

# Brain amyloid $\beta$ protein and memory disruption in Alzheimer's disease

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**Abstract:** The development of amyloid-containing neuritic plaques is an invariable characteristic of Alzheimer's diseases (AD). The conversion from monomeric amyloid  $\beta$  protein ( $A\beta$ ) to oligomeric  $A\beta$  and finally neuritic plaques is highly dynamic. The specific  $A\beta$  species that is correlated with disease severity remains to be discovered. Oligomeric  $A\beta$  has been detected in cultured cells, rodent and human brains, as well as human cerebrospinal fluid. Synthetic, cell, and brain derived  $A\beta$  oligomers have been found to inhibit hippocampal long-term potentiation (LTP) and this effect can be suppressed by the blockage of  $A\beta$  oligomer formation. A large body of evidence suggests that  $A\beta$  oligomers inhibit N-methyl-D-aspartate receptor dependent LTP; additional receptors have also been found to elicit downstream pathways upon binding to  $A\beta$  oligomers. Amyloid antibodies and small molecular compounds that reduce brain  $A\beta$  levels and block  $A\beta$  oligomer formation are capable of reversing synaptic dysfunction and these approaches hold a promising therapeutic potential to rescue memory disruption.

**Keywords:** Alzheimer, amyloid, oligomer, long-term potentiation, NMDA

## Amyloid $\beta$ protein and Alzheimer's disease

Alzheimer's disease (AD) is pathologically characterized by the presence of intracellular neurofibrillary tangles and extracellular neuritic plaques.<sup>1</sup> Neurofibrillary tangles are mainly composed of hyperphosphorylated tau protein while neuritic plaques are formed by a gradual accumulation of amyloid  $\beta$  protein ( $A\beta$ ).  $A\beta$  is produced by the sequential cleavage of amyloid precursor protein (APP) by  $\beta$ -secretase and  $\gamma$ -secretase.<sup>1-3</sup> The APP gene encodes three different splicing isoforms, APP770, APP751 and APP695. The ectodomain of APP contains Kunitz protease inhibitor region (KPI) that accounts for the major difference in precursor sizes. Earlier studies have shown that KPI containing APP are present in dystrophic neuritis and may be associated with  $A\beta$  production and senile plaque formation.<sup>4</sup> The final cleavage by the  $\gamma$ -secretase determines the length of  $A\beta$  peptides. Among various  $A\beta$  isoforms, the most common ones are 40-residue  $A\beta$  ( $A\beta$ 40) and 42-residue  $A\beta$  ( $A\beta$ 42). The  $\gamma$ -secretase complex contains four components, presenilin 1 (PS1), nicastrin, anterior pharynx defective-1 (APH-1) and presenilin enhancer-2 (PEN-2).<sup>5</sup> Two aspartate residues located at the transmembrane domain 6 and 7 of PS1 constitute the active site of the  $\gamma$ -secretase.<sup>6</sup> Nicastrin was found to associate with the C-terminal fragments of APP and required for  $\gamma$ -secretase activity.<sup>7,8</sup> Genetic screens in *Caenorhabditis elegans* revealed two additional components of the  $\gamma$ -secretase components, APH-1,<sup>9</sup> and PEN-2.<sup>10</sup>

