (PS2), nicastrin (NCT), anterior pharynx-defective phenotype 1 (Aph-1) and presenilin enhancer 2 (Pen-2) [41]. The complex formation is initiated with a sub complex that forms between NCT and Aph-1 which then binds to one of the PS proteins [42]. Pen-2 is used to activate the complex by selective binding [43]. Either PS can be used in this process, with a 67% amino acid homology, including two separate aspartate residues, asp257 and asp385, considered essential to catalytic ability, both have the ability to cleave APP [44, 45]. Consequently, the decrease in availability of the  $\gamma$ -secretase components results in a reduction of overall functionality, which can be targeted for use when considering inhibitors and the vulnerabilities of this complex [46]. Mutation to either the presenilins, whether it be PS1 or PS2, can be seen as a potential threat to the production of  $A\beta_{40-42}$ , as one can compensate for the other.

The  $\gamma$ -secretase, being the consistent piece of the APP cleavage puzzle, is actually responsible for the diseased state in which plaques are formed. There are upwards of 100 missense mutations identified in the presenilins, mostly creating the difference in cleaved fragments of APP [47]. The  $A\beta$  peptide created will be influenced with a varying overall length of between 38-42 amino acids [19]. The majority of these mutations influence a higher number of  $A\beta_{42}$  peptides, which have been found to be more amyloidogenic and neurotoxic [48, 49]. The wild-type presenilin generates  $A\beta_{40}$ , which is considered to be less neurotoxic even though it is present in amyloid plaques.  $\gamma$ -secretase cuts downstream from the transmembrane domain into the  $\epsilon$ -site of APP and therefore slightly shortens the AICD cleaving domain [50].  $A\beta_{42}$  provides the basis for oligomerisation, fibrillation and plaque generation, even with  $A\beta_{40}$  being found with a limited ability to protect neurons in mouse models [51]. The ratio of  $A\beta_{42}/A\beta_{40}$  increases after the AD age of onset but is still relatively low. However, with a binding affinity to  $A\beta_{40}$ , fibrils can, as a consequence, bind together, meaning there does not need to be a high concentration to be effective [52].

The  $A\beta$  fragments are not always cleaved to the extracellular space either. There are a number of conformations that  $A\beta$  fragments can take including monomers, which are found free in neurons as a consequence of APP cleavage. Studies have also shown that fragments can form the shape of  $\alpha$ -helices, random coils and even as  $\beta$ -sheets in a neutral pH to add to the complexity [53-56]. These individual fragments maintain the ability to block synaptic neurotransmitter transfer and instigate an apoptotic response via activation of the p53 promoter leading to cell death [57]. Alternatively, soluble  $A\beta$  oligomers have been referred to as  $A\beta$ -derived diffusible ligands (ADDLs) that have the ability to aggregate into protofibrils, spherical structures of 7-10 nm wide, that can have the ability to interrupt nerve signal transduction leading to cell death [58]. Initial investigations into the  $A\beta$  fibrils showed there was a toxic response from the neurons in which they were attached [59].

To target the  $A\beta$  fibrils, gene knockout studies of PS1 have found adverse reactions relating to formation of the axial skeleton, neurogenesis and neuronal survival causing affected mice to die late in embryogenesis [60]. These reactions however, can be attributed to a

dysfunction of the Notch signalling pathway because of its involvement with cell proliferation, apoptosis and myelin formation, not to mention it is a substrate of  $\gamma$ -secretase [61]. This indicates that there are a number of associated risks with nullifying  $\gamma$ -secretase that with any inhibition, the complex will invariably prevent the cleavage of two regulatory pathways.

### 3.2. Neuroinflammation

The increase of intra and extracellular  $A\beta$  has a direct effect on the complement system and the recruitment of microglia, instigating the activation of inflammatory mechanisms, a trademark of stress on an Alzheimer's brain [62]. The microglia, derived from the mesenchyme and transferred to the CNS where proliferation occurs, are classified as the macrophages of the brain and regulate apoptotic cell abundance [63]. Stress incurred, mainly by amyloid plaque formation, triggers an immune response which leads to the activation of astrocytes and microglia to dismantle the ailing cell. The  $A\beta$  fragment has the ability to activate this process via the receptor for advanced glycation end products (RAGE), a multi-ligand member of the immunoglobulin family that is increased in production in an AD brain, and by CD40, an inflammatory signalling receptor [64-66]. The general immune response is aimed at eliminating  $A\beta$ , removing disease ridden cells and restoring tissue integrity but does more damage when it becomes of a chronic nature. The activation of reactive oxygen species (ROS), prostaglandins, and pro-inflammatory cytokines are a characteristic of the chronic inflammation and neuronal dysfunction in AD [67].

The onset of AD encourages the migration of microglia to the plaque affected areas. Cultured microglia, from elderly human patients, showed this migration as they coupled with  $A\beta$  for the purpose of deconstruction [68]. Similar findings were discovered in sections of cortex tested in vitro for migration but growing evidence suggests that once endocytosed, the microglia struggle to breakdown the  $A\beta$ , causing stress and functional changes to the cell [68-70]. Additionally, the APP mRNA translation is upregulated in the event of trauma, nerve damage and brain ischemia, which can be beneficial for the release of more AICD, but can also result in the production of more  $A\beta$  [71-73]. BACE1 has also been found to be regulated by  $A\beta_{42}$  via the c-Jun N-terminal kinase (JNK) pathway, otherwise known as the stress-activated signalling pathway which is important for the mediation of pro-inflammatory cytokines [74, 75]. This reiterates the important influence that a variety of stressors have when responding to the amyloidogenic pathway and that there are many different pathways that can be involved in the pathogenesis of amyloid plaques.

All aspects of the amyloid plaque progression relates back to the effectiveness of the BACE1 enzyme. Taking into consideration alternative BACE1 substrates, the proteolytic cleavage of APP can be regulated with an inhibitor. The  $A\beta$  concentration increases as the disease gets worse but could be diverted with the use of an effective pharmaceutical intervention. The possibilities and implications will not be recognised until BACE1 is inhibited successfully *in vivo*.

## 4. BACE1

### 4.1. BACE1 production and natural degradation

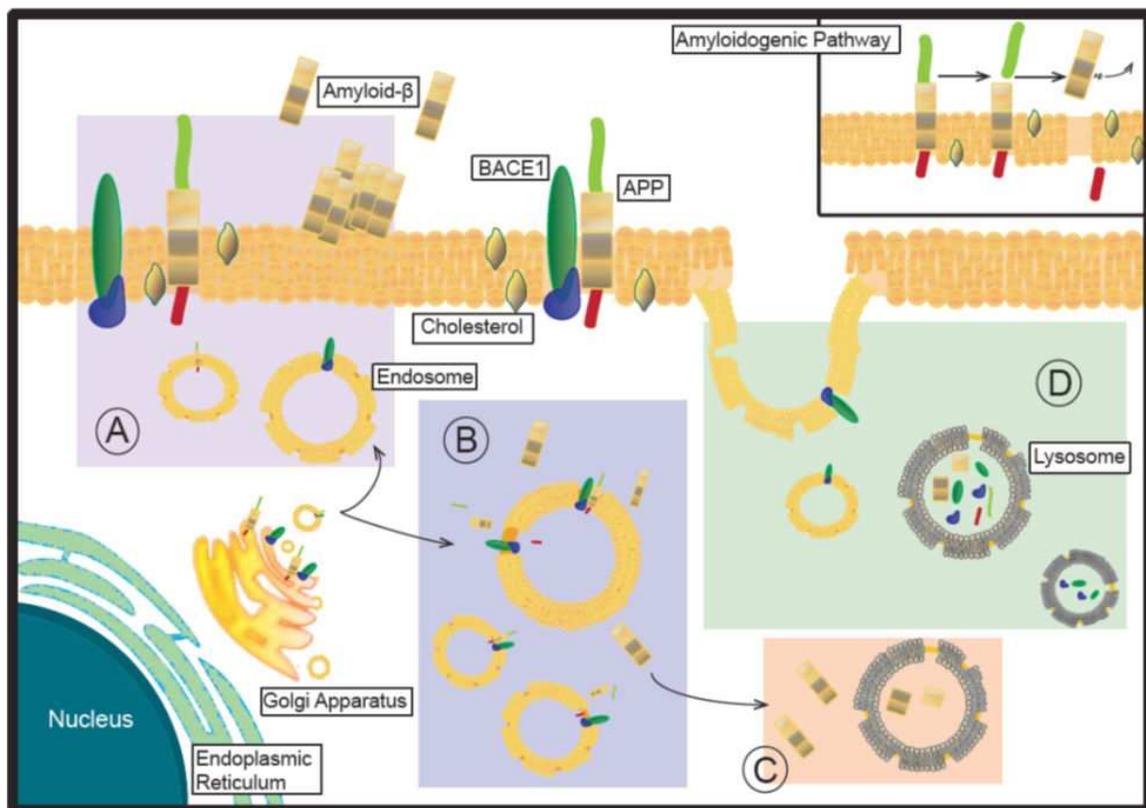
The BACE1 enzyme (also called  $\beta$ -secretase, Asp2 or memapsin2) is developed in the endoplasmic reticulum from a 501 amino acid, 60 kDa, immature precursor protein. The transfer from the endoplasmic reticulum to the Golgi is dependent on the prodomain, which is removed by the proprotein convertase, furin [76]. At this stage the immature BACE1 can already cleave APP so is not a true zymogen but the removal of the prodomain can double the proteolytic effectiveness whilst increasing structural stability [77, 78]. The immature BACE1 is deacetylated and transformed in the golgi by post-translational modification into a type 1 transmembrane protein [79]. A bi-lobal structure is formed with two aspartate motifs (D93TG and D289SG in a D-T/S-G-T/S conformation), which forms the active site that stimulates water molecules to hydrolyse APP peptide bonds, a defining characteristic of aspartic proteases [80, 81]. The proteolytic ability of this motif is only limited by site-directed mutagenesis of the aspartate residues [81]. The active site remains an important structure of this enzyme because of its supposed vulnerability. However, although it has been the focus of inhibition studies since its highly contested discovery in 1999, no effective pharmaceutical options have been elucidated since [34, 82-85]. The distinctiveness surrounding BACE1 is exposed when comparing it to other aspartic proteases and the contrasting characteristics of a C-terminal cytosolic tail and a transmembrane domain.

