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Amyloid-modifying therapies for Alzheimer's disease: therapeutic progress and its implications

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Abstract Alzheimer's disease (AD) is the most prevalent form of dementia, affecting an estimated 4.8 million people in North America. For the past decade, the amyloid cascade hypothesis has dominated the field of AD research. This theory posits that the deposition of amyloid-beta protein (Aβ) in the brain is the key pathologic event in AD, which induces a series of neuropathological changes that manifest as cognitive decline and eventual dementia. Based on this theory, interventions that reduce Aβ burden in the brain would be expected to alleviate both the neuropathological changes and dementia, which characterize AD. Several diverse pharmacological strategies have been developed to accomplish this. These include inhibiting the formation of Aβ, preventing the aggregation of Aβ into insoluble aggregates, preventing the entry of Aβ into the brain from the periphery and enhancing the clearance of Aβ from the central nervous system. To date, no amyloid-modifying therapy has yet been successful in phase 3 clinical trials; however, several trials are currently underway. This article provides a review of the status of amyloid-modifying therapies and the implications for the amyloid cascade hypothesis.

Keywords Beta-amyloid · Amyloid cascade hypothesis · Alzheimer's disease · Vaccination · Secretase · Cognition

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Introduction

Alzheimer's disease (AD) is an age-related neurodegenerative disorder afflicting an estimated 4.8 million people in North America. AD is defined by cholinergic cell loss, neurofibrillary tangles (NFT), and beta-amyloid (Aβ)-containing plaques in the hippocampus and neocortex, accompanied by cognitive decline, behavioral disturbance, and dementia. At present, there are no effective disease-modifying therapies, and the cause of the disease is unknown. The leading theory of the disease is currently the amyloid cascade hypothesis, which posits that deposition of Aβ into plaques is the causative pathological event in AD (Hardy and Allsop 1991).

According to the amyloid cascade hypothesis, the primary cause of AD is neurodegenerative processes in selective brain regions. The initial trigger is the accumulation and likely aggregation of the $A\beta_{1-42}$ peptide. This in turn promotes the deposition of senile plaques and

leads to neurodegeneration through multiple mechanisms that include induction of oxidative stress, caspase activation, and hyperphosyhorylation of tau protein (Nakagawa et al. 2000; Takashima et al. 1998; Zheng et al. 2002). Cognitive decline and subsequent dementia are, in turn, a consequence of these neuropathological events targeting the brains cognitive circuitry.

Aβ protein is derived from the amyloid precursor protein (APP), which is cleaved initially by either α- or β-secretase and subsequently by γ-secretase (Selkoe 1996). Aβ is produced via the β-secretase cleavage pathway; α-secretase cleaves in the center of the Aβ domain of APP, precluding its formation, and instead leads to the formation of the benign peptides sAPPα and p3 (Selkoe 1996). The missense mutations are known to be associated with early-onset, autosomal dominant AD (Bertram and Tanzi 2008), occurring either in the gene encoding APP itself or in the presenilin enzymes, which form the active site of γ-secretase (Goate et al. 1991; Levy-Lahad et al. 1995; Sherrington et al. 1995). These mutations affect the expression or processing of APP, in the case of presenilins, favoring a shift toward the production of the more hydrophobic amyloidgenic fragment, $A\beta_{42}$ (Kumar-Singh et al. 2006). Cumulatively, these mutations have further implicated Aβ in the pathogenesis of AD (Bertram and Tanzi 2008; Citron et al. 1992).

If formation of amyloid plaques is a causal pathological event in AD, then reducing amyloid burden by either suppressing the formation of Aβ, inhibiting its aggregation into plaques, or enhancing its clearance should be effective disease-modifying therapies. Compounds that intervene at each of these three stages have been developed and tested (Table 1). However, to date, no anti-amyloid therapy has successfully affected cognition in large-scale clinical trials.

This article reviews progress in the development of amyloid-modifying therapies for AD. The focus is on compounds specifically targeting beta-amyloid; we do not cover a broader range of compounds that may also have beta-amyloid-reducing properties but are not directly linked to beta-amyloid processing or elimination.

Pathways in beta-amyloid synthesis, deposition, and clearance

Aβ protein is derived from the APP, which is cleaved initially by either α- or β-secretase and subsequently by γ-secretase (Selkoe 1996). Aβ is produced via the β-secretase cleavage pathway; α-secretase cleaves in the center of the Aβ domain of APP, precluding its formation, and instead leads to the formation of the benign peptides sAPPα and p3 (Selkoe 1996). Cleavage of APP and initial processing of Aβ occurs at the cell surface, before Aβ is released into the extracellular space. Aggregation of Aβ is mediated by electrostatic interactions leading to the deposition of insoluble senile plaques. It has also been suggested that systemic circulating Aβ may be a precursor for brain Aβ (Pluta et al. 1996; Selkoe 1996; Zlokovic 2004). Circulating Aβ enters the brain passively through the blood–brain barrier (Stewart et al. 1992) and via active transport mediated by the receptor for advanced glycation end-products (RAGE) (Mackic et al. 1998). Different amyloid species exhibit varying degrees of toxicity, and as we will discuss, identifying the truly toxic forms of Aβ will be necessary in order to develop effective disease-modifying therapies for AD.

Suppression of Aβ synthesis

Reduction in Aβ production can be achieved by inhibition of APP expression, reduction in either β- or γ-secretase, or up-regulation of α-secretase. Suppression of APP expression, however, is not likely to have other undesirable effects. Transgenic mice with APP deletions or modifications show impaired spatial memory, lower synaptic density, and high rates of early post-natal lethality relative to wild-type littermates (Dawson et al. 1999; Magara et al. 1999; Muller et al. 1994; von Koch et al. 1997; Zheng et al. 1995). These studies indicate that APP is critically involved in brain development, neuronal architecture, and migration, although its postnatal importance is thus far unknown. Interventions that modify activity of any of the three enzymes that process APP, therefore, rather than suppress its expression, have been developed as therapeutic agents for AD.

γ-Secretase inhibitors

The catalytic action of γ-secretase is mediated by two highly homologous presenilins, PS-1 and PS-2 (Wolfe et al. 1999). Conditional genetic inactivation of PS-1 was found to inhibit Aβ accumulation and plaque formation in transgenic APP mice (Yu et al. 2001).