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The temporal sequence of deposition for different A $\beta$  species is critical for understanding the pathogenesis of neuritic plaques in brains.<sup>11</sup> While the number of neuritic plaques may not be correlated to the severity of dementia in a linear fashion, the levels of A $\beta$ 42 are closely associated with the disease.<sup>12</sup> Among a subset of nondemented subjects who carry classic AD pathology, as determined by A $\beta$  immunoreactive plaques and thioflavin histofluorescent plaques, the concentration of insoluble A $\beta$  is similar to those from AD patients. The soluble pool of A $\beta$ , which could be both extracellular and intracellular, differentiates AD from nondemented subjects and shows a strong inverse correlation with synapse loss.<sup>13</sup> Measuring the soluble and insoluble A $\beta$  pools from another subset of AD and control subjects indicated that levels of total and insoluble A $\beta$  differentiate AD from control subjects although not the disease severity. Likewise, the soluble pool of A $\beta$  is increased threefold in AD subjects and correlates with disease severity.<sup>14</sup> Pathological comparison of nondemented subjects, or those at the very early stage of dementia, with demented subjects suggests that an increase in A $\beta$ 40 and A $\beta$ 42 correlates with the progression of dementia and precedes apparent tau pathology in the frontal cortex of the brain.<sup>15</sup> Plasma A $\beta$ 42 is also elevated in patients carrying familial Alzheimer disease-linked mutations in PS1, PS2 and APP genes.<sup>16</sup> All autosomal dominant mutations have been found in PS and APP genes, and missense mutations in PS and APP genes account for the majority of early onset familial AD cases. Most of these familial AD (FAD) patients have a very early onset of disease, reflecting an increase of both peripheral and cerebral deposition of A $\beta$ 42. Among most AD patients, evidence suggests that a small pool of soluble A $\beta$  may contain the toxic form of A $\beta$  that causes neurodegeneration. This pool of A $\beta$  may contribute to the large static pool of insoluble A $\beta$  and form neuritic plaques over time.

## Oligomeric A $\beta$ and A $\beta$ 42

Biochemical analysis of the soluble pool of A $\beta$  has revealed a fraction of A $\beta$  specimens to migrate as 6–8 kDa on electrophoresis gel. Extracted from aged human cortical samples, these sodium dodecyl sulfate (SDS)-stable A $\beta$  species corresponding to the size of dimers are detected by Western blot analysis using antibodies against A $\beta$ . Similar SDS-stable A $\beta$  dimers are also detected in brain lysates from PDGF-driven hAPP PDAPP transgenic mice that over express human APP gene.<sup>17</sup>

The A $\beta$  dimer and higher molecular weight A $\beta$  trimer have been similarly discovered in the media of Chinese

hamster ovary (CHO) cells expressing human APP.<sup>18</sup> These oligomeric A $\beta$  have been immunoprecipitated with a number of A $\beta$  antibodies, and the authenticity of A $\beta$  peptide forming the dimer and trimer has been confirmed by amino acid sequencing.<sup>18</sup> When CHO cells coexpress APP and a FAD-linked mutant PS1 or PS2 gene, oligomeric A $\beta$  has been detected in the culture media that contains high levels of A $\beta$ 42 monomers. These results clearly indicate that increase in A $\beta$ 42 monomers facilitate the aggregation and formation of A $\beta$  oligomers.<sup>12,19</sup>

The significance of A $\beta$ 42 has been elucidated in an earlier report on the biochemical and pathological comparison of AD brains.<sup>20</sup> A third of brains with no congophilic angiopathy have been found to be carrying a majority of A $\beta$  species ending at residue 42.<sup>20</sup> The same amount of A $\beta$ 42 has also been found in brains with substantial congophilic angiopathy, although these brains contain far more A $\beta$ 40. Immunohistochemical staining has revealed that A $\beta$ 42 is primarily located in the senile plaques, while A $\beta$ 40 is mainly in blood vessel walls.<sup>20</sup> The association of A $\beta$ 42 in neuritic plaques and disease onset appears to be the outcome of oligomer formation at the early stages. Biophysical analysis of synthetic A $\beta$  peptides ending at 40 and 42 pinpoints the critical amino acids at residue 41 and 42, isoleucine-41 (Ile-42) and alanine-42 (Ala-42). A $\beta$ 40 monomers and oligomers can quickly reach equilibrium; however, A $\beta$ 42 preferentially forms pentamer/hexamer followed by subsequent conversion to early protofibrils. The addition of residue Ile-41 to A $\beta$ 40 facilitates the formation of pentamer/hexamer and the residue Ala-42 is needed for further formation of protofibrils.<sup>21</sup> The major subcellular compartment that contains dimeric A $\beta$  is the lipid raft, which carries a quarter of the brain's A $\beta$ 40 and A $\beta$ 42. Dimeric A $\beta$  is detected in lipid rafts from transgenic (Tg) Tg2576 mouse brains at 6 months when no amyloid plaque are present although memory impairment starts to emerge. The levels of dimeric A $\beta$  continue to increase, and then apolipoprotein E (ApoE) and phosphorylated tau start to accumulate in the lipid rafts. When animals are two years or older, a 500 fold increase of dimeric A $\beta$  has been found in the lipid rafts. Importantly, similar increases of dimeric A $\beta$ , ApoE and phosphorylated tau are also found in brains of AD patients.<sup>22</sup>