The transfer from the Golgi to the cell membrane is mediated by transport vesicles because BACE1 is membrane bound. The serine and di-leucine residues of BACE1 act as a signal for the Golgi-localized  $\gamma$ -ear-containing ARF binding protein 1 (GGA1), a sorting protein that aids the link between the transporting endosome and the cell membrane [86]. The endosome provides an optimal transport vesicle because of its internal acidic nature (approximately pH 4.5), a fundamental for the conformational shape and functionality of BACE1, but also because it is much more stable environment for transporting proteins [87, 88]. Changes in pH below 4.0 can have a negative effect on Wat1, a molecule considered to be the nucleophile with the ability to attack the carbonyl carbon of a peptide bond in the active site resulting in the loss of functionality. Changes at the other end of the scale, above that of pH 7.0, render the enzyme inactive and unable to cleave a substrate [87] (Figure 2A,B,C).

The active site placement is also vulnerable as it remains exposed to the extracellular space once it makes the transition to the lipid bi-layer of the membrane where it concludes its intracellular transport. BACE1 becomes susceptible to post-translational modification, protein to protein interactions or even inhibitor attachment because of its availability. It is here, however, that the BACE1 enzyme comes into contact with the membrane bound APP [89]. In some instances, the help of increased cellular cholesterol producing lipid rafts helps improve the availability of APP for BACE1. So what is considered a negative of vulnerability of the enzyme actually serves an important purpose in that a specific environment is created for APP to become more available. The lipid rafts are formed as an essential membrane stabilizer of intermediate space but have also controversially, improved signal transduction and intracellular trafficking ability of the cell [90].

BACE1 is found in a number of tissues throughout the body but majority of expression is found in the brain [91]. The levels of this expression have been of much debate due to conflicting reports separating different tissues in which the samples were taken, animal or human models and the use of controls [92]. The increase in expression could then be argued as a reason for the uncontrollable aggregation of A $\beta$  but reports of little or no increase in expression of BACE1 instigates the influence of another factor is involved [93].

The natural degradation of BACE1 has been found to be involved the ubiquitin proteasome pathway. BACE1 can be transported from endosomes to lysosomes by ubiquitination with help from the sorting proteins, ADP ribosylation factor 6 (ARF6) and GGA3, another member of the GGA family [94-97]. The degradation by lysosomes is still ambiguous with an increase in BACE1 protein available in an AD brain, whilst there is little to no increase in mRNA levels. The failed or impaired lysosome could be a contributing factor to the increase in cellular A $\beta$  or BACE1 could be getting recycled back into endosomes [93] (Figure 2D).



**Figure 2.** BACE1 Trafficking. BACE1 is constructed in the ER and processed, with the removal of the proBACE1 domain, in the Golgi. A) BACE1 is a membrane bound protease and so is either exocytosed from the Golgi or transported to an endosome with the help of the sorting protein GGA1. From here, the cleavage of APP to produce extracellular A $\beta$  takes place in the lipid raft region of the membrane. The synthesis of A $\beta$  occurs through the amyloidogenic (pathogenic) pathway. B) Processing of APP can otherwise occur whilst present in the endosome and so can release A $\beta$  into the cytoplasm. C) The A $\beta$  can then either aggregate together or be endocytosed by lysosomes and degraded. D) Once APP proteolysis has occurred, BACE1 is either internalized to endosomes via GGA1 and ARF6 or labelled with ubiquitin and endocytosed. With assistance from the sorting protein, GGA3, the lysosomes will either recycle or degrade BACE1.

Recently, a natural inhibition of BACE1 has been found via the sAPP $\alpha$  fragment produced from the non-amyloidogenic pathway [98]. The sAPP $\alpha$ , once produced from APP, has been found to implicate its own cleavage. The findings showed that sAPP $\alpha$  can bind to BACE1 and interfere with the APP cleavage in a mouse model. This resulted in an overall reduction in A $\beta$  formation under physiological conditions. The consequence of this pathway being impaired in any way, could result in a decrease in BACE1 production thus reducing the cleavage of further units of APP. The sAPP $\alpha$  production or cellular concentration could be a therapeutic intervention strategy in future research.

#### 4.2. BACE2 and closely related proteins

BACE2 is a closely associated  $\beta$ -secretase to BACE1. Sequence analysis show amino acid residue similarities of ~45% and structural comparisons of 75% homology [99]. BACE2 is also classified as an aspartic protease with the ability to cleave APP at the  $\beta$ -site [100]. *In vitro* studies have also implicated BACE2 as a possible competitor of BACE1 for the cleavage of the APP protein but it is not formally expressed in excess in the brain and does not compensate for the loss of BACE1 in gene knockout models in mice [89, 101]. In fact, over expression of BACE2 has actually been found to reduce A $\beta$  production in primary neuronal cultures derived from APP transgenic mice. This could be caused by the ability of BACE2 to cleave APP before BACE1 or because BACE2 cleaves a longer 79 residue A $\beta$  fragment from APP, which is closer to the cleaved fragment of  $\alpha$ -secretase than that of BACE1 [89, 99].

Apart from the sequence homology, BACE2 is not primarily expressed in the brain. It is more commonly expressed in the colon, kidney and pancreas, showing that whilst theoretically still having the ability to increase the pathogenesis of AD it is not considered a threat to A $\beta$  generation [100, 102]. BACE1 is the main amyloidogenic enzyme and still remains the major contributor to amyloid plaque formation but does show some sequence homology with a number other aspartic proteases including renin and cathepsin D. These homologs are generally used for selectivity testing with *in vitro* assays to confirm no unwanted binding occurs.

#### 4.3. Substrates of BACE1

The BACE1 protein is not solely defined to the cleavage of APP and has the ability to cleave other proteins like amyloid like precursor proteins 1 and 2, APP $\epsilon$  (which is another closely related *N*-terminal product of APP), neuregulin-1 and -3 involved in axon myelination,  $\beta$ -subunits of voltage gated sodium channels required for neuronal action potentials, P-selectin glycoprotein ligand-1 (PSGL-1), which regulates leukocyte adhesion in the inflammatory process, the interleukin 1 receptor type-II (IL1-R2) and the low density lipoprotein receptor-related protein 1 (LLRP1), which is a multifunctional endocytic and signalling receptor [1, 2, 103-106]. With the use of an unbiased, quantitative proteomics method, the identification of 64 new potential substrates were elucidated. The majority of these were type I transmembrane proteins but an added 3 glycoposphatidylinositol anchors and one type II transmembrane protein were also identified, all of which are membrane bound [107]. This suggests that BACE1 has a significant purpose in normal cellular functions but a number of these are

yet to be characterised *in vivo* and subsequently cannot be verified as a substrate until this time. With the interaction between BACE1 and APP being widely of interest in the prevention of AD, the actual damage by completely inhibiting the enzyme from normal functioning could prove detrimental in its own right. APP may not even be the primary substrate of the enzyme. Therefore, partial inhibition of BACE1 is all that may be required as an intervention therapy to normalize the enhanced BACE1 activity seen in AD patients.

Alternatively, both of the amyloidogenic creating enzymes, BACE1 and  $\gamma$ -secretase, have alternative purposes relating to other substrates. Gene knockout studies in mouse models produced the reaction of hyperactivity, premature death and seizure like behaviours for BACE1 whilst presenilin -/- mice displayed early neurodegenerative behaviours and an increase of  $A\beta$  species which could possibly be due to the large number of substrates in which both are effective [108-111]. The  $\gamma$ -secretase complex is essential for the Notch signalling pathway, which is important for the development of the nervous system and this would also be in deficit if inhibited completely [112]. Likewise, the  $\gamma$ -secretase complex is also required for the non-amyloidogenic pathway, suggesting that the complete inhibition or deletion of these proteins will be detrimental to the homeostatic processing related to normal development and function.

## 5. Challenges

### 5.1. Past BACE1 inhibitors

Inhibition of BACE1 has been the focus of many studies since it was discovered. The main interest in this enzyme remains with the obvious involvement in plaque formation but also to the positive intervention target for which it provides. Firstly, mouse models have shown that without BACE1,  $A\beta$  is no longer produced, which indicates that if a product can reduce the enzymatic ability of BACE1 then plaques will not be formed [109]. The enzyme has been characterised and 3-D structure produced, which will allow for inhibitor modelling [80]. BACE1 also has the ability to bind to a wide variety of peptidic structures, while specific binding is an attribute there is still access to the active site for potential targeting [113]. BACE1 is a part of the large aspartic protease family, which have had the success of at least two members (renin and HIV protease-1(HIV-1) inhibited with success [114]. This shows confidence that BACE1 is a viable option for inhibition and, if successful, could influence the production of  $A\beta$ .

Drug intervention of AD is limited between therapeutic relief of symptoms and the prevention of the underlying etiology of the disease [115]. The treatment to minimise symptoms is the only viable option for sufferers of AD. This method slows the decline of cognition, function and behaviour but only masks the underlying neurodegeneration taking place [116]. The drugs currently available to treat AD are divided into two categories of cholinesterase inhibitors and receptor antagonists [115].

Cholinesterase inhibitors attempt to prevent the metabolism of acetylcholine allowing the neurotransmitter to maintain effect in the neuron [117]. Cholinesterase inhibiting drugs like

donepezil, galantamine and revastigmine are only effective in the early stages of AD by allowing the retention of acetylcholine [115]. By inhibiting the hydrolysis of acetylcholine by cholinesterase, once the neurotransmitter has crossed the synapse, the cholinergic neuron remains active [117]. This action becomes redundant when inadvertent progression of NFTs interfere with the normal signal transmission of the axon [15]. Cholinesterase inhibitors have shown that they can actually increase the amount of phosphorylated tau which can further the progression of AD, minimising the effectiveness of the drug [118, 119].