Table 1 Summary of amyloid-based interventions advanced to clinical trials

Drug name	Description	Company	Phase	Reference
Alters Aβ production				
Lovastatin	HMG-co reductase inhibitor; Alters APP metabolism	Merck	IIa/b	Friedhoff et al. 2001
Simvastatin	HMG-co reductase inhibitor; Alters APP metabolism	Merck	II/III	Carlsson et al. 2008
Atorvastatin	HMG-co reductase inhibitor; Alters APP metabolism	Pfizer	Inactive	Sparks et al. 2005; Jones et al. 2008
(EHT 0202)	α-Secretase upregulator	ExonHit Therapeutics	IIa/b	Marcade et al. 2008
BMS-708163	γ-Secretase inhibitor	Bristol-Myers Squibb	IIa/IIb	
LY450139 dihydrate	γ-Secretase inhibitor	Eli Lilly & Co.	III	Siemers et al. 2006, 2007
r-flurbiprofen	γ-Secretase modulator	Myriad Pharmaceuticals Inc.	Discontinued	Wilcock et al. 2008
CTS-21166	B-Secretase inhibitor	CoMentis	I	No published results
Alters Aβ Aggregation				
Tramiprosate	Aβ antagonist, inhibits the aggregation of Aβ	Alzhemed (QU)	Discontinued	Aisen et al. 2006, 2007
Enhances Aβ clearance				
AAB-001	Humanized monoclonal anti-Aβ antibody; enhances clearance of Aβ	Elan Wyeth	III	Gilman 2005, Salloway 2009
MABT5102	Humanized monoclonal anti-Aβ antibody; enhances clearance of Aβ	Genetech Inc	Recruiting for phase I	No published resutls
ACC-001	Humanized monoclonal anti-Aβ antibody; enhances clearance of Aβ	Elan Wyeth	IIa/IIb	Ryan and Grundman 2009
AN-1792 (AIP 001)	Synthetic aggregated $A\beta_{42}$, induces clearance of Aβ	Elan Pharmaceuticals	Disoncontinued	Bayer et al. 2005; Orgogozo et al. 2003
IVIg (Gammagard®, intravenous immunoglobulin)	Contains natural Aβ antibodies	Baxter Healthcare	IIa/b	Dodel et al. 2004
Cognishunt®	Increases CSF turnover	NA	IIa/b	Silverberg et al. 2004, 2008
Prevents entry of Aβ into the CNS				
PF-04494700	RAGE inhibitor; prevents entry of Aβ into the CNS from the periphery	Pfizer	II	No published results

Since cleavage by γ-secretase is needed to liberate Aβ from the β-secretase cleavage product of APP, decreasing γ-secretase activity would also inhibit the liberation of Aβ; this has been attempted through both classical inhibition and allosteric modulation.

Classical γ-secretase inhibitors

The classical γ-secretase inhibitor, [*N*-[*N*-(3, 5-difluorophenacetyl)-L-alanyl]-*S*-phenylglycine *t*-butyl ester] (DAPT) interacts with the catalytic site formed between the two catalytic subunits of γ-secretase. In both PDAPP and Tg2576 transgenic mice, acute DAPT administration has been reported to dose-dependently decrease cortical Aβ levels, improve contextual fear conditioning, and reduce age-dependent deficits in the Morris water maze (Comery et al. 2005; Dovey et al. 2001; Lanz et al. 2003; Saura et al. 2005). Dewachter et al. (2002), however, reported that inactivation of γ-secretase subunits fail

to rescue object recognition deficits observed in APP-expressing mice. Moreover, Saura et al. (2004) found that disruption of PS-1 and PS-2 in non-amyloid expressing rats induces mild but detectable impairments in spatial learning and contextual fear conditioning.

In addition to APP, γ-secretases process other inter-membrane proteins, most notably the cell receptor Notch. Regulated cleavage via the notch receptor is critical to development, differentiation, and architecture of several cell types (Louvi and Artavanis-Tsakonas 2006). Compounds that modulate Notch processing are therefore likely to alter differentiation in tissues whose architecture is governed by Notch signaling. Correspondingly, intestinal goblet cell metaplasia (Milano et al. 2004), thymus atrophy (Gounari et al. 2005; Radtke et al. 1999) deficient T cell fate specification (Gounari et al. 2005; Radtke et al. 1999), and decrease in lymphocytes (Sandy and Maillard 2009) have been reported in experimental models in which Notch signaling has been disturbed. DAPT has been found to inhibit notch signaling through this non-presenilin-specific mechanism of action (Cai et al. 2008), which presents a major limitation of γ-secretase inhibition. Recent studies have also demonstrated a role of presenilins in activity-dependent Ca^{2+} release, which has implications for neurotransmitter release and synaptic plasticity (Zhang et al. 2009).

In addition to the presenilin subunits, the γ-secretase complex contains Aph1 components, which affects the conformation of the presenilin catalytic subunit (De Strooper 2003). Mutations in the Aph1 gene have been shown to enhance the production of more amyloidgenic fragments of Aβ, leading to increased amyloid burden and hippocampal-dependent learning deficits (Serneels et al. 2009). Both Aph1 and the presenilins are subject to alternative splicing, contributing to the structural complexity and difficulty in the design of selective γ-secretase inhibitors (Hebert et al. 2004; Serneels et al. 2009; Shirotani et al. 2004). However, optimization of γ-secretase inhibitors has lead to the development of several azepine compounds (Olson and Albright 2008). Preclinical studies with one of the earliest members of this class, LY411575, established a therapeutic window where Aβ levels are reduced by 69% with negligible adverse events (Hyde et al. 2006). Further chemical modifications to improve in vivo inhibition have resulted in a series of related compounds that are currently under development (Annaert and De Strooper 1999; Peters et al. 2007).