## A $\beta$ oligomers and memory impairment

A disconnection of memory impairment and plaque formation has been explored in transgenic mice overexpressing wild type (wt) or mutant APP. Loss of synaptophysin-immunoreactive

(SYN-IR) presynaptic terminals in specific brain regions has been correlated to cognitive decline in AD and used as an index for comparing wt and mutant APP transgenic mice. In transgenic mice expressing high levels of wt APP, there is no plaque formation even when large amounts of A $\beta$ 42 peptides are generated. This causes a significant decrease of SYN-IR presynaptic terminals, which is inversely correlated to A $\beta$ 42 levels.<sup>23</sup> In transgenic mice overexpressing FAD linked (APP V717F) mutant APP, SYN-IR presynaptic terminals decrease before the appearance of amyloid plaques.<sup>24</sup> Significant deficits in synaptic transmission have been detected by electrophysiological recordings from the hippocampus of these mutant transgenic mice in the absence of plaques. When the Swedish mutation is introduced to APP, those transgenic mice generate more A $\beta$  peptide with relatively lower expression levels of Swedish mutant APP. The young transgenic mice do not develop plaques although they do show increased synaptic transmission deficits.<sup>24</sup> Apparently, A $\beta$  peptides exhibit neurotoxicity independent of plaque formation.

Although the neurotoxic effect of A $\beta$ 42 is observed in very young mice, there is a significant delay in any behavioral effect upon injection of aggregated A $\beta$ 42 into the hippocampi of rats. After rats were trained in two-lever operant chambers under an alternating lever cyclic-ratio schedule, aggregated A $\beta$ 42 was injected into the CA3 area of the hippocampus. Severe deficits were shown in behavioral tests at 30 days post injection, with much greater symptoms at 50 days post injection.<sup>25</sup> Upon intracerebroventricular injection of A $\beta$ 42 into rat brains, low-frequency stimulation induces long-term depression (LTD), while the low-frequency stimulation alone is not sufficient. Therefore, A $\beta$ 42 promotes a long-lasting reduction in synaptic strength and causes disruption of the processing that relies on hippocampal synaptic plasticity.<sup>26</sup>

The synthetic A $\beta$  peptides have been used to generate low molecular weight oligomers like A $\beta$ -derived diffusible ligands (ADDLs). At nanomolar concentrations, ADDLs are neurotoxic *in vitro*. ADDLs are found to inhibit hippocampal long-term potentiation (LTP) and affect neural signal transduction.<sup>27</sup> Synthetic ADDLs have been correlated to synapse loss and memory failure and their properties are highly similar to those A $\beta$  oligomers detected in brains using antibodies raised against synthetic A $\beta$  oligomers. When compared to control brains, more than a 70 fold increase of oligomer A $\beta$  has been found in the frontal cortex of AD patients. Both synthetic ADDLs and brain derived oligomeric A $\beta$  are found attached to cultured hippocampal neurons and bound to dendrite surfaces.<sup>28</sup> When rat hippocampal slices

were preincubated with ADDLs, Tetanus-induced LTP and reversal of LTD were strongly inhibited, while LTD was not affected.<sup>29</sup>