The other pharmaceutical option is memantine, an uncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist, which prevents binding of the primary excitatory neurotransmitter in the brain, glutamate [120, 121]. As a result, glutamatergic overstimulation can occur causing neuronal cell damage by increased localized calcium stores or excitotoxicity [122]. Both drug options have important side effects and can only offer therapeutic relief of symptoms and are not useful as a long-term intervention strategy.

Therapeutic relief by drugs like donepezil, galantamine and memantine are required to optimize the productivity of the brain whilst the disease progresses and cannot translate to a definite cure. The production of A $\beta$  is the basis of senile plaques and should be investigated as an important therapeutic target. Theories suggest that by inhibiting BACE1 before it cleaves APP, the formation of the beta amyloid residues would be reduced [123, 124]. To prevent this process, inhibition would be paramount between the translation of BACE1 and its binding to APP, before sAPP $\beta$  has been cleaved.

Modern drug discovery for a pharmaceutical intervention aims to hinder the BACE1 enzymatic activity by exactly this process. Notable methods look at high-throughput screening (HTS), fragment-based drug discovery (FBDD) and substrate-based inhibitors [125]. However, none of these methods have been successful in therapeutic trials. The more complex approach, generally taken by larger pharmaceutical companies, relate to either the HTS strategy or a FBDD approach. Both processes use a large library of complex compounds in order to find a suitable hit followed by a long modification process to refine it into a suitable chemical. Initial use of HTS sourced complex, high-molecular weight, compounds that were difficult to manipulate. Potential therapeutic candidates showed strong oral availability and good brain permeability but could not provide the standards of potency and selectivity for therapeutic trials [125, 126]

The HTS method has been substituted by the FBDD approach because it uses smaller and more specific compounds [127]. The screening of a fragment library is more appealing because a higher hit ratio is produced and the options show favourable drug properties. The main problem with the hit compounds is again, the low potency and selectivity. Often, fragments that showed promise were too small to be effective and have not provided any real inhibition with the effectiveness required for therapeutic trials.

A large range of inhibitors are being researched including statins, primary, secondary and tertiary amines, hydroethylamines (HEA) of many different conformations, arylamino compounds and acyclic acylguanidines. Most of these compounds seek to act as transition state mimics with a reduced peptidic structure that preclude them from the same scrutiny

as other peptidic structures. To their detriment, hydroxyethylamine derivatives had poor brain exposure, mainly because of p-glycoprotein (PgP) mediated efflux [128-130]. Bristol-Myers Squibb produced a HEA-derivative that contained an indole and a 7-azaindole carboxamide but struggled to maintain brain A $\beta$  levels *in vivo* [131]. Unfortunately, the inhibitor showed a high potency *in vitro* that had an IC<sub>50</sub> of 10 nM and a low affinity for BACE2 and cathepsin D.

Another study has looked to hybridize the HEA isomere and replace the sulfonate ester present in one molecule with the sulphonamide of another [132]. The result was a compound that had high potency IC<sub>50</sub> of 15 nM to BACE1 that was able to bind to the hydrophobic space of the active site. Unfortunately, this compound was able to bind to BACE2 which minimised the selectivity to BACE1. After problems with HEA structures and brain penetration, Merck decided to use a macrocyclic inhibitor, produced by cross linking the P1 and P3 sidechains of an isophthalamide-based inhibitor. This process helped improve potency but a bolus of 100 mg/kg fed to APP-YAC mouse model could only obtain A $\beta$  decreases of 25% in the first hour and 10% after the third [133].

From another perspective, BACE1 inhibitors have been manufactured from peptide sequences by a substrate-based method. The initial peptide sequences showed promise with high potency and selectivity but did not progress as a viable pharmaceutical target because of the large, unstable products it produced. Branded OM99-2, this peptide was originally used to determine many functions and shapes of BACE1 including the active site. Unfortunately, it was too bulky to cross the BBB but maintained appropriate potency. Revision of this method looked specifically at the structure and function of BACE1 in order to manufacture a peptide sequence that would exploit its weaknesses [134]. It was designed to competitively inhibit the binding regions and shut down the enzymatic properties. The finished product was a long peptide upwards of 18 amino acid residues, which produced a high potency but was not stable enough for use in therapeutic trials.

These studies made it clear that the peptidic structure would fulfil the desired properties required for an inhibitor, the only issue being BBB transport which has motivated the research into small molecules that can freely penetrate the brain. The potential inhibitor needs to be lipophilic enough to permeate the BBB by passive diffusion via the use of a transporter that can maintain entry to the CNS without exiting the same way. Another issue with the peptide inhibitors is the added convolution of ubiquity throughout the body. Since amino acids, peptides and proteins are the building blocks of the human form and function, there is the added complication of anonymity with other proteins and the possibility of unwanted binding causing adverse reactions. If a severe enough reaction to the peptide is apparent, it would be more detrimental than beneficial.

## 5.2. Crossing the blood brain barrier

The BBB is a network of brain capillaries that regulate the transfer of nutrients and co-factors that are important to the functioning and maintenance of the brain. The conformation begins with the lumen that is lined with a monolayer of capillary endothelial cells, held together with tight gap junctions. This monolayer is complemented by pericytes for the pur-

pose of BBB-specific gene expression and inducing polarisation of the astrocyte end-feet with the surrounding basal lamina [135]. The tight junctions are known to prevent the paracellular diffusion of polar molecules from systemic circulation and brain parenchyma [136]. Complementing the endothelium are a number of compounds that defend against foreign transfer like cytochrome p450 and glutathione S-transferase in conjunction with transporters and the multidrug resistance associated proteins 1 and 2 (Mrp1/2) [137-139].

The defence mechanisms that protect the brain from systemic circulation become a challenging interference when fabricating an inhibitor, especially when it comes to testing the administration of localised drug performance. The molecular weight threshold, which is a relevant property of all membranes, is <450 Da [140]. A molecular model can be used to determine the effect of molecular size on membrane permeability and should be a consideration when investigating an inhibitor [141]. This creates a challenge that goes beyond the production of protein specific intervention strategies. In the case of BACE1, there are a number of different challenges, as previously mentioned, influencing the inhibition of APP cleavage. With the influence of the BBB, the drug will be required to maintain an ability to inhibit BACE1 but also maintain solubility across the tight junctions of vessel epithelium.

The natural functioning of the BBB is to maintain the tight junctions, for which it is idiosyncratic, but in order to transport a required drug, the concept of BBB disruption can be considered. The relaxing of these junctions with hyperosmotic chemicals, for example, could be enough to encourage transport to the brain. The only concern with this method is due to the unfavourable uptake of plasma proteins, like albumin, which is toxic to astrocytes [142]. Consequently, it could also affect drug retention and enhance unwanted migration of otherwise rejected contaminants.

Another drastic, invasive technique involves drilling into the cranium and administering the treatment via intracerebroventricular injection, intracerebral implantation and convection enhanced diffusion. While all are incredibly invasive and risky, the required responses from the drug once applied are generally not that positive [143]. Intracerebroventricular injection specifically has been found to cause haemorrhage after the insertion of a needle into the brain as an adverse reaction but has otherwise shown good responses [144]. This suggests these invasive techniques are questionable and should be used with extreme caution.

The interesting, non-invasive, concept of lipid carriers has the most potential. The carriers are attached to water soluble inhibitors, that cannot otherwise penetrate the BBB, and turn them lipid soluble [145]. This will allow the transfer into the BBB but once across, the requirement to either shed the carrier or be able to function with it attached in order to maintain functionality, becomes apparent. The naturally occurring, 60 amino acid, galanin-like peptide (GALP) has the ability to cross the BBB where needed with the use of a saturable transport system [146]. The glucose transporter (GLUT1) is another natural carrier system that is used frequently as glucose is the main energy source of the brain [147]. If this method

can be transformed for BACE1 inhibitors, the transfer of larger, more potent molecules with an increased selectivity, could be achieved.

### 5.3. Transporters

The ability to transport potential inhibitors across the blood brain barrier can determine the success or failure of the potential therapeutic intervention. The BBB has motivated the research base to investigate small <450 Da molecules that have the potential to pass the barrier with minimal scrutiny. The issue being a lack of potency and selectivity *in vivo* which indicates that a greater effectiveness could be achieved with a larger style inhibitor. The potential for transporters could revolutionize the future of drug production by providing a medium in which the larger more effective inhibitors can cross this barrier.

The earlier transporters aimed to cross the BBB by utilising the already formed channels and receptors. The idea was to couple an inhibitor with the specific ligand of a surface receptor like that of the H1 histone, insulin, insulin-like growth factors or transferrin [148-151]. These systems can also work by binding the inhibitor to antibodies that identify surface epitopes already present in the BBB. The coupling of an 18mer peptide to transferrin receptor specific antibodies were used to infiltrate the BBB via the transferrin receptor for the inhibition of the rev gene of HIV-1 [152]. This study demonstrated a 15-fold increase in transfer across the BBB. This system can be modified to display a number of different biotinylated peptides specifically but could also be manipulated for other effective compounds.

A more advanced method of targeted liposomes progresses from the inhibitor-ligand structure to a colloidal carrier system. The concept still utilizes BBB receptors and channels but is improved with an increase in the concentration of the inhibitor that it can carry. The liposomal system can hold >10,000 drug molecules [153]. The main question regarding this system is the avoidance of opsonins which are members of the complement system and immunoglobulins that cover the colloidal particle and are able to activate phagocytosis. This method has already been used to transfer monoclonal antibodies across the BBB [153]. The negative of this system however, is the vulnerability to macrophages via opsonisation and the lack of selectivity that liposomes have in regards to the brain and BBB. The targeted liposome system has already been used successfully to transfer sodium borocaptate in defence against malignant glioma, which suggests that further research could provide a neurodegenerative diseases template [154].