Eli Lilly completed phase II clinical trials with two such compounds, LY451395 and LY2062430, in June 2003 and May 2008, respectively. The results were not published, and no further studies are planned. Another related compound, LY450139, has been tested in phase I and II clinical trials. In a study of 31 healthy adults completed in 2007, LY450139 was well tolerated but did induce headaches (Siemers et al. 2007). An initial phase II safety study conducted over 14 weeks in 43 AD patients reported daily administration of LY450139 to be well-tolerated, with the exception of seven withdrawals related to hair color change ($n=3$), drug rash ($n=3$), and GI distress ($n=1$) (Fleisher et al. 2008). Despite its good central nervous system (CNS) penetrance, reductions in cerebrospinal fluid (CSF) Aβ were not significant after LY450139 treatment. However, the study authors have proposed that the interval between drug administration and CSF collection was not sufficient to detect changes (Fleisher et al. 2008). There was also an initial dose-dependent decrease in plasma Aβ levels, followed by an increase that did not return to baseline levels within a 24-h dosing interval (Henley et al. 2009). No group differences were found in the Alzheimer's disease Assessment Scale cognitive subscale (ADAS-Cog) score or activities of daily living score (Siemers et al. 2006), which has been attributed to the 14-week trial interval (Henley et al. 2009). By suppressing Aβ formation, LY1450139 should delay the onset of AD. Some researchers have therefore suggested that a large-scale, phase II trial lasting at least 18 months and initiated in early stages of AD as a preventative therapy would be required to detect cognitive effects (Henley et al. 2009).

Based on these results, Eli Lilly began two phase III trials in 2008, one using PET and MRI to examine Aβ levels, brain volume, metabolism, and tau levels and a separate study assessing effects on behavior and cognition using a battery of neuropsychiatric indices. Completion is anticipated for spring 2012. Eli Lilly is also recruiting for phase I and II studies of an additional γ-secretase inhibitor, LY2811376, with the primary outcome measure being the four-item agitation/aggression subscale of the neuropsychiatric inventory; results are expected November, 2010.

Bristol-Myers Squibb has completed phase I trials with a member of its series of γ-secretase inhibitors,

BMS-708163, in July 2009. While no results have been published, recruitment is underway for two parallel phase II trials; no completion date has been projected.

γ-Secretase modulators

Another strategy of altering γ-secretase activity was initiated after epidemiological studies suggested an inverse relationship between long-term nonsteroidal anti-inflammatory drug (NSAID) administration and incidence of AD (Andersen et al. 1995; Cornelius et al. 2004). Subsequent work showed that some NSAID analogues preferentially inhibit the formation of $A\beta_{42}$ and do not affect notch processing, which opened a new therapeutic window (Weciggen et al. 2003a,b). The mechanisms underlying these γ-secretase modulators is postulated to involve allosteric stabilization of the enzyme in a conformation that favors the formation of non-amyloidgenic fragments from APP, without affecting Notch processing (Beher et al. 2004; Okochi et al. 2006; Weciggen et al. 2003a,b).

r-Flurbiprofen, an NSAID derivative and selective amyloid lowering agent, was developed by Myriad Genetics for the treatment of AD. In preclinical studies, r-flurbiprofen lowered levels of Aβ in the brains of Tg2576 mice by an average of 70% (Eriksen et al. 2003). Clinical trials in which patients received r-flurbiprofen found the drug to be well tolerated. These phase II studies reported that, among patients with mild AD at the time of enrolment, those treated with r-flurbiprofen exhibited more stable ADAS-Cog scores than did patients assigned to the placebo group, while there was no significant treatment affect for patients with moderate AD (Wilcock et al. 2008). Subsequent phase III trials, however, were terminated in February 2008, due to failure to prove efficacy. Several theories arose to explain this disappointing failure after initially promising results. While clearance of plaques correlated with CSF concentrations of the drug, r-flurbiprofen, exhibits low brain penetrance and weak pharmacological activity (Galasko et al. 2007), which has been proposed to account for poor clinical efficacy of the drug. It has also been suggested that γ-secretase may not be an appropriate target for AD, raising doubts as to the causative role of the Aβ fragment in AD, or the idea that preventing the formation of more Aβ fragments is insufficient to exert clinical effects on cognition (Imbimbo 2009).

To date, clinical trials have failed to demonstrate efficacy of the NSAIDS ibuprofen, naproxen, and celecoxib in enhancing AD-associated cognitive impairment (Group et al. 2007; Pasqualetti et al. 2009). However, these trials were initiated in patients with mild to moderate AD, whereas the effect observed in epidemiological studies may reflect a protective effect of NSAIDS use before AD onset.

β-Secretase inhibitors

Cleavage of APP by β-secretase, an aspartic protease, is the initiating step in the proteolysis of APP into amyloidgenic fragments. Compounds that inhibit β-secretase-mediated, amyloidgenic cleavage of APP in vivo would thus be expected to reduce Aβ burden and avoid the side effects of γ-secretase inhibitors. In vitro, inhibition of β-secretase effectively reduces Aβ40 and Aβ42 deposition (Cai et al. 2001), and knockout of β-secretase in vivo produces viable, fertile mice (Luo et al. 2001; Roberds et al. 2001). In transgenic models of AD, knockout of β-secretase eliminates Aβ overproduction and rescues hippocampus-dependent memory deficits (Cai et al. 2001; Luo et al. 2001; Ohno et al. 2004, 2006).

The first β-secretase inhibitor to advance to clinical trials was CTS-21166, a small molecule inhibitor developed by CoMentis, which selectively binds the active site of β-secretase and prevents cleavage of APP allosterically (Ghosh et al. 2008). In a phase I study of 56 healthy male volunteers completed in February 2008, CTS-21166 was found to be well tolerated and displayed low inter-subject pharmacokinetic variability. While the study design included measurements of plasma Aβ, results have not yet been published, and no phase II studies have been initiated. Schering-Plough (now Merck) has also undertaken a joint venture with Pharmacopeia (now Ligand Pharmaceuticals, Inc.) to optimize the medicinal chemistry of a novel β-secretase inhibitor, although no clinical trial results have yet been released.

While no adverse effects have yet been reported with beta-secretase inhibitors, recent research has identified important targets of beta-secretase, which may have clinical implications (Kim et al. 2005; Lichtenthaler et al. 2003; Wong et al. 2005). Among the most important of these findings has been that beta-secretase regulates myelination in both the

central and peripheral nervous system through the regulated cleavage of neuregulin-1 (Hu et al. 2008; Hu et al. 2006). The cleavage product of neuregulin-1 signals to oligodendrocytes and Schwann cells to regulate myelin thickness (Bao et al. 2003; Hu et al. 2006), and BACE knockouts exhibit hypomyelination and delayed re-myelination after sciatic nerve crush injury when compared to their wild-type littermates (Hu et al. 2006, 2008). Although the function of BACE is most evident during development, impaired neuregulin signaling may have widespread effects on neuronal survival and maintenance (Mei and Xiong 2008). Moreover, given that BACE1 is required for remyelination and that remyelination is impaired with age (Kovacic et al. 2009), the use of BACE inhibitors in an elderly population may have more complications than in a sample of healthy volunteers. While the cleavage routes of neuregulin and APP are sufficiently different that selective inhibitors could be developed (Hu et al. 2008), this complication underscores the need to fully elucidate the physiological roles of enzymes to fully anticipate in vivo effects of enzyme inhibition.