A $\beta$  oligomers are formed inside cells and subsequently secreted into the media. Microinjection of A $\beta$  oligomer-containing cell media clearly inhibits hippocampal LTP in rats. The inhibitory effect could be blocked by removing all A $\beta$ s with A $\beta$  antibodies. However, inhibition could not be blocked in media pretreated with insulin-degrading enzyme that specifically targets A $\beta$  monomers, though not oligomers. On the other hand,  $\gamma$ -secretase inhibitors used to treat cells at dosages which slightly reduce A $\beta$  monomer production lead to a reduction in oligomeric A $\beta$  formation. Media from  $\gamma$ -secretase inhibitor-treated cells contain A $\beta$  monomers, although not oligomers, and fail to disrupt LTP.<sup>30</sup> The low number of oligomers, including the dimers, trimers or tetramers, but not monomers, are found to: block the hippocampal LTP; do not affect presynaptic vesicle release; and fail to affect LTP in juvenile mice and brain-derived neurotrophic-factor-induced LTP in the adult hippocampus.<sup>31</sup> Importantly, A $\beta$  dimers isolated from brains of AD subjects have shown the same effect.<sup>32</sup>

In APP transgenic mice Tg2576, little or no neuronal cell loss appears concurrently with the accumulation of A $\beta$  and memory impairment. Tg2576 mice younger than 6 months old have normal memory, middle-aged mice (6–14 months old) show memory deficits in the absence of neuronal loss and striking neuropathology, and mice at 14 months old develop abundant neuritic plaques. While small A $\beta$  dimers and trimers derived from cultured cells are known to specifically disrupt cognitive function,<sup>33</sup> a unique form of A $\beta$  oligomer, termed A $\beta$ \*56, has been purified from the brains of middle-aged (6–14 month old) Tg2576 mice that impairs memory function once it is administered to young rats. Because Tg2576 mice start to develop plaques after 14 months, the accumulation of soluble A $\beta$ \*56 in the brains of middle-aged Tg2576 mice may be responsible for cognitive deficit, which is independent of plaque formation.<sup>34</sup> However, it is not clear whether A $\beta$ \*56 is unique to Tg2576 or a universal A $\beta$  oligomer species that can be identified across most middle aged transgenic mouse lines that develop neuritic plaques at a later time.

## From A $\beta$ to synaptic deficits: possible mechanisms

A number of mechanisms of action have been proposed for the effect of A $\beta$  on synaptic signaling. In cultured cortical neurons, A $\beta$  has been found to promote the endocytosis of N-methyl-D-aspartate (NMDA) receptors and the

reduction of A $\beta$  by  $\gamma$ -secretase inhibitor which suppresses the internalization of NMDA receptors. On the other hand, lower levels of cell surface NMDA receptors are found in APP transgenic mice that carry high levels of A $\beta$ .  $\alpha$ -7 nicotinic receptors (nAChRs), protein phosphatase 2B and the striatal-enriched protein (STEP) tyrosine phosphatase that are required for endocytosis of NMDA receptors; which is correlated to the dephosphorylation of the NMDA receptor-2B at Tyr1472.<sup>35</sup> A $\beta$  has been shown to inhibit NMDA receptor dependent LTP although not the NMDA receptor independent LTP or LTD. This inhibition requires the activation of microglia and involves inducible nitric oxide synthase (iNOS) and superoxide. These inflammatory related reactions seem to be necessary for the A $\beta$  mediated inhibition of LTP; which could be prevented in the presence of minocycline (inhibition of microglia activation), nicotinamide adenine dinucleotide phosphate-H oxidase inhibitor, or in iNOS knock out mice.<sup>36</sup>