Alternatively, fullerenes are an allotropic form of carbon that form an arrangement of 60 atoms into the shape of a hollow sphere that is 1 nanometre in diameter and can be coupled with an inhibitor [155]. These fullerene systems have shown promise in the fight against chronic multiple sclerosis [156]. Whilst still in the early stages of research, recovery has been attributed to a reduction in axon loss and demyelination in the spinal region of the CNS in a mouse model. Another study has shown a reduction of infarct size in gerbils and rats with the use of a hexasulfonated C60 that was administered intravenously [157, 158]. Further

research is required for the use in human studies but there is anticipation for its use against the aggregation of A $\beta$ .

## 6. Future perspectives

There are a number of exciting directions that future research will take to determine a safe and effective treatment for AD. The regulation of internal pathways for the natural management of BACE1 activity is of key interest as it involves the crossroad from compound based, active site mimics. The recent study identifying the sAPP $\alpha$  as a BACE1 modulator could motivate the investigation of the broader pathway rather than focussing on the enzyme itself [98]. Pathway regulation could reduce side effects and associated pathologies because of the broad spectrum enzymes that envelope amyloidogenesis.

The implication of A $\beta$  signalling in plaque formation is another concept for investigation for the possibility of a feedback system. The promotion of A $\beta$  proliferation could increase the ability for a plaque to form as there is the availability to encourage the process. The application of a potential BACE1 inhibitor is rudimentary to the prevention of this disease because of the potential to bypass the amyloidogenic pathway. With minimal A $\beta$  production the pressure released on the natural inflammatory defences would allow for the metabolism of impeding plaques.

$\gamma$ -secretase inhibitors have already been progressing to phase III clinical trials which seek to influence the production of A $\beta$  but maintain a number of important consequences that surround this process [159]. The implication of the Notch signalling pathway, important in axon myelination and apoptosis, could be more detrimental than plaque formation because of the regulatory purpose in which it serves [112].

Merck have developed the MK-8931 inhibitor, which is currently undergoing phase II clinical trials [160]. It has already shown an ability to reduce A $\beta_{40}$ , A $\beta_{42}$  and sAPP $\beta$  levels, is well tolerated *in vivo* and shows minimal adverse reactions, which is encouraging for the <450 Da compounds. Eli Lilly and AstraZeneca are also involved with BACE1 inhibitor clinical trials, which will increase competition once results come to fruition. The opposing mentality behind BACE1 inhibitors, as small <450 Da compounds, could be the answer to years of attempts. The crossing of the barrier is not a major obstacle for this method but show alternative hurdles of selectivity and bioavailability. This has created a race for a pharmaceutically able inhibitor.

Alternatively, the membrane transporters concept for the advantage of allowing the transfer of a range of different inhibitors, in a variety of doses, could produce a platform for a number of different brain based diseases without being overly invasive. The BBB is an ominous obstacle for a number of the earlier peptidic inhibitors, whilst showing a lot of promising pharmaceutical attributes. The combination of the two technologies could provide the difference required for drug penetration and selectivity.

Once an inhibitor has been potentiated, the focus can move into research looking at plaque clearing and neurogenesis that will aid regeneration of the AD brain. One option is the utilization of stem cells to replace lost brain mass by utilizing multi-potent adult neural stem cells found in the subgranular and subventricular zones. The CNS has the ability to regenerate a number of neuronal cell lines with astrocytes, oligodendrocytes, and functional neurons [161]. By utilizing these cell lines the brain could be "rebuilt" to maintain the structures affected by the plaque formation. However, this would be implicated by numerous ethical challenges.

## 7. Conclusions

The onset of AD is relatively unknown, but early stages of the disease are met with the accumulation of amyloid plaques. The amyloidogenic pathway, mediated by the proteolytic cleavage of APP, is the major focus for the alleviation of AD. BACE1, the defining enzyme of this process, is responsible for the cleavage of the A $\beta$  fragment. The determinant of fragment length, 38-42 amino acids, is coordinated by  $\gamma$ -secretase. The focus is placed specifically on fragments A $\beta_{40}$  and A $\beta_{42}$  because of the influence they maintain over plaque formation and an increased affinity for aggregation.

Whilst there is an indication that  $\gamma$ -secretase maintains the ability to determine the severity of plaque formation, there are a number of reasons to avoid inhibition, mainly because of its involvement in the Notch pathway and the extreme consequences which brings to light the cost outweighing the means. This is a hallmark of plaque formation as the enzymes involved have a number of substrates in different pathways that do not involve AD. This motivates the notion of regulation, as opposed to complete inhibition, to aid the process alleviating majority of the proteolysis.

The recent association between the cleaved product of APP by  $\alpha$ -secretase, sAPP $\alpha$ , having an influence on the amyloidogenic pathway adds another perspective to AD and the mechanisms relating to brain homeostasis. It prompts the idea of maintaining normal processing as opposed to initiating inhibition. The BACE1 cleavage of APP is not a response, or trigger, to AD progression but is fundamental in A $\beta$  production, which implies that normal conservation is a natural process and regulation can be achieved with an external stimulus.

A large obstacle with potential BACE1 inhibitors is the ability to maintain the pharmaceutical properties between *in vitro* and *in vivo* testing, namely the BBB. The transfer between the Tight junctions of brain endothelium and the neuronal cells has defined the way BACE1 inhibitors are engaged. The change from basic peptidic structures to that of small <450 Da chemicals has solved one hurdle but has inadvertently created others. The original *in vitro* studies of peptidic inhibitors maintained positive selectivity for BACE1 but were too bulky for transport. The application of a BACE1 inhibitor, whatever structure it is formed from, will be rudimentary in treating and alleviating the devastating prognosis of AD in the years to come.

## Author details

Justin Read and Cenk Suphioglu

NeuroAllergy Research Laboratory (NARL), School of Life and Environmental Sciences, Deakin University, Geelong, Victoria, Australia

## References

- [1] Hu X, Hicks CW, He W, Wong P, Macklin WB, Trapp BD, et al. Bace1 Modulates Myelination in the Central and Peripheral Nervous System. *Nature Neuroscience*. 2006;9(12):1520-5.
- [2] Wong H-K, Sakurai T, Oyama F, Kaneko K, Wada K, Miyazaki H, et al.  $\beta$  Subunits of Voltage-gated Sodium Channels Are Novel Substrates of  $\beta$ -Site Amyloid Precursor Protein-cleaving Enzyme (BACE1) and  $\gamma$ -Secretase. *Journal of Biological Chemistry*. 2005;280(24):23009-17.
- [3] Choi DW. Excitotoxic cell death. *Journal of Neurobiology*. 1992;23(9):1261-76.
- [4] Brun A, Englund E. Regional Pattern of Degeneration in Alzheimer's Disease: Neuronal Loss and Histopathological Grading. *Histopathology*. 1981;5:549-64.
- [5] Brookmeyer R, Johnson E, Ziegler-Graham K, Arrighi HM. Forecasting the Global Burden of Alzheimer's Disease. *Alzheimer's and Dementia*. 2007;3(3):186-91.
- [6] WHO. Dementia: a public health priority. Geneva: World Health Organization and Alzheimer's Disease International. [http://www.who.int/mental\\_health/publications/dementia\\_report\\_2012/en/index.html](http://www.who.int/mental_health/publications/dementia_report_2012/en/index.html). (accessed 7 July 2012).
- [7] Rice DP, Fillit HM, Max W, Knopman DS, Lloyd JR, Duttagupta S. Prevalence, Costs, and Treatment of Alzheimer's Disease and Related Dementia: a Managed Care Perspective. *The American Journal of Managed Care*. 2001;7(8):809-18.
- [8] Malone DC, McLaughlin TP, Wahl PM, Leibman C, Arrighi M, Cziraky MJ, et al. Burden of Alzheimer's Disease and Association With Negative Health Outcomes. *The American Journal of Managed Care*. 2009;15(8):481-8.
- [9] Wenk GL. Neuropathologic Changes in Alzheimer's Disease. *The Journal of Clinical Psychiatry*. 2003;64 Suppl 9:7-10.
- [10] Sultzer DL, Brown CV, Mandelkern MA, Mahler ME, Mendez MF, Chen ST, et al. Delusional Thoughts and Regional Frontal/Temporal Cortex Metabolism in Alzheimer's Disease. *American Journal Of Psychiatry*. 2003;160:341-9.
- [11] Bruen PD, McGeown WJ, Shanks MF, Venneri A. Neuroanatomical Correlates of Neuropsychiatric Symptoms in Alzheimer's Disease. *Brain*. 2008;131(9):2455-63.