α-Secretase enhancers

Up-regulation of α-secretase both prevents the formation of the amyloid fragment by physical cleavage and shifts the equilibrium between processing pathways away from the amyloidgenic β-secretase pathway. Furthermore, the product of the α-secretase pathway, sAPPα, has shown anti-apoptotic and neuroprotective effects against oxidative stress (OS) and prevents against Aβ-induced cytotoxicity in vitro and in animal models (reviewed by Turner et al. 2003). In both aged humans and rodents, activity of α-secretase decreases in an age-dependent manner, and this shift in APP processing has been proposed to be associated with age-dependent cognitive decline (Almkvist et al. 1997; Andersen et al. 1995; Nistor et al. 2007; Sennvik et al. 2000). Using transgenic mice expressing human APP, genetic up-regulation of alpha-secretase homologue, ADAM10 has been reported to reduce by 30% reduced Aβ burden and rescue hippocampus-dependent memory deficits. By contrast, catalytic inactivation of this enzyme increased central plaque pathology (Postina et al. 2004). This evidence cumulatively provides support for the pharmacological up-regulation of α-secretase as a viable strategy for AD. Two classes of interventions that are based on α-secretase up-regulation are $GABA_A$ modulating compounds and HMG-co-reductase inhibitors.

α-Secretase facilitators

ExonHit Therapeutics has developed EHT 0202, a pyrazolopyridine compound that is believed to increase co-localization of APP and α-secretase at the plasma membrane, thereby facilitating cleavage of APP by α-secretase and protecting against Aβ-induced neurotoxicity in cortical cell culture (Marcade et al. 2008). Guinea pigs treated with clinically relevant dose of EHT0202 (10 mg/kg) exhibited a 20% reduction in total Aβ and a three-fold increase in sAPPα relative to controls. This neuroprotective effect is abolished by the selective $GABA_A$ antagonists, picrotoxin and bicuculine, indicating that modulation of $GABA_A$ signaling by EHT0202 is a necessary component of its therapeutic mechanism (Marcade et al. 2008).

While the mechanisms linking $GABA_A$ signaling to sAPPα generation and neuroprotection remain incompletely understood, the modulation of synaptic activity and stabilization of calcium levels have been implicated. Alpha secretase activity is strongly regulated by several neurotransmitters (Ulus and Wurtman 1997) and is dependent on neuronal and synaptic activity (Farber et al. 1995; Kamenetz et al. 2003; Marcello et al. 2007; Nitsch et al. 1993; Nitsch and Growdon 1994; Walker et al. 1988). Moreover, toxicity of Aβ is at least partially mediated through excitotoxicity and excessive Ca^{2+} entry mediated by stimulated glutamate receptors (Gu et al. 2003; Mark et al. 1995; Mattson et al. 1991; Paula-Lima et al. 2003). Modulation of the $GABA_A$ receptor, therefore, by altering synaptic and excitatory transmission would have both symptomatic and disease modifying effects. This inhibitory mechanism cannot entirely account for the protective effects of $GABA_A$ signaling on Aβ toxicity, since $GABA_B$ receptor agonists do not protect against Aβ toxicity (Marcade et al. 2008). The link between $GABA_A$ signaling and sAPPα generation is an area of active investigation (Gu et al. 2003; Louzada et al. 2004).

Initial clinical studies demonstrated that EHT0202 is well tolerated up to 120 mg/day, with infrequent minor adverse events in elderly volunteers. No

dropouts were reported at any dose, and cognitive improvement in scopolamine-induced working memory impairment was evident at a dose of 80 mg daily. A phase II study to assess safety, tolerability, and effect on cognition of EHT0202 as an adjunct to acetylcholinesterase inhibitors for AD has recently been completed, but to date, no results have been published.

HMG-Co-reductase inhibitors

Epidemiological studies have shown lower rates of incident AD among patients administered chronic statin therapy, an effect not observed with non-statin cholesterol-lowering drugs (Haag et al. 2009; Zamrini et al. 2004). These observations have supported the use of HMG-co-reductase inhibitors (statins) for AD. While large prospective cohort studies have reported decreased levels of NFTs as well as lower rates of incident AD in statin users after controlling for age, gender, and APOE4 status (Sparks et al. 2008), other epidemiological studies have not found differences in cognitive function and Aβ burden attributable to statin treatment (Arvanitakis et al. 2008; Tokuda et al. 2001).

Analysis of APP isoforms in human subjects prescribed Simvastatin® therapy suggests that statin therapy alters brain metabolism of APP, favoring a shift toward the non-amyloidgenic pathway without affecting levels of Aβ in the CSF or plasma (Hoglund et al. 2005). In vitro, sufficient depletion of cholesterol can completely abolish the production of Aβ from APP (Buxbaum et al. 2002), and levels of Aβ are significantly reduced in brain homogenates of statin-treated guinea pigs relative to controls (Fassbender et al. 2001).

Randomized clinical trials have reported significantly lower serum concentrations of Aβ in Lovostatin®-treated patients after 3 months of therapy relative to controls (Friedhoff et al. 2001). A similar study with Simvastatin® demonstrated significant decreases in levels of Aβ and tau in the CSF after 4 months of 40 mg Simvastatin®, which was accompanied by improvement in select indices of verbal fluency and working memory (Carlsson et al. 2008).

Concerns about the safety of statins that penetrate the b;ood–brain barrier (BBB) were raised by Sparks et al. (2002). They suggested that excessive inhibition of cholesterol synthesis by statins at the neuron-membrane may further exacerbate degeneration of neurons, given the critical role of cholesterol in neuronal membrane fluidity and biophysical properties (Sparks et al. 2002). Atorvastatin® does not penetrate the BBB and would thus not be expected to have this adverse effect (Sparks et al. 2002).