In differentiated cultures of hippocampal neurons, synthetic ADDLs bind to excitatory pyramidal neurons although not  $\gamma$ -aminobutyric acid (GABA)-ergic neurons and are associated with postsynaptic density complexes containing NMDA receptors. It decreases expression of memory-related receptors (NMDA and ephrin-B2) and causes abnormal spine morphology and a significant decrease in spine density.<sup>37</sup> The NMDA-evoked cell firing rate has been studied in CA1 neurons in the rat and A $\beta$ 42 significantly increases NMDA responses, an effect which is irreversible.<sup>38</sup> The peptide A $\beta$ 25–35 shows a similar effect. On the other hand dendritic spine loss in the presence of A $\beta$  oligomers is reversible once A $\beta$  is eliminated by antibodies. When exposed to a physiological concentration of A $\beta$  oligomers (a picomolar concentration), the density of dendritic spines decreases, and the active synapses are reduced. Furthermore, the activity of NMDA receptors is required for A $\beta$  oligomer to affect dendritic spine morphological changes, and NMDA receptor-mediated calcium influx into dendritic spines decrease in the presence of A $\beta$  oligomers.<sup>39</sup>

Application of A $\beta$  to astrocytes *in vitro* leads to an inhibition of glutamate uptake along with rapid depolarization of astroglial membranes. Infusion of A $\beta$  via microdialysis in the rat magnocellular nucleus basalis (MBN) leads to an acute increase of the extracellular concentration of excitatory amino acid neurotransmitters and enhances the intracellular accumulation of Ca<sup>2+</sup> in the injection area. The effect of A $\beta$  in the MBN can be suppressed by the NMDA receptor channel blocker dizocilpine maleate MK-801, suggesting multiple

occurring events upon A $\beta$  insult, eg, astroglial depolarization, extracellular glutamate accumulation, intracellular Ca<sup>2+</sup> increase, and NMDA receptor activation.<sup>40</sup> On the other hand, an earlier study shows that MK-801 at the dose that produces a similar inhibition of NMDA potentials shows no effect on LTP, while A $\beta$  produces a significant inhibition of NMDA receptor-mediated synaptic potentials.<sup>41</sup> Therefore, it is possible that A $\beta$  affects multiple pathways leading to the inhibition of LTP, and it is important to understand whether these downstream pathways are among the main contributors to the effect of A $\beta$  on LTP.

Besides NMDA receptors, A $\beta$  also promotes endocytosis of synaptic  $\alpha$ -amino-3-hydroxyl-5-methyl-4-isoxazole-propionate receptors (AMPA), which causes the loss of dendritic spines.<sup>42</sup> Consistent with this finding, double knock-in mice expressing mutant APP and presenilin genes show age-dependent downscaling of AMPAR-mediated evoked currents. Accordingly, age-related deficits in LTP/LTD are also found in the double knock-in mice.<sup>43</sup>

A receptor for advanced glycation end products (RAGE) dependent pathway has been proposed for A $\beta$  mediated synaptic dysfunction. Inhibition of LTP by A $\beta$  is abolished in slices from anti-RAGE antibody-treated wild type mice. Furthermore, no inhibition has been observed in slices from RAGE knock-out mice or transgenic mice expressing a dominant negative mutant of RAGE. Since activation of p38 mitogen-activated protein kinase (p38 MAPK) phosphorylation is decreased by antibodies against RAGE, suppression of LTP inhibition has been achieved by blocking p38 MAPK. These studies illustrate a RAGE-dependent pathway that involves A $\beta$  induced activation of p38 MAPK to impair LTP.<sup>44</sup> On the other hand, A $\beta$  has been shown to activate the complement cascade and generate complement component C5-derived anaphylatoxin C5a, which activates MAPK and mediates neuroprotection.<sup>45,46</sup> The complex pathways downstream of A $\beta$  reflect direct and indirect activation of MAPK pathways<sup>47</sup> and may have different effect on synaptic function.