- [12] Fountoulakis M, Kossida S. Proteomics-driven Progress in Neurodegeneration Research. *Electrophoresis*. 2006;27(8):1556-73.
- [13] Cai H, Wang Y, McCarthy D, Wen H, Borchelt DR, Price DL, et al. BACE1 is the Major  $\beta$ -secretase for Generation of A $\beta$  Peptides by Neurons. *Nature Neuroscience*. 2001;4(3):233-4.
- [14] Spillantini MG, Goedert M. Tau Protein Pathology in Neurodegenerative Diseases. *Trends in Neurosciences*. 1998;21(10):428-33.
- [15] Lebouvier T, Scales TME, Williamson R, Noble W, Duyckaerts C, Hanger DP, et al. The Microtubule-Associated Protein Tau is Also Phosphorylated on Tyrosine. *Journal of Alzheimer's Disease*. 2009;18(1):1-9.
- [16] Selkoe DJ. Alzheimer Disease: Mechanistic Understanding Predicts Novel Therapies. *Annals of Internal Medicine*. 2004;140(8):627-38.
- [17] Eikelenboom P, Hack C, Rozemuller J, Stam F. Complement Activation in Amyloid Plaques in Alzheimer's Dementia. *Virchows Archiv B Cell Pathology Zell-pathologie*. 1988;56(1):259-62.
- [18] Tuppo EE, Arias HR. The Role of Inflammation in Alzheimer's Disease. *The International Journal of Biochemistry & Cell Biology*. 2005;37(2):289-305.
- [19] Wolfe MS. When Loss is Gain: Reduced Presenilin Proteolytic Function Leads to Increased A $\beta$ 42/A $\beta$ 40. *EMBO Reports*. 2007;8(2):136-40.
- [20] Arendt T. Synaptic Degeneration in Alzheimer's Disease. *Acta Neuropathologica*. 2009;118(1):167-79.
- [21] Francis PT. Glutamatergic Systems in Alzheimer's Disease. *International Journal of Geriatric Psychiatry*. 2003;18(S1):S15-S21.
- [22] Nixon RA, Wegiel J, Kumar A, Yu WH, Peterhoff C, Cataldo A, et al. Extensive Involvement of Autophagy in Alzheimer Disease: An Immuno-Electron Microscopy Study. *Journal of Neuropathology & Experimental Neurology*. 2005;64(2):113-22.
- [23] Martins IJ, Berger T, Sharman MJ, Verdile G, Fuller SJ, Martins RN. Cholesterol Metabolism and Transport in the Pathogenesis of Alzheimer's Disease. *Journal of Neurochemistry*. 2009;111(6):1275-308.
- [24] Bibel M, Barde Y-A. Neurotrophins: Key Regulators of Cell Fate and Cell Shape in the Vertebrate Nervous System. *Genes & Development*. 2000;14(23):2919-37.
- [25] Morfini GA, Burns M, Binder LI, Kanaan NM, LaPointe N, Bosco DA, et al. Axonal Transport Defects in Neurodegenerative Diseases. *The Journal of Neuroscience*. 2009;29(41):12776-86.

- [26] Hardy J, Duff K, Hardy KG, Perez-Tur J, Hutton M. Genetic Dissection of Alzheimer's Disease and Related Dementias: Amyloid and its Relationship to Tau. *Nature Neuroscience*. 1998;1(5):355-8.
- [27] Varani L, Hasegawa M, Spillantini MG, Smith MJ, Murrell JR, Ghetti B, et al. Structure of Tau Exon 10 Splicing Regulatory Element RNA and Destabilization by Mutations of Frontotemporal Dementia and Parkinsonism Linked to Chromosome 17. *Proceedings of the National Academy of Sciences*. 1999;96(14):8229-34.
- [28] Glenner GG, Wong CW. Alzheimer's Disease: Initial Report of the Purification and Characterization of a Novel Cerebrovascular Amyloid Protein. *Biochemical and Biophysical Research Communications*. 1984;120(3):885-90.
- [29] Tanzi R, Gusella J, Watkins P, Bruns G, St George-Hyslop P, Van Keuren M, et al. Amyloid Beta Protein Gene: cDNA, mRNA Distribution, and Genetic Linkage Near the Alzheimer Locus. *Science*. 1987;235(4791):880-4.
- [30] Kang J, Lemaire H-G, Unterbeck A, Salbaum JM, Masters CL, Grzeschik K-H, et al. The Precursor of Alzheimer's Disease Amyloid A $\beta$  Protein Resembles a Cell-surface Receptor. *Nature*. 1987;325(6106):733-6.
- [31] Lee J, Retamal C, Cuitiño L, Caruano-Yzermans A, Shin J-E, van Kerkhof P, et al. Adaptor Protein Sorting Nexin 17 Regulates Amyloid Precursor Protein Trafficking and Processing in the Early Endosomes. *Journal of Biological Chemistry*. 2008;283(17):11501-8.
- [32] Jorissen E, Prox J, Bernreuther C, Weber S, Schwanbeck R, Serneels L, et al. The Disintegrin/Metalloproteinase ADAM10 Is Essential for the Establishment of the Brain Cortex. *The Journal of Neuroscience*. 2010;30(14):4833-44.
- [33] Selkoe DJ. The Molecular Pathology of Alzheimer's Disease. *Neuron*. 1991;6(4):487-98.
- [34] Vassar R, Bennett BD, Babu-Khan S, Kahn S, Mendiaz EA, Denis P, et al.  $\beta$ -Secretase Cleavage of Alzheimer's Amyloid Precursor Protein by the Transmembrane Aspartic Protease BACE. *Science*. 1999;286(5440):735-41.
- [35] Kuhn P-H, Wang H, Dislich B, Colombo A, Zeitschel U, Ellwart JW, et al. ADAM10 is the Physiologically Relevant, Constitutive  $\alpha$ -secretase of the Amyloid Precursor Protein in Primary Neurons. *The EMBO Journal*. 2010;29(17):3020-32.
- [36] Alves da Costa C, Sunyach C, Pardossi-Piquard R, Sévalle J, Vincent B, Boyer N, et al. Presenilin-Dependent  $\gamma$ -Secretase-Mediated Control of p53-Associated Cell Death in Alzheimer's Disease. *The Journal of Neuroscience*. 2006;26(23):6377-85.
- [37] Madeira A, Pommet J-M, Prochiantz A, Allinquant B. SET Protein (TAF1 $\beta$ , I2PP2A) is Involved in Neuronal Apoptosis Induced by an Amyloid Precursor Protein Cytoplasmic Subdomain. *The FASEB Journal*. 2005;19(13):1905-07.

- [38] Müller T, Concannon CG, Ward MW, Walsh CM, Tirniceriu AL, Tribl F, et al. Modulation of Gene Expression and Cytoskeletal Dynamics by the Amyloid Precursor Protein Intracellular Domain (AICD). *Molecular Biology of the Cell*. 2007;18(1):201-10.
- [39] Dulin F, Léveillé F, Ortega JB, Mornon J-P, Buisson A, Callebaut I, et al. p3 Peptide, a Truncated Form of A $\beta$  Devoid of Synaptotoxic Effect, Does Not Assemble Into Soluble Oligomers. *FEBS Letters*. 2008;582(13):1865-70.
- [40] Walsh DM, Klyubin I, Fadeeva JV, Cullen WK, Anwyl R, Wolfe MS, et al. Naturally Secreted Oligomers of Amyloid $\beta$  Protein Potently Inhibit Hippocampal Long-term Potentiation in vivo. *Nature*. 2002;416(6880):535-9.
- [41] De Strooper B. Aph-1, Pen-2, and Nicastrin with Presenilin Generate an Active  $\gamma$ -Secretase Complex. *Neuron*. 2003;38(1):9-12.
- [42] Takasugi N, Tomita T, Hayashi I, Tsuruoka M, Niimura M, Takahashi Y, et al. The Role of Presenilin Cofactors in the  $\gamma$ -secretase Complex. *Nature*. 2003;422(6930):438-41.
- [43] LaVoie MJ, Fraering PC, Ostaszewski BL, Ye W, Kimberly WT, Wolfe MS, et al. Assembly of the  $\gamma$ -Secretase Complex Involves Early Formation of an Intermediate Subcomplex of Aph-1 and Nicastrin. *Journal of Biological Chemistry*. 2003;278(39):37213-22.
- [44] Wolfe M, Xia W, Ostaszewski B, Diehl T, Kimberly W, Selkoe D. Two Transmembrane Aspartates in Presenilin-1 Required for Presenilin Endoproteolysis and  $\gamma$ -secretase Activity. *Nature*. 1999;398(6727):513-7.
- [45] Levy-Lahad E, Wasco W, Poorkaj P, Romano D, Oshima J, Pettingell W, et al. Candidate Gene for the Chromosome 1 Familial Alzheimer's Disease Locus. *Science*. 1995;269(5226):973-7.
- [46] Francis R, McGrath G, Zhang J, Ruddy DA, Sym M, Apfeld J, et al. aph-1 and pen-2 Are Required for Notch Pathway Signaling,  $\gamma$ -Secretase Cleavage of  $\beta$ APP, and Presenilin Protein Accumulation. *Developmental Cell*. 2002;3(1):85-97.
- [47] Tanzi RE, Bertram L. Twenty Years of the Alzheimer's Disease Amyloid Hypothesis: A Genetic Perspective. *Cell*. 2005;120(4):545-55.
- [48] Suzuki N, Cheung T, Cai X, Odaka A, Otvos L, Eckman C, et al. An Increased Percentage of Long Amyloid Beta Protein Secreted by Familial Amyloid Beta Protein Precursor (Beta APP717) Mutants. *Science*. 1994;264(5163):1336-40.
- [49] Burdick D, Soreghan B, Kwon M, Kosmoski J, Knauer M, Henschen A, et al. Assembly and Aggregation Properties of Synthetic Alzheimer's A4/beta Amyloid Peptide Analogs. *Journal of Biological Chemistry*. 1992;267(1):546-54.