A clinical trial to assess the efficacy of Atorvastatin enrolled 120 patients with mild to moderate AD and reported that a dose of 80 mg/day decreased cholesterol levels and produced improvement in ADAS-Cog, geriatric depression scale (GDS) and mini-mental state examination (MMSE), although at 6 months, this effect only reached significance for the GDS and ADAS-Cog (Sparks et al. 2005). No further studies have been announced, and trials for Simvastatin® are currently underway for AD.

Prevention of Aβ aggregation

An important caveat of altering the activity of endogenous proteins, namely, APP and the secretases, is the likelihood of unintended physiological consequences. Self-assembly of Aβ is the first exclusively pathological event in the processing of APP, and inhibition of this aggregation may be able to disrupt the amyloid cascade without adverse side effects.

3-Amino-1-propanesulfonic acid (3-APS; Tramiprosate®) was developed by Neurochem to inhibit Aβ aggregation. In neuronal cell culture, 3-APS preferentially binds soluble Aβ 40 and 42 peptides, preventing the conformational transitions necessary for aggregation into β-pleated sheets (Gervais et al. 2007). When administered to transgenic mice, 3-APS crossed the BBB and lowered Aβ deposition by 30%; cerebral levels of soluble and insoluble Aβ 40-42 were also reduced by 20–30%, whereas reductions in plasma Aβ were as great as 60% (Gervais et al. 2007). In clinical trials, 3-APS was well tolerated in phase I studies, with only mild nausea, vomiting, and diarrhea occurring in some patients (Aisen et al. 2007). Initial phase II studies of 58 patients reported a dose-dependent decrease in CSF levels of Aβ and a stabilization of cognitive function, as indexed by MMSE and clinical dementia rating scale scores over 3 years with 3-APS therapy (Aisen et al. 2006). However, concerns were raised about the actions of 3-APS® on tau, based on in vitro studies demonstrating that the drug facilitates aggregation of the

cytoskeletal protein (Santa-Maria et al. 2007). Despite initially promising results, two concurrent phase III clinical trials were prematurely discontinued in December 2008 due to failure to prove efficacy.

Scyllo-inositol is a naturally occurring cyclohexane-hexol stereoisomer that has been formulated and marketed as ELND005 (ADZ-103) by Elan. The compound has been shown to interfere with the electrostatically-stabilized hair-pin turn structure of Aβ oligomers. Studies using cultures of primary human neurons have shown that scyllo-inositol interacts with low-molecular weight Aβ fragments in vitro, forming a complex that prevents the further aggregation of Aβ into plaques and its neurotoxic interactions of Aβ with cellular lipids and proteins (McLaurin et al. 1998; McLaurin et al. 2000; Townsend et al. 2006). In rodent studies, ADZ-103 has been reported to neutralize the inhibitory effect of intracerebrovascular infusion of Aβ oligomers on hippocampal long-term potentiation and hippocampal-dependent memory tasks (McLaurin et al. 2006; Townsend et al. 2006). When administered orally to TgCRND8 mice, scyllo-inositol decreased plaque burden and enhanced spatial reference learning in the Morris water maze test (McLaurin et al. 2006). Because of its small size, scyllo-inositol easily penetrates the blood–brain barrier and is able to interact with Aβ oligomers in the CNS. When administered orally to mice previously infused with Aβ oligomers, scyllo-inositol dose-dependently enhanced performance on the alternating lever cyclic ration assay, which is a test of complex reference memory (Townsend et al. 2006).

Elan has developed a formulation of scyllo-inositol, ELND005, for which two phase II clinical trials are ongoing. The primary outcome measures are safety, tolerability, as well as unspecified assessments of cognitive function. Results of the 18-month studies are anticipated in May 2010 and April 2011.

Inhibition of the Aβ transporter

One of the most recent therapeutic strategies for AD is the inhibition of the neuronal RAGE, which is expressed on brain endothelial cells and transports Aβ across the BBB from the plasma into the CNS (Mackic et al. 1998). In vitro, binding of insoluble Aβ fragments to RAGE induces macrophage activation and cytotoxicity (Du Yan et al. 1997), while the addition of an anti-RAGE antibody improves neuronal survival after Aβ exposure (Sturchler et al. 2008). In AD patients, RAGE expression is enhanced in brain regions affected by AD pathology (Miller et al. 2008).

The RAGE inhibitor, PF-04494700 (formerly TTP488) has been developed by Pfizer. While no preclinical or phase I studies have been published to date, Pfizer is currently recruiting for phase II studies. In addition to its role in Aβ transport and cytotoxicity, RAGE mediates the damaging effect of advanced glycation end products (Sakurai et al. 2003), and RAGE inhibition might therefore ameliorate AD pathology through Aβ-independent mechanisms. A phase II safety study of PF-04494740 was successfully completed in 2006; a completion date for a larger phase II study to assess efficacy and further safety has been projected for March 2011.

Enhancement of Aβ clearance

The previously discussed pharmacotherapies are based on the principle that inhibiting Aβ formation and its deposition into plaques will improve cognitive function of AD. However, when patients present clinically with cognitive dysfunction, plaque deposition is advanced. This suggests a need for compounds capable of removing Aβ deposits once they have formed, rather than simply precluding their formation. Increasing Aβ efflux from the CNS has been attempted both by vaccination and by mechanically increasing CSF turnover. In clinical studies of interventions purported to enhance amyloid clearance, measures of Aβ in the periphery and CNS are taken via serum and CSF measurements. However, these two measures do not necessarily correlate with each other, with cognitive function, or provide an accurate reflection of central plaque pathology.

Active vaccination

Active immunization involves inoculation with a full-length antigen of interest, in this case, the Aβ peptide, and inducing an individual immune response to Aβ. In a pioneering study in PDAPP mice, Schenk et al. found that monthly vaccination of transgenic mice beginning before the onset of plaque pathology with synthetic human $A\beta_{42}$ resulted in complete protection

against plaque deposition in seven out of nine vaccinated mice, while the other two showed dramatically decreased amyloid burden relative to controls. This effect was not observed in animals administered SAP*, a non-Aβ-related peptide associated with amyloid conditions (Schenk et al. 1999). Initial studies demonstrated that active vaccination of APP-expressing mice restored performance in the Morris water maze and radial arm maze to the level of wild-type littermates (Janus et al. 2000; Morgan et al. 2000). However, more recent studies using larger groups of PDAPP mice have failed to find an effect on spatial learning after active immunization in mice expressing extensive amyloid deposition (Chen et al. 2007). Moreover, investigations in higher mammalian species, namely the aged canine, have not supported the use of active vaccination to improve cognition (Head and Torp 2002; Vasilevko and Head 2009). In this investigation, aged canines, which express spontaneous plaque deposition and cognitive impairment with advancing age, were immunized with fibrillar Aβ or vehicle while undergoing extensive cognitive testing over 2.5 years. While plaque burden was preferentially decreased in the PFC of animals receiving regular immunizations, these animals exhibited only preservation of executive function relative to vehicle-treated animals; no effects on spatial memory, complex learning, or attention were observed (Head and Torp 2002).