Recently, cellular prion protein (PrP(C)) has been found to bind to A $\beta$  oligomers, and the infectious prion protein PrP(Sc) conformation is not required for the interaction.<sup>48,49</sup> The binding of A $\beta$  oligomers to PrP(C) can be blocked by anti-PrP antibodies, and the inhibitory effect on LTP is suppressed.<sup>48</sup> Whether PrP(C) plays a critical role in recognizing A $\beta$  oligomers and mediating downstream synaptic response is not clear, as conflicting results have been reported on LTP in A $\beta$  oligomer-treated hippocampal slices from PrP null mice.<sup>48,49</sup>

Pharmacological inhibition of ion translocation by the Na<sup>+</sup>, K<sup>+</sup>-adenosine triphosphatase has been observed in the presence of micromolar concentrations of A $\beta$ 1-42.<sup>50</sup> Inhibition of calcineurin activity by FK506 or cyclosporine A reveals the calcineurin-dependent inhibition of LTP by A $\beta$ 42.<sup>51</sup> The disruptions in Ca<sup>2+</sup> signaling are caused not only by A $\beta$  oligomers but also by amyloid-type oligomers like prions and polyglutamine. Amyloid oligomers increase intracellular Ca<sup>2+</sup>, and Ca<sup>2+</sup> signals induced by A $\beta$ 42 oligomers are derived from extracellular and intracellular Ca<sup>2+</sup> sources. Treating a fluorescent dye loaded neuroblastoma cell line with A $\beta$  oligomers leads to an increase in membrane permeability and the leakage of anionic dye. When membrane permeability is increased in the presence of A $\beta$  or other oligomers, cells with a high transmembrane concentration gradient of Ca<sup>2+</sup> ion are extremely vulnerable.<sup>52</sup>

Additional pathways that are implicated in A $\beta$  mediated synaptic deficits include altered serine/threonine protein kinase (Akt), glycogen synthase kinase3 $\beta$  (GSK3 $\beta$ ) together with the phosphatase and tensin homolog (PTEN). Exposing organotypic slice culture to synthetic A $\beta$ 25–35 peptide leads to an initial increase then decrease in phosphorylation of Akt and GSK3 $\beta$ , followed by an increase of PTEN protein after one day of exposure.<sup>53</sup>

In animals, analysis of APP transgenic mice reveals an upregulation of nAChRs along with a decrease of the MAPK in hippocampus. The phosphorylation of cyclic adenosine monophosphate regulatory element binding (CREB) protein is decreased by high levels of A $\beta$ 42. Therefore, A $\beta$ 42 may depend on nAChRs to downregulate MAPK and the phosphorylation of CREB protein.<sup>54</sup> Accordingly, activation of extracellular signal-regulated kinase/MAPK, Ca<sup>2+</sup>/calmodulin-dependent kinase II, and the phosphatidylinositol 3-kinase-activated protein Akt/protein kinase B is disrupted in the presence of soluble A $\beta$ . The insulin receptor family of tyrosine kinase has been found to mediate A $\beta$  induced synaptic deficits and soluble A $\beta$  binds to insulin receptors and affects its autophosphorylation in the presence of insulin. The A $\beta$  mediated kinase inhibition is similar to that achieved by an antagonist of the insulin receptor family of tyrosine kinases.<sup>55</sup>

## Rescuing synaptic deficits: amyloid based therapeutic intervention

Multiple approaches have been applied to decrease A $\beta$  oligomer-induced synaptic dysfunction. Cyclohexanehexol stereoisomers have been identified to prevent the formation

of A $\beta$  oligomers and reduce AD-like pathologies in transgenic mouse brains. Impaired cognition and synaptic function are reduced in the presence of cyclohexanehexol inhibitors.<sup>56</sup> Among these stereoisomers, small molecular weight scyllo-inositol is found to suppress the inhibitory effect of A $\beta$  oligomers on LTP in mouse hippocampus. Cerebroventricular injection of A $\beta$  oligomers derived from APP overexpressing CHO cells into rat impairs learned performance on a complex lever-pressing task. This impairment can be rescued in animals drinking scyllo-inositol containing water.<sup>57</sup> Apparently, small molecular compounds that block the oligomer formation suppress the inhibitory effect of A $\beta$  on LTP.<sup>58,59</sup>