- [50] Sato T, Dohmae N, Qi Y, Kakuda N, Misonou H, Mitsumori R, et al. Potential Link between Amyloid  $\beta$ -Protein 42 and C-terminal Fragment  $\gamma$  49–99 of  $\beta$ -Amyloid Precursor Protein. *Journal of Biological Chemistry*. 2003;278(27):24294-301.
- [51] Zou K, Kim D, Kakio A, Byun K, Gong J-S, Kim J, et al. Amyloid  $\beta$ -protein (A $\beta$ )<sub>1–40</sub> Protects Neurons from Damage Induced by A $\beta$ <sub>1–42</sub> in Culture and in Rat Brain. *Journal of Neurochemistry*. 2003;87(3):609-19.
- [52] Duering M, Grimm MOW, Grimm HS, Schröder J, Hartmann T. Mean Age of Onset in Familial Alzheimer's Disease is Determined by Amyloid Beta 42. *Neurobiology of Aging*. 2005;26(6):785-8.
- [53] Coles M, Bicknell W, Watson AA, Fairlie DP, Craik DJ. Solution Structure of Amyloid  $\beta$ -Peptide(1–40) in a Water–Micelle Environment. Is the Membrane-Spanning Domain Where We Think It Is? *Biochemistry*. 1998;37(31):11064-77.
- [54] Crescenzi O, Tomaselli S, Guerrini R, Salvadori S, D'Ursi AM, Temussi PA, et al. Solution Structure of the Alzheimer Amyloid  $\beta$ -peptide (1–42) in an Apolar Microenvironment. *European Journal of Biochemistry*. 2002;269(22):5642-8.
- [55] Zhang S, Iwata K, Lachenmann MJ, Peng JW, Li S, Stimson ER, et al. The Alzheimer's Peptide A $\beta$  Adopts a Collapsed Coil Structure in Water. *Journal of Structural Biology*. 2000;130(2–3):130-41.
- [56] Barrow C, Zagorski M. Solution Structures of Beta Peptide and its Constituent Fragments: Relation to Amyloid Deposition. *Science*. 1991;253(5016):179-82.
- [57] Ohyagi Y, Asahara H, Chui D-H, Tsuruta Y, Sakae N, Miyoshi K, et al. Intracellular A $\beta$ <sub>42</sub> Activates p53 Promoter: a Pathway to Neurodegeneration in Alzheimer's Disease. *The FASEB Journal*. 2004;19(2):255-57.
- [58] Hartley DM, Walsh DM, Ye CP, Diehl T, Vasquez S, Vassilev PM, et al. Protofibrillar Intermediates of Amyloid  $\beta$ -Protein Induce Acute Electrophysiological Changes and Progressive Neurotoxicity in Cortical Neurons. *The Journal of Neuroscience*. 1999;19(20):8876-84.
- [59] Yankner B, Dawes L, Fisher S, Villa-Komaroff L, Oster-Granite M, Neve R. Neurotoxicity of a Fragment of the Amyloid Precursor Associated with Alzheimer's Disease. *Science*. 1989;245(4916):417-20.
- [60] Shen J, Bronson RT, Chen DF, Xia W, Selkoe DJ, Tonegawa S. Skeletal and CNS Defects in Presenilin-1-Deficient Mice. *Cell*. 1997;89(4):629-39.
- [61] De Strooper B, Saftig P, Craessaerts K, Vanderstichele H, Guhde G, Annaert W, et al. Deficiency of Presenilin-1 Inhibits the Normal Cleavage of Amyloid Precursor Protein. *Nature*. 1998;391(6665):387-90.
- [62] Wyss-Coray T, Mucke L. Inflammation in Neurodegenerative Disease—A Double-Edged Sword. *Neuron*. 2002;35(3):419-32.

- [63] Rezaie P, Male D. Mesoglia & Microglia-A Historical Review of the Concept of Mononuclear Phagocytes Within the Central Nervous System. *Journal of the History of the Neurosciences*. 2002;11(4):325.
- [64] Yan SD, Roher A, Schmidt AM, Stern DM. Cellular Cofactors for Amyloid  $\beta$ -Peptide-Induced Cell Stress: Moving from Cell Culture to in vivo. *The American Journal of Pathology*. 1999;155(5):1403-11.
- [65] Yan SD, Chen X, Fu J, Chen M, Zhu H, Roher A, et al. RAGE and Amyloid- $\beta$  Peptide Neurotoxicity in Alzheimer's Disease. *Nature*. 1996;382(6593):685-91.
- [66] Tan J, Town T, Paris D, Mori T, Suo Z, Crawford F, et al. Microglial Activation Resulting from CD40-CD40L Interaction After  $\beta$ -Amyloid Stimulation. *Science*. 1999;286(5448):2352-5.
- [67] Mrak RE, Griffin WST. Glia and Their Cytokines in Progression of Neurodegeneration. *Neurobiology of Aging*. 2005;26(3):349-54.
- [68] Walker DG, Lue LF. Investigations with Cultured Human Microglia on Pathogenic Mechanisms of Alzheimer's Disease and Other Neurodegenerative Diseases. *Journal of Neuroscience Research*. 2005;81(3):412-25.
- [69] Bard F, Cannon C, Barbour R, Burke R-L, Games D, Grajeda H, et al. Peripherally Administered Antibodies Against Amyloid  $\beta$ -peptide Enter the Central Nervous System and Reduce Pathology in a Mouse Model of Alzheimer Disease. *Nature Medicine*. 2000;6(8):916-9.
- [70] Majumdar A, Chung H, Dolios G, Wang R, Asamoah N, Lobel P, et al. Degradation of Fibrillar Forms of Alzheimer's Amyloid  $\beta$ -peptide by Macrophages. *Neurobiology of Aging*. 2008;29(5):707-15.
- [71] Banati RB, Gehrman J, Czech C, Mönning U, Jones LL, König G, et al. Early and Rapid de novo Synthesis of Alzheimer  $\beta$ A4-Amyloid Precursor Protein (APP) in Activated Microglia. *Glia*. 1993;9(3):199-210.
- [72] Shi J, Yang SH, Stubble L, Day AL, Simpkins JW. Hypoperfusion Induces Overexpression of  $\beta$ -amyloid Precursor Protein mRNA in a Focal Ischemic Rodent Model. *Brain Research*. 2000;853(1):1-4.
- [73] Ciallella JR, Ikonovic MD, Paljug WR, Wilbur YI, Dixon CE, Kochanek PM, et al. Changes in Expression of Amyloid Precursor Protein and Interleukin-1 $\beta$  After Experimental Traumatic Brain Injury in Rats. *Journal of Neurotrauma*. 2002;19(12):1555-67.
- [74] Guglielmotto M, Monteleone D, Giliberto L, Fornaro M, Borghi R, Tamagno E, et al. Amyloid- $\beta$ <sub>42</sub> Activates the Expression of BACE1 Through the JNK Pathway. *Journal of Alzheimer's Disease*. 2011;27(4):871-83.

- [75] Davies C, Tournier C. Exploring the Function of the JNK (c-Jun N-terminal Kinase) Signalling Pathway in Physiological and Pathological Processes to Design Novel Therapeutic Strategies. *Biochemical Society Transactions* 2012;40(1):85-9.
- [76] Benjannet S, Elagoz A, Wickham L, Mamarbachi M, Munzer JS, Basak A, et al. Post-translational Processing of  $\beta$ -Secretase ( $\beta$ -Amyloid-converting Enzyme) and its Ecto-domain Shedding. *Journal of Biological Chemistry*. 2001;276(14):10879-87.
- [77] Creemers JWM, Ines Dominguez D, Plets E, Serneels L, Taylor NA, Multhaup G, et al. Processing of  $\beta$ -Secretase by Furin and Other Members of the Proprotein Convertase Family. *Journal of Biological Chemistry*. 2001;276(6):4211-7.
- [78] Shi X-P, Chen E, Yin K-C, Na S, Garsky VM, Lai M-T, et al. The Pro Domain of  $\beta$ -Secretase Does Not Confer Strict Zymogen-like Properties but Does Assist Proper Folding of the Protease Domain. *Journal of Biological Chemistry*. 2001;276(13):10366-73.
- [79] Haniu M, Denis P, Young Y, Mendiaz EA, Fuller J, Hui JO, et al. Characterization of Alzheimer's  $\beta$ -Secretase Protein BACE. *Journal of Biological Chemistry*. 2000;275(28):21099-106.
- [80] Hong L, Koelsch G, Lin X, Wu S, Terzyan S, Ghosh AK, et al. Structure of the Protease Domain of Memapsin 2 ( $\beta$ -Secretase) Complexed with Inhibitor. *Science*. 2000;290(5489):150-3.
- [81] Charlwood J, Dingwall C, Matico R, Hussain I, Johanson K, Moore S, et al. Characterization of the Glycosylation Profiles of Alzheimer's  $\beta$ -Secretase Protein Asp-2 Expressed in a Variety of Cell Lines. *Journal of Biological Chemistry*. 2001;276(20):16739-48.
- [82] Hussain I, Powell D, Howlett DR, Tew DG, Meek TD, Chapman C, et al. Identification of a Novel Aspartic Protease (Asp 2) as  $\beta$ -secretase. *Molecular and Cellular Neurosciences*. 1999;14(6):419-27.
- [83] Sinha S, Anderson JP, Barbour R, Basi GS, Caccaveffo R, Davis D, et al. Purification and Cloning of Amyloid Precursor Protein  $\beta$ -secretase from Human Brain. *Nature*. 1999;402(6761):537-40.
- [84] Yan R, Blenkowski MJ, Shuck ME, Miao H, Tory MC, Pauley AM, et al. Membrane-anchored Aspartyl Protease with Alzheimer's Disease  $\beta$ -secretase Activity. *Nature*. 1999;402(6761):533-7.
- [85] Lin X, Koelsch G, Wu S, Downs D, Dashti A, Tang J. Human Aspartic Protease Memapsin 2 Cleaves the  $\beta$ -secretase Site of  $\beta$ -amyloid Precursor Protein. *Proceedings of the National Academy of Sciences of the United States of America*. 2000;97(4):1456-60.