In 2002, Elan and Wyeth developed the first synthetic analogue of the aggregated human Aβ peptide, AN-1792. Preclinical testing with AN-1792 has demonstrated significant reductions (>99% relative to controls) in plaque burden and neuropathology in PDAPP mice at 13 months of age when vaccination began at 6 weeks (Schenk et al. 2000). Immunization was also associated with enhanced performance in hippocampus-dependent memory tasks and concomitant decrease in Aβ deposits (Janus et al. 2000; Morgan et al. 2000; Sigurdsson et al. 2001). In a phase I trial to assess safety and immunogenicity, AN-1792 was found to be well tolerated (Bayer et al. 2005). In the treatment group, 58.8% of patients generated antibody titers and showed less deterioration in disability for dementia (DAD) score after 84 weeks of treatment (Bayer et al. 2005). Subsequently, 372 patients were enrolled in a parallel phase II/III study, which was terminated due to the occurrence of encephalitis in 6% of patients less than 6 months after immunization (Gilman et al. 2005; Orgogozo et al. 2003). This phenomenon was not correlated with Aβ titers and is thought to be related to the infiltration of T cells into the CNS (Orgogozo et al. 2003). Despite discontinuation, long-term follow-up studies revealed that patients who developed the highest levels of anti-AN1792 titers exhibited the most extensive plaque clearance, which was seemingly associated with phagocytosis (Ferrer et al. 2004; Nicoll et al. 2003). However, even in regions that were subject to amyloid clearance, tau pathology and neuropil threads were not different from that seen in non-immunized controls (Masliah et al. 2005; Nicoll et al. 2003). Extensive clearance was only observed after 60 months post-immunization, and neurodegeneration continued in spite of amyloid clearance (Masliah et al. 2005; Nicoll et al. 2006; Patton et al. 2006). Although not statistically significant, patients expressing the most robust initial titer response to the Aβ vaccine showed faster clinical progression of AD (Holmes et al. 2008). However, in separate studies, production of titers has been associated with lesser decline on the DAD score among immunized subjects (Vellas et al. 2009). If the response to AN1792 is indeed associated with faster disease progression, the observation that Aβ could stimulate an immune response or increases the potentially damaging oligomeric forms of Aβ during active plaque disintegration have been proposed as candidate mechanisms for this decline (Holmes et al. 2008). In a follow-up study, patients who passed away between 4 and 5 months after the last immunization showed higher density of cerebrovascular Aβ, cortical microhemorrhages, and microvascular lesions than un-immunized controls , which has been attributed in some instances to the dissolution of Aβ plaques that were then drained vial the parivascular pathway (Boche et al. 2008).

Another strategy developed by Elan and Wyeth was the administration of ACC-001, an Aβ fragment attached to a carrier protein intended to help induce antibody generation. In phase I trials with ACC-001, an acute dose was found to be well tolerated and was shown to cross the BBB to increase clearance of Aβ from the CNS (Ryan and Grundman 2009). Recruitment for multiple phase II trials is currently ongoing.

In addition to the incidence of microhemorrhages and encephalitis, there are concerns that immunization with full-length Aβ may induce toxicity through the

same mechanisms as endogenous Aβ or that it may seed fibril formation. Second generation or passive vaccines have thus been developed and are expected to circumvent these latter adverse events.

Passive vaccination

In an attempt to reduce the safety risks with vaccines, Elan-Wyeth developed a passive immunization strategy, which entailed direct administration of the antibodies to the antigen of interest. Multiple studies have been conducted to assess the mechanisms of action of peripherally administered antibodies, although no consensus yet exists. Transport of antibodies through the BBB and decoration of plaques have been found to be associated with plaque clearance in AD patients (reviewed by Brody and Holtzman 2008). However, phagocytosis of plaques is not the exclusive mechanism of antibody-mediated plaque clearance. Aβ-recognizing antibodies that lack the fragment necessary for astrocyte-mediated phagocytosis are still able to induce clearance of Aβ plaques in vivo, which suggests that the binding of antibodies to plaques may disrupt their aggregated conformation and lead to clearance by dissolution (Bacskai et al. 2002). However, studies in PDAPP mice have demonstrated that entry of monoclonal antibodies into the CNS is not necessary for reduction of Aβ deposits and that antibodies may reduce Aβ burden by shifting the equilibrium from the CNS to the plasma compartment (DeMattos et al. 2001).

The first of synthetic human monoclonal antibodies to advance to clinical trials is AAB-001. In phase II studies, the subjects treated with AAB-001 showed a non-significant improvement on the ADAS-Cog scale and neuropsychological test battery. However, after stratifying the study population by APOE4 status, the drug was found to have a small but positive effect (Salloway et al. 2009). An improvement of 2.3 points on the ADAS-Cog scale was found among carriers of the allele, although this was not significant across all subscales, and the post hoc nature of this stratification has been viewed skeptically (Strobel 2008). There were also potential safety issues, with 26.7% of patients in the high-dose groups experiencing vasogenic edema as revealed by serial MRI; this complication was more frequent in carriers of the APOE4 allele (Salloway et al. 2009). Phase III trials were initiated on the basis of encouraging trends toward reduced levels of Aβ and phosphorylated tau, a potential indicator of tau pathology, in the CSF, and less brain-volume loss in treated patients (Salloway et al. 2009; Wilcock 2010). The use of CSF measurements to assess the disease-modifying ability of amyloid therapies is reinforced by a recent study supported by several pharmaceutical companies and the NIH, which indicated that a CSF biomarker signature involving AB42 and total tau levels predict conversion from mild cognitive impairment to AD (Shaw et al. 2009). More recently, Genentech Inc. has also developed a humanized monoclonal antibody, MABT5102; a phase I trial is currently in progress.

