Disease-modifying treatments for Alzheimer's disease

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Daniela Galimberti and Elio Scarpini

***Abstract*:** The first drugs developed for Alzheimer's disease (AD), acetylcholinesterase inhibitors (AChEI), increase acetylcholine levels, previously demonstrated to be reduced in AD. To date, four AChEI are approved for the treatment of mild-to-moderate AD. A further therapeutic option available for moderate-to-severe AD is memantine. These treatments are symptomatic, whereas drugs under development are intended to modify the pathological steps leading to AD, thus acting on the evolution of the disease. For this reason they are have been termed 'disease-modifying' drugs. To block the progression of the disease they have to interfere with the pathogenic steps responsible for the clinical symptoms, including the deposition of extracellular amyloid beta (Aβ) plaques and of intracellular neurofibrillary tangles, inflammation, oxidative damage, iron deregulation and cholesterol metabolism. In this review, new perspectives will be discussed. In particular, several approaches will be described, including interference with Aβ deposition by anti-Aβ aggregation agents, vaccination, γ-secretase inhibitors or selective Aβ-lowering agents; interference with tau deposition by methylthioninium chloride; and reduction of inflammation and oxidative damage.

***Keywords*:** Alzheimer's disease, amyloid, disease-modifying drugs, inflammation, tau protein

Correspondence to:
Daniela Galimberti, PhD
Department of Neurological Sciences, 'Dino Ferrari' Center, University of Milan, Fondazione Cà Granda, IRCCS Ospedale Maggiore Policlinico, Via F. Sforza 35, 20122, Milan, Italy
daniela.galimberti@unimi.it

Elio Scarpini, MD
Department of Neurological Sciences, 'Dino Ferrari' Center, University of Milan, Milan, Italy

Alzheimer's disease: pathogenesis and symptomatic treatments

Alzheimer's disease (AD) is the most common cause of dementia in the elderly, with a prevalence of 5% after 65 years of age, increasing to about 30% in people aged 85 years or older. It is characterized clinically by progressive cognitive impairment, including impaired judgement, decision-making and orientation, often accompanied in later stages by psychobehavioural disturbances and language impairment. Mutations in genes encoding for amyloid precursor protein (*APP*), presenilin 1 (*PSEN1*) and presenilin 2 (*PSEN2*) account for about 5% of cases and are characterized by an early onset (before 65 years of age). So far, 32 different mutations causing amino acid changes in putative sites for the cleavage of the protein have been described in the *APP* gene in 85 families, together with 178 mutations in *PSEN1* and 14 in *PSEN2* (see http://www.molgen.ua.ac.be/).

The two major neuropathological hallmarks of AD are extracellular amyloid beta (Aβ) plaques and intracellular neurofibrillary tangles (NFTs). The production of Aβ, which is a crucial step in AD pathogenesis, is the result of cleavage of APP, which is overexpressed in AD [Griffin, 2006]. Aβ forms highly insoluble and proteolysis-resistant fibrils known as senile plaques (SP). NFTs are composed of the tau protein. In healthy subjects, tau is a component of microtubules, which are the internal support structures for the transport of nutrients, vesicles, mitochondria and chromosomes within the cell. Microtubules also stabilize growing axons necessary for the development and growth of neurites [Griffin, 2006]. In AD, tau protein is abnormally hyperphosphorylated and forms insoluble fibrils, causing deposits within the cell.

A number of additional pathogenic mechanisms have been described, possibly overlapping with Aβ plaques and NFT formation, including inflammation [Galimberti *et al.* 2008], oxidative damage [Reddy *et al.* 2009], iron deregulation [Adlard and Bush, 2006] and cholesterol metabolism [Stefani and Liguri, 2009].

The first drugs developed for AD, acetylcholinesterase inhibitors (AChEI), were designed to increase acetylcholine levels, previously demonstrated to be reduced in AD [Lawrence and

Sahakian, 1998]. To date, four AChEI have been approved for the treatment of mild-to-moderate AD: tacrine (First Horizon Pharmaceuticals), donepezil (Pfizer), rivastigmine (Novartis) and galantamine (Janssen) [Farlow, 2002]. Donepezil is now also approved for severe AD. Although tacrine was the first drug approved for AD, in 1993, it is rarely used, due to hepatotoxicity.

A meta-analysis of 13 randomized, double-blind, placebo-controlled trials with donepezil, rivastigmine and galantamine was considered by the Cochrane Dementia and Cognitive Improvement Group's Specialized Register. Conclusions were that the three AChEI are effective for mild-to-moderate AD, although it is not possible to identify patients who will respond to treatment. There is no evidence that treatment with an AChEI is not cost-effective. Despite the slight variations in the mode of action of the three AChEI, there is no evidence of any differences among them with respect to efficacy. There appear to be fewer adverse effects associated with donepezil than rivastigmine. It may be that galantamine and rivastigmine match donepezil in tolerability if a careful and gradual titration routine over more than 3 months is used. Titration with donepezil is more straightforward, and the lower dose may be worth considering [Birks, 2006].

A further therapeutic option for moderate-to-severe AD is memantine. This drug is an uncompetitive, moderate-affinity *N*-methyl-D-aspartate (NMDA) antagonist believed to protect neurons from excitotoxicity. A recent meta-analysis