- [86] Walter J, Fluhner R, Hartung B, Willem M, Kaether C, Capell A, et al. Phosphorylation Regulates Intracellular Trafficking of  $\beta$ -Secretase. *Journal of Biological Chemistry*. 2001;276(18):14634-41.
- [87] Shimizu H, Tosaki A, Kaneko K, Hisano T, Sakurai T, Nukina N. Crystal Structure of an Active Form of BACE1, an Enzyme Responsible for Amyloid  $\beta$  Protein Production. *Molecular and Cellular Biology*. 2008;28(11):3663-71.
- [88] Pasternak SH, Bagshaw RD, Guiral M, Zhang S, Ackerley CA, Pak BJ, et al. Presenilin-1, Nicastrin, Amyloid Precursor Protein, and  $\gamma$ -Secretase Activity are Co-localized in the Lysosomal Membrane. *Journal of Biological Chemistry*. 2003;278(29):26687-94.
- [89] Yan R, Munzner JB, Shuck ME, Bienkowski MJ. BACE2 Functions as an Alternative  $\alpha$ -Secretase in Cells. *Journal of Biological Chemistry*. 2001;276(36):34019-27.
- [90] Lingwood D, Simons K. Lipid Rafts As a Membrane-Organizing Principle. *Science*. 2010;327(5961):46-50.
- [91] Lin X, Koelsch G, Wu S, Downs D, Dashti A, Tang J. Human Aspartic Protease Memapsin 2 Cleaves the  $\beta$ -secretase Site of  $\beta$ -amyloid Precursor Protein. *Proceedings of the National Academy of Sciences*. 2000;97(4):1456-60.
- [92] Zhao J, Fu Y, Yasvoina M, Shao P, Hitt B, O'Connor T, et al.  $\beta$ -Site Amyloid Precursor Protein Cleaving Enzyme 1 Levels Become Elevated in Neurons around Amyloid Plaques: Implications for Alzheimer's Disease Pathogenesis. *The Journal of Neuroscience*. 2007;27(14):3639-49.
- [93] Coulson DTR, Beyer N, Quinn JG, Brockbank S, Hellemans J, Irvine GB, et al. BACE1 mRNA Expression in Alzheimer's Disease Postmortem Brain Tissue. *Journal Of Alzheimer's Disease: JAD*. 2010;22(4):1111-22.
- [94] Davies BA, Lee JRE, Oestreich AJ, Katzmann DJ. Membrane Protein Targeting to the MVB/Lysosome. *Chemical Reviews*. 2009;109(4):1575-86.
- [95] Kang EL, Cameron AN, Piazza F, Walker KR, Tesco G. Ubiquitin Regulates GGA3-mediated Degradation of BACE1. *Journal of Biological Chemistry*. 2010;285(31):24108-19.
- [96] Koh YH, von Arnim CAF, Hyman BT, Tanzi RE, Tesco G. BACE Is Degraded via the Lysosomal Pathway. *Journal of Biological Chemistry*. 2005;280(37):32499-504.
- [97] Sannerud R, Declerck I, Peric A, Raemaekers T, Menendez G, Zhou L, et al. ADP Ribosylation Factor 6 (ARF6) Controls Amyloid Precursor Protein (APP) Processing by Mediating the Endosomal Sorting of BACE1. *Proceedings of the National Academy of Sciences*. 2011;108(34):E559-E68.
- [98] Obregon D, Hou H, Deng J, Giunta B, Tian J, Darlington D, et al. Soluble Amyloid Precursor Protein- $\alpha$  Modulates  $\beta$ -secretase Activity and Amyloid- $\beta$  Generation. *Nature Communicationa*. 2012;3:777.

- [99] Sun X, Wang Y, Qing H, Christensen MA, Liu Y, Zhou W, et al. Distinct Transcriptional Regulation and Function of the Human BACE2 and BACE1 Genes. *The FASEB Journal*. 2005;19(7):739-49.
- [100] Farzan M, Schnitzler CE, Vasilieva N, Leung D, Choe H. BACE2, a  $\beta$ -secretase Homolog, Cleaves at the  $\beta$  Site and Within the Amyloid- $\beta$  Region of the Amyloid- $\beta$  Precursor Protein. *Proceedings of the National Academy of Sciences*. 2000;97(17):9712-7.
- [101] Luo Y, Bolon B, Damore MA, Fitzpatrick D, Liu H, Zhang J, et al. BACE1 ( $\beta$ -secretase) Knockout Mice do not Acquire Compensatory Gene Expression Changes or Develop Neural Lesions Over Time. *Neurobiology of Disease*. 2003;14(1):81-8.
- [102] Bennett BD, Babu-Khan S, Loeloff R, Louis J-C, Curran E, Citron M, et al. Expression Analysis of BACE2 in Brain and Peripheral Tissues. *Journal of Biological Chemistry*. 2000;275(27):20647-51.
- [103] von Arnim CAF, Kinoshita A, Peltan ID, Tangredi MM, Herl L, Lee BM, et al. The Low Density Lipoprotein Receptor-related Protein (LRP) is a Novel  $\beta$ -Secretase (BACE1) Substrate. *Journal of Biological Chemistry*. 2005;280(18):17777-85.
- [104] Li Q, Südhof TC. Cleavage of Amyloid- $\beta$  Precursor Protein and Amyloid- $\beta$  Precursor-like Protein by BACE 1. *Journal of Biological Chemistry*. 2004;279(11):10542-50.
- [105] Lichtenthaler SF, Dominguez D-i, Westmeyer GG, Reiss K, Haass C, Saftig P, et al. The Cell Adhesion Protein P-selectin Glycoprotein Ligand-1 is a Substrate for the Aspartyl Protease BACE1. *Journal of Biological Chemistry*. 2003;278(49):48713-9.
- [106] Kuhn P-H, Marjaux E, Imhof A, De Strooper B, Haass C, Lichtenthaler SF. Regulated Intramembrane Proteolysis of the Interleukin-1 Receptor II by  $\alpha$ -,  $\beta$ -, and  $\gamma$ -secretase. *Journal of Biological Chemistry*. 2007;282(16):11982-95.
- [107] Hemming ML, Elias JE, Gygi SP, Selkoe DJ. Identification of  $\beta$ -Secretase (BACE1) Substrates Using Quantitative Proteomics. *PLoS ONE*. 2009;4(12):e8477.
- [108] Dominguez D, Tournoy J, Hartmann D, Huth T, Cryns K, Deforce S, et al. Phenotypic and Biochemical Analyses of BACE1- and BACE2-deficient Mice. *Journal of Biological Chemistry*. 2005;280(35):30797-806.
- [109] Hitt B, Jaramillo T, Chetkovich D, Vassar R. BACE1-/- Mice Exhibit Seizure Activity that does not Correlate with Sodium Channel Level or Axonal Localization. *Molecular Neurodegeneration*. 2010;5(1):31.
- [110] Siman R, Reaume AG, Savage MJ, Trusko S, Lin Y-G, Scott RW, et al. Presenilin-1 P264L Knock-In Mutation: Differential Effects on A $\beta$  Production, Amyloid Deposition, and Neuronal Vulnerability. *The Journal of Neuroscience*. 2000;20(23):8717-26.
- [111] Flood DG, Reaume AG, Dorfman KS, Lin Y-G, Lang DM, Trusko SP, et al. FAD Mutant PS-1 Gene-Targeted Mice: Increased A $\beta$ 42 and A $\beta$  Deposition Without APP Overproduction. *Neurobiology of Aging*. 2002;23(3):335-48.

- [112] Louvi A, Artavanis-Tsakonas S. Notch Signalling in Vertebrate Neural Development. *Nature Reviews Neuroscience*. 2006;7(2):93-102.
- [113] Grüninger-Leitch F, Schlatter D, Kung E, Nelböck P, Döbeli H. Substrate and Inhibitor Profile of BACE ( $\beta$ -Secretase) and Comparison with Other Mammalian Aspartic Proteases. *Journal of Biological Chemistry*. 2002;277(7):4687-93.
- [114] Nguyen J-T, Hamada Y, Kimura T, Kiso Y. Design of Potent Aspartic Protease Inhibitors to Treat Various Diseases. *Archiv der Pharmazie*. 2008;341(9):523-35.
- [115] Hansen RA, Gartlehner G, Webb AP, Morgan LC, Moore CG, Jonas DE. Efficacy and Safety of Donepezil, Galantamine, and Rivastigmine for the Treatment of Alzheimer's Disease: A Systematic Review and Meta-analysis. *Dove Medical Press*. 2008;3(2):211-25.
- [116] Ghosh AK, Kumaragurubaran N, Hong L, Koelsh G, Tang J. Memapsin 2 (Beta-Secretase) Inhibitors: Drug Development. *Current Alzheimer Research*. 2008;5(2):121-31.
- [117] Román GC, Kalaria RN. Vascular Determinants of Cholinergic Deficits in Alzheimer Disease and Vascular Dementia. *Neurobiology of Aging*. 2006;27(12):1769-85.
- [118] Hellström-Lindahl E, Moore H, Nordberg A. Increased Levels of Tau Protein in SH-SY5Y Cells After Treatment with Cholinesterase Inhibitors and Nicotinic Agonists. *Journal of Neurochemistry*. 2000;74(2):777-84.
- [119] Chalmers K, Wilcock G, Vinters H, Perry E, Perry R, Ballard C, et al. Cholinesterase Inhibitors may Increase Phosphorylated Tau in Alzheimer's Disease. *Journal of Neurology*. 2009;256(5):717-20.
- [120] Danysz W, Parsons C, Möbius H-J, Stöffler A, Quack G. Neuroprotective and Symptomatological Action of Memantine Relevant for Alzheimer's Disease — a Unified Glutamatergic Hypothesis on the Mechanism of Action. *Neurotoxicity Research*. 2000;2(2):85-97.
- [121] Orrego F, Villanueva S. The Chemical Nature of the Main Central Excitatory Transmitter: A Critical Appraisal Based Upon Release Studies and Synaptic Vesicle Localization. *Neuroscience*. 1993;56(3):539-55.
- [122] Lipton SA, Rosenberg PA. Excitatory Amino Acids as a Final Common Pathway for Neurologic Disorders. *New England Journal of Medicine*. 1994;330(9):613-22.
- [123] Luo X, Yan R. Inhibition of BACE1 for Therapeutic use in Alzheimer's Disease. *International Journal of Clinical and Experimental Pathology*. 2010;3(6):618-28.
- [124] Guo T, Hobbs DW. Development of BACE1 Inhibitors for Alzheimer's Disease. *Current Medicinal Chemistry*. 2006;13(15):1811-29.
- [125] Baxter EW, Conway KA, Kennis L, Bischoff F, Mercken MH, De Winter HL, et al. 2-Amino-3,4-dihydroquinazolines as Inhibitors of BACE-1 ( $\beta$ -Site APP Cleaving En-