An alternative means of passive vaccination is the administration of intravenous immunoglobulin (IVIg), which is composed of pooled plasma from healthy donors and contains endogenous antibodies that bind Aβ deposits. In vitro, IVIg inhibited plaque formation, reduced Aβ-induced neurotoxicity, and enhanced microglial activation toward Aβ deposits (Du et al. 2003; Istrin et al. 2006). Four of five patients that completed a pilot study with IVIg showed a significant 3.7 point improvement in ADAS-Cog score after 6 months of treatment (Dodel et al. 2004). Moreover, all patients showed reduction of CSF levels of Aβ at levels ranging from17.3% to 43.5%; however, there was no reported correlation between cognitive improvement and reduction of CSF Aβ (Dodel et al. 2004). Baxter Healthcare Corporation is currently recruiting for phase II trials to assess the effect of 10% human IVIG on cognition and global function in patients with mild to moderate AD.

Ventriculo-peritoneal shunt

The COGNIshunt® is a ventriculo-peritoneal shunt, which differs only from shunts used to treat hydrocephalus in that it has been engineered to allow much lower drainage of the CSF (Silverberg et al. 2004). By improving CSF circulation and turnover, the COGNIshunt® is hypothesized to increase clearance of Aβ peptides, aggregated tau, and other inflammatory mediators from the CNS (Silverberg et al. 2004).

In 2002, Baxter Healthcare began an open label phase II trial, in which 29 patients with mild to moderate AD were implanted with the COGNIshunt®. In spite of reports of seizure, shunt infection, and headache, which are commonly observed in other shunt procedures, all patients recovered and no dropouts were reported. One

year following implantation, total scores on the Mattis Dementia Rating Scale had significantly improved; this stabilization of cognitive function was associated with reductions in CSF Aβ (1–42) and tau (Silverberg et al. 2002). While this study was been criticized for not being double blind or placebo controlled, a phase III trial was initiated in 2001 that enrolled 215 patients with moderate to severe AD. This trial was prematurely terminated due to failure to prove efficacy, and no further clinical trials are in progress (Silverberg et al. 2008).

Limitations of amyloid-based models and therapeutics

Thus far, select amyloid-modifying therapies have effectively improved cognition in animal models and reduced Aβ burden in controlled trials but have not demonstrated efficacy in clinical trials. The consistent failures achieved raise questions about the value of the preclinical model systems, the time course of interventions, and the validity of the amyloid hypothesis.

Despite their lack of predictive validity for amyloid-modifying therapies, transgenic mice are still widely used as a preclinical system to assess the efficacy of AD pharmacotherapies. The lack of predictive validity could arise from the fact that many models were engineered to overexpress human APP and thus model cerebral amyloidsis, rather than recapitulate the hallmarks of AD. Even the manipulations of transgenic models engineered to express neurofibrillary tangles as well as Aβ deposition may not translate to humans or may only apply to patient populations with known genetic predisposition to AD (Clavaguera et al. 2009; Lewis et al. 2001). Moreover, in humans, AD affects higher order cognitive processes, such as executive function and episodic memory, for which no validated test battery exists for murine species. For these reasons, some researchers have begun to move toward non-genetically manipulated species, namely, aged primates and canines as a pharmacological screen for AD therapeutics because they exhibit spontaneous, age-dependent plaque deposition and associated cognitive decline, which can be reliably assessed using extensive test batteries (Cotman and Head 2008; Papaioannou et al. 2001; Walker et al. 1988). The advantages of spontaneous, higher mammalian models are underscored by studies of active vaccination, which better predicted human response than did studies with transgenic mice.

Another barrier in translating preclinical research to human trials is the time course of intervention in relation to the onset of AD. In animal studies, Aβ-modifying compounds are often given before the onset of Aβ deposition or prove to be less effective if given after deposition has begun. Epidemiological studies demonstrating a reduced risk of antecedent AD with chronic NSAID or statin therapy may also reflect the effect of long-term prophylactic use rather than as a first-time intervention for patients already presenting with mild to moderate AD. Likewise, it has been suggested that vaccination against Aβ should optimally be delivered before extensive amyloid deposition (Lemere et al. 2006). One strategy to overcome this barrier is to initiate clinical trials of amyloid-modifying therapies in patients with symptoms of mild cognitive impairment (MCI), which is characterized by a memory deficit without dementi and is a major risk factor for AD (Golob et al. 2001; Zaudig 1992). Clinical trials exploring the use of existing AD therapies have recently been initiated in MCI patients.

Even though some approaches, specifically vaccination, have effectively lowered amyloid burden clinically, there has been no therapy that has had significant clinical effects on cognition. This has led some researchers to question the validity of the amyloid cascade hypothesis. Two further salient criticisms are that, in models, overexpression of amyloid plaques does not induce the other pathological hallmarks of the disease (McGowan et al. 2006), and dense Aβ deposits are reported in cognitively healthy older adults (reviewed by Joseph et al. 2001). Familial forms of AD are associated with mutations that alter the processing and deposition of Aβ and have been pointed to as support of the amyloid cascade hypothesis. Critics of the amyloid cascade hypothesis counter that these mutations account for <2% of AD cases and that these mutations confer greater susceptibility to oxidative and apoptotic insults (Castellani et al. 2006; in 't Veld et al. 1998; Mohmmad Abdul et al. 2006; Mohmmad Abdul et al. 2004). In addition, molecular investigation of sporadic AD suggests that common upstream factors cause both increased Aβ burden and hyperphosphorylated tau (Small and Duff 2008). Two alternatives to the amyloid cascade hypothesis are the OS hypothesis and the dual pathway hypotheses.