The rescue of LTP inhibition by A $\beta$  oligomers can also be achieved by a monoclonal antibody against A $\beta$ . Earlier studies have shown that a monoclonal antibody against the mid region of A $\beta$  (m266) reverses memory deficits in an object recognition task and a holeboard learning and memory task.<sup>60</sup> Injection of monoclonal A $\beta$  antibody after intracerebroventricular injection of A $\beta$  secreted from APP overexpressing cells prevents the inhibition of LTP by A $\beta$  oligomers. Interestingly, active immunization shows partial effects that correlate positively with levels of A $\beta$  antibodies.<sup>61</sup> In another study, the memory loss in Tg2576 mice can be fully reversed upon intraperitoneally dosing with BAM-10, an antibody recognizing N-terminus of A $\beta$ . BAM-10 neutralizes A $\beta$  assemblies in the brain and prevents them from disrupting cognitive function.<sup>62</sup> Furthermore, soluble A $\beta$  oligomer induced spine loss could be rescued by A $\beta$  specific antibodies; this reversible event provides a promising approach for therapeutic intervention of AD.<sup>39</sup>

The most advanced clinical trial with antibody based A $\beta$  reduction therapy is at Phase III with bapineuzumab, a humanised anti-amyloid- $\beta$  monoclonal antibody. This was based on earlier findings that vaccination with a synthetic A $\beta$  reduces plaque load in transgenic mice.<sup>63</sup> However, active vaccination with AN1792 ( $\beta$ -amyloid [A $\beta$ ]1-42) failed clinical trial, and recent studies with passive vaccine bapineuzumab showed some effect on A $\beta$  load. Three ascending dose groups at 0.5, 1 and 2 mg/kg of bapineuzumab were administered up to 6 times in mild to moderate AD patients at 13 weeks apart. Positron emission tomography (PET) scanning using Carbon-11-labelled Pittsburgh compound B (<sup>11</sup>C-PiB) was carried out to measure cortical fibrillar amyloid- $\beta$  load in AD patients. Administration of bapineuzumab for 78 weeks led to a reduction of <sup>11</sup>C-PiB retention. Currently there is no imaging based method to measure soluble A $\beta$  oligomers in human brains,

and it is not clear why bapineuzumab only improves cognitive function in apolipoprotein-E4 non-carriers.

Non-vaccine based therapeutic approaches include  $\gamma$ -secretase inhibitors<sup>64</sup> and modulators. R-flurbiprofen is a non-steroidal anti-inflammatory drug and a  $\gamma$ -secretase modulator, and treatment of transgenic mice with R-flurbiprofen efficiently reduces A $\beta$  levels.<sup>65</sup> However, R-flurbiprofen failed Phase III clinical trials for the treatment of AD. Semagacestat<sup>66–69</sup> is a  $\gamma$ -secretase inhibitor and was shown to be effective in reducing plasma A $\beta$  during a recent Phase II clinical trial. Begacestat, a  $\gamma$ -secretase modulator, reduced both A $\beta$ 40 and A $\beta$ 42 in transgenic mouse brains and reversed cognitive deficits.<sup>70</sup>

## Conclusion

The amyloid hypothesis illustrates a multi-step cascade originating from excessive A $\beta$  generation to final neuropathological hallmarks found in brains of Alzheimer's patients.<sup>71</sup> While potential targets have been explored to block the cascade and prevent the onset of the disease, identification and validation of A $\beta$  species that are most toxic to neurons continues to be a challenge. A $\beta$  oligomers physically or functionally interact with multi-components that play intrinsic roles in pathways leading to LTP inhibition, and potential targets related to these pathways show tremendous promise for therapeutic intervention. Current efforts focus on removing monomeric and oligomeric A $\beta$  to rescue synaptic dysfunction, and these approaches are highly promising as they have reversed A $\beta$ -induced synaptic deficits. In conclusion, reducing oligomeric A $\beta$  formation as well as minimizing its toxicity to synapses will provide enhanced protection against neuronal loss and eventually delay memory impairment.

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## Disclosure

The author reports no conflicts of interest relevant to this research.

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