- zyme): Use of Structure Based Design to Convert a Micromolar Hit into a Nanomolar Lead. *Journal of Medicinal Chemistry*. 2007;50(18):4261-4.
- [126] Cole DC, Manas ES, Stock JR, Condon JS, Jennings LD, Aulabaugh A, et al. Acylguanidines as Small-Molecule  $\beta$ -Secretase Inhibitors. *Journal of Medicinal Chemistry*. 2006;49(21):6158-61.
- [127] Edwards PD, Albert JS, Sylvester M, Aharony D, Andisik D, Callaghan O, et al. Application of Fragment-Based Lead Generation to the Discovery of Novel, Cyclic Amidine  $\beta$ -Secretase Inhibitors with Nanomolar Potency, Cellular Activity, and High Ligand Efficiency. *Journal of Medicinal Chemistry*. 2007;50(24):5912-25.
- [128] Hussain I, Hawkins J, Harrison D, Hille C, Wayne G, Cutler L, et al. Oral Administration of a Potent and Selective Non-peptidic BACE-1 Inhibitor Decreases  $\beta$ -cleavage of Amyloid Precursor Protein and Amyloid- $\beta$  Production in vivo. *Journal of Neurochemistry*. 2007;100(3):802-9.
- [129] Iserloh U, Pan J, Stamford AW, Kennedy ME, Zhang Q, Zhang L, et al. Discovery of an Orally Efficacious 4-phenoxyprolidine-based BACE-1 Inhibitor. *Bioorganic and Medicinal Chemistry Letters*. 2008;18(1):418-22.
- [130] Kortum SW, Benson TE, Bienkowski MJ, Emmons TL, Prince DB, Paddock DJ, et al. Potent and Selective Isophthalamide S2 Hydroxyethylamine Inhibitors of BACE1. *Bioorganic and Medicinal Chemistry Letters*. 2007;17(12):3378-83.
- [131] Marcin LR, Higgins MA, Zusi FC, Zhang Y, Dee MF, Parker MF, et al. Synthesis and SAR of Indole-and 7-azaindole-1,3-dicarboxamide Hydroxyethylamine Inhibitors of BACE-1. *Bioorganic & Medicinal Chemistry Letters*. 2011;21(1):537-41.
- [132] Stachel SJ, Coburn CA, Steele TG, Jones KG, Loutzenhiser EF, Gregro AR, et al. Structure-Based Design of Potent and Selective Cell-Permeable Inhibitors of Human  $\beta$ -Secretase (BACE-1). *Journal of Medicinal Chemistry*. 2004;47(26):6447-50.
- [133] Stachel SJ, Coburn CA, Sankaranarayanan S, Price EA, Pietrak BL, Huang Q, et al. Macrocyclic Inhibitors of  $\beta$ -Secretase: Functional Activity in an Animal Model. *Journal of Medicinal Chemistry*. 2006;49(21):6147-50.
- [134] Tung JS, Davis DL, Anderson JP, Walker DE, Mamo S, Jewett N, et al. Design of Substrate-Based Inhibitors of Human  $\beta$ -Secretase. *Journal of Medicinal Chemistry*. 2001;45(2):259-62.
- [135] Armulik A, Genove G, Mae M, Nisancioglu MH, Wallgard E, Niaudet C, et al. Pericytes Regulate the Blood-brain Barrier. *Nature*. 2010;468(7323):557-61.
- [136] Brightman MW, Reese TS. Junctions Between Intimately Apposed Cell Membranes in the Vertebrate Brain. *The Journal of Cell Biology*. 1969;40(3):648-77.

- [137] Ghersi-Egea JF, Leninger-Muller B, Suleman G, Siest G, Minn A. Localization of Drug-metabolizing Enzyme Activities to Blood-Brain Interfaces and Circumventricular Organs. *Journal of Neurochemistry*. 1994;62(3):1089-96.
- [138] Meyer RP, Gehlhaus M, Knoth R, Volk B. Expression and Function of Cytochrome p450 in Brain Drug Metabolism. *Current Drug Metabolism*. 2007;8(4):297-306.
- [139] Bauer B, Hartz AMS, Lucking JR, Yang X, Pollack GM, Miller DS. Coordinated Nuclear Receptor Regulation of the Efflux Transporter, Mrp2, and the Phase-II Metabolizing Enzyme, GST[pi], at the Blood-brain Barrier. *Journal of Cerebral Blood Flow Metabolism*. 2008;28(6):1222-34.
- [140] Fischer H, Gottschlich R, Seelig A. Blood-Brain Barrier Permeation: Molecular Parameters Governing Passive Diffusion. *Journal of Membrane Biology*. 1998;165(3):201-11.
- [141] Träuble H. The Movement of Molecules Across Lipid Membranes: A Molecular Theory. *Journal of Membrane Biology*. 1971;4(1):193-208.
- [142] Nadal A, Fuentes E, Pastor J, McNaughton PA. Plasma Albumin is a Potent Trigger of Calcium Signals and DNA Synthesis in Astrocytes. *Proceedings of the National Academy of Sciences of the United States of America*. 1995;92(5):1426-30.
- [143] Pardridge WM. Blood-brain Barrier Delivery. *Drug Discovery Today*. 2007;12(1-2):54-61.
- [144] Tuszynski MH, Thal L, Pay M, Salmon DP, U HS, Bakay R, et al. A Phase 1 Clinical Trial of Nerve Growth Factor Gene Therapy for Alzheimer Disease. *Nature Medicine*. 2005;11(5):551-5.
- [145] Bodor N, Prokai L, Wu W, Farag H, Jonalagadda S, Kawamura M, et al. A Strategy for Delivering Peptides into the Central Nervous System by Sequential Metabolism. *Science*. 1992;257(5077):1698-700.
- [146] Kastin AJ, Akerstrom V, Hackler L. Food Deprivation Decreases Blood Galanin-like Peptide and its Rapid Entry into the Brain. *Neuroendocrinology*. 2001;74(6):423-32.
- [147] Simpson IA, Carruthers A, Vannucci SJ. Supply and Demand in Cerebral Energy Metabolism: the Role of Nutrient Transporters. *Journal of Cerebral Blood Flow Metabolism*. 2007;27(11):1766-91.
- [148] Duffy KR, Pardridge WM. Blood-brain Barrier Transcytosis of Insulin in Developing Rabbits. *Brain Research*. 1987;420(1):32-8.
- [149] Fishman JB, Rubin JB, Handrahan JV, Connor JR, Fine RE. Receptor-mediated Transcytosis of Transferrin Across the Blood-brain Barrier. *Journal of Neuroscience Research*. 1987;18(2):299-304.
- [150] Reinhardt RR, Bondy CA. Insulin-like Growth Factors Cross the Blood-brain Barrier. *Endocrinology*. 1994;135(5):1753-61.

- [151] Tadayoni BM, Friden PM, Walus LR, Musso GF. Synthesis, in vitro Kinetics and in vivo Studies on Protein Conjugates of AZT: Evaluation as a Transport System to Increase Brain Delivery. *Bioconjugate Chemistry*. 1993;4(2):139-45.
- [152] Penichet ML, Kang Y-S, Pardridge WM, Morrison SL, Shin S-U. An Antibody-Avidin Fusion Protein Specific for the Transferrin Receptor Serves as a Delivery Vehicle for Effective Brain Targeting: Initial Applications in Anti-HIV Antisense Drug Delivery to the Brain. *The Journal of Immunology*. 1999;163(8):4421-6.
- [153] Huwyler J, Wu D, Pardridge WM. Brain Drug Delivery of Small Molecules Using Immunoliposomes. *Proceedings of the National Academy of Sciences*. 1996;93(24):14164-9.
- [154] Doi A, Kawabata S, Iida K, Yokoyama K, Kajimoto Y, Kuroiwa T, et al. Tumor-specific Targeting of Sodium Borocaptate (BSH) to Malignant Glioma by Transferrin-PEG Liposomes: a Modality for Boron Neutron Capture Therapy. *Journal of Neuro-Oncology*. 2008;87(3):287-94.
- [155] Bosi S, Da Ros T, Spalluto G, Prato M. Fullerene Derivatives: an Attractive Tool for Biological Applications. *European Journal of Medicinal Chemistry*. 2003;38(11-12):913-23.
- [156] Basso AS, Frenkel D, Quintana FJ, Costa-Pinto FA, Petrovic-Stojkovic S, Puckett L, et al. Reversal of Axonal Loss and Disability in a Mouse Model of Progressive Multiple Sclerosis. *The Journal of Clinical Investigation*. 2008;118(4):1532-43.
- [157] Huang SS, Tsai SK, Chih CL, Chiang L-Y, Hsieh HM, Teng CM, et al. Neuroprotective Effect of Hexasulfobutylated C60 on Rats Subjected to Focal Cerebral Ischemia. *Free Radical Biology and Medicine*. 2001;30(6):643-9.
- [158] Yang DY, Wang MF, Chen IL, Chan YC, Lee MS, Cheng FC. Systemic Administration of a Water-soluble Hexasulfonated C60 (FC4S) Reduces Cerebral Ischemia-induced Infarct Volume in Gerbils. *Neuroscience Letters*. 2001;311(2):121-4.
- [159] Henley DB, May PC, Dean RA, Siemers ER. Development of Semagacestat (LY450139), a Functional  $\gamma$ -secretase Inhibitor, for the Treatment of Alzheimer's Disease. *Expert Opinion on Pharmacotherapy*. 2009;10(10):1657-64.
- [160] Forman M, Tseng J, Palcza J, Leempoels J, Ramael S, Krishna G, et al. The Novel BACE Inhibitor MK-8931 Dramatically Lowers CSF A $\beta$  Peptides in Healthy Subjects: Results from a Rising Single Dose Study (PL02.004) 64th American Academy of Neurology Annual Meeting; 2012; New Orleans: Neurology.
- [161] Ming GL, Song H. Adult Neurogenesis in the Mammalian Central Nervous System. *Annual Review of Neuroscience*. 2005;28:223-50.