According to the OS hypothesis, OS is induced by upstream pathogenic factors and directly causes tau

hyperphoshorylation leading to NFTs and cellular oxidative damage-causing apoptosis (Castellani et al. 2006; Lee et al. 2006; Seabrook et al. 2007). Support for this model comes from in vivo studies of transgenic mice in which OS precedes Aβ deposition (Drake et al. 2003); this phenomenon has also been found in sporadic and familial AD (Nunomura et al. 1999, 2000, 2004). An extension of this reasoning is that Aβ protects against oxidative stress by acting as a redox sensor (Cuajungco et al. 2000; Lee et al. 2006; Yan et al. 1995). In support of this mechanism, neurons respond to OS by increasing Aβ production (Yan et al. 1995), which is associated with a decrease in OS (Dickson et al. 1992; Nunomura et al. 1999, 2000). Aβ deposition is associated with decreases in OS in AD patients (Nunomura et al. 2001), and in aged canines, dietary prevention of OS protects against Aβ deposition (Cotman et al. 2002). It has also been shown that an oxidative event is critical for Aβ neurotoxicity (Rottkamp et al. 2001). Genetic mutations associated with AD also confer greater sensitivity to reactive oxygen species (Mohmmad Abdul et al. 2004, 2006), suggesting that OS may be causally involved in familial cases of AD.

The dual pathway hypothesis posits that Aβ and tau are mechanistically interlinked, and while no parsimonious explanation of this relationship has been offered, APOE4 and glycogen synthase kinase 3 have been offered as potential up-stream regulators. Both of these proteins are typically disrupted in sporadic AD, and either independently or in concert, they influence both plaque and tangle burden. For a review, see Small and Duff (2008).

Another consideration is the possible discrepancy between the species of Aβ that is responsible for neurotoxicity and the species of Aβ that is targeted by amyloid-modifying interventions. Aβ exits as monomers, soluble oligomers, insoluble fibrils, and large senile plaques. There has been a recent suggestion among experts that the soluble oligomeric form is responsible for Aβ-induced neurotoxicity, which has been proposed to account for the lack of correlation between senile plaques and cognitive function (Viola et al. 2008). Relative to the fibrillized and monomeric species of Aβ, oligomers inhibit neuronal viability in vitro 10- and 40-fold, respectively (Dahlgren et al. 2002) and to colocalize with synaptic proteins (Lacor et al. 2004). Further support of the oligomeric hypothesis includes observed elevated $A\beta_{42}$ oligomer ratios in AD patients and patients with mild cognitive impairment relative to healthy aged individuals (Englund et al. 2009; Kokubo et al. 2005; Lacor et al. 2007). Moreover, the oligomeric form of Aβ has been shown to accumulate in an age-dependent manner and to potently interfere with hippocampal LTP and synaptic plasticity (Lacor et al. 2007; Oddo et al. 2006). In addition to the neurotoxic actions of the Aβ fragment, the N-terminal domain of the APP has been shown to have neurotoxic effects in isolation. These effects are mediated through interaction with death-receptor 6 (DR6), a capase-associated receptor expressed along the length of axons. Trophic deprivation increases the expression of DR6 and the surface shedding of the APP ligand, leading to the degeneration of axons. This mechanism has been suggested to account for regulated pruning during development and to be hijacked in AD. In this way, the suppression of APP would be expected to circumvent this independent mechanism of AD pathology (Nikolaev et al. 2009). Identifying and eliminating the neurotoxic form of the amyloid protein will therefore be necessary before clinical benefit is realized.

A final consideration when designing amyloid-modifying therapies is the potential deleterious effects of Aβ removal, given the beneficial functions of Aβ as a potential BBB sealant, regulator of cholesterol signalling, and metal chelator. Aβ spans the plasma membrane, binds to molecules in the plasma membrane, and aggregates under conditions of oxidative stress (Atwood et al. 1998). These properties confer the ability for Aβ to act as a sealant molecule with maintains the integrity of the BBB in areas subject to oxidative damage (Atwood et al. 2002). The association of microhemorrhage with the removal of Aβ via vaccination with AN1792 trials supports this view (Boche et al. 2008). The regulation of cholesterol homeostasis by APP and Aβ signaling is necessary to maintain fluidity of the neuronal membrane (Liu et al. 1998). High concentrations of Cu and Zn have been reported in senile plaques, and Cu and Fe both have high affinities for Aβ (Nakamura et al. 2007). Free Cu catalyzes the formation of ROS (Mathie et al. 2006), while free Zn kills neurons directly (Yokoyama et al. 1986) and indirectly by increasing susceptibility of neurons to excitotoxic insults (Weiss et al. 1993), and both ions disrupt the lipid bilayer of the plasma membrane (Lau et al. 2006). This neuro-

toxicty is attenuated by Aβ-mediated metal chelation in vivo (Cherny et al. 2001; Lee et al. 2004). It has also been speculated that Aβ aggregation is not spontaneous but is dependent on Zn and Cu (Huang et al. 2004). The hypothesis that chelation of these trace elements could prevent the deposition of Aβ plaques has been supported by the ability of the heavy-metal chelator, PBT2, to rapidly decrease brain levels of Aβ and restore cognitive deficits in plaque-expressing mice (Adlard et al. 2008). PBT2 is a heavy metal chelator that crosses the BBB; its administration is associated with higher plasma levels of Zn and lower levels of Aβ than placebo and is well tolerated in phase I trials (Ritchie et al. 2003). In phase II trials, although PBT2 was associated with significant decreases in CSF Aβ levels, subjects treated with PBT2 showed clinical improvement on only two indices of cognitive function (Lannfelt et al. 2008); no further studies are planned.

This emerging body of research, which questions the role of Aβ in AD, fundamentally challenges the basis of existing disease-modifying therapies for AD. It is therefore not surprising that drugs that work solely by lowering Aβ through any mechanism would have limited effectiveness in attenuating disease progression and clinical outcome.

Concluding remarks

To date, no intervention that inhibits the formation and deposition of Aβ and its entry into the CNS or enhances its clearace has moved beyond phase III trials. The consistent failure of amyloid-modifying therapies to stabilize cognition in clinical trials with AD patients, despite significantly altering levels of Aβ in the plasma and CSF compartments, indicates that either more time is needed to refine this kind of therapy, that amyloid-based therapies may need to be used preventatively to demonstrate clinical benefits on cognition, or that the role of Aβ in AD pathogenesis are incompletely understood. It is therefore necessary to re-examine the hypotheses underlying these interventions, specifically the role of the various species of Aβ, their pharmacological modification, and downstream effects. Enhanced understanding of the pathogenesis of AD, in addition to allowing refinement of therapeutic interventions, will lead to the development of novel or enhanced pre-clinical model systems, which more accurately recapitulate AD and are more predictive of human response. Both avenues will need to progress significantly if a clinically effective, disease-modifying therapy for AD is to be realized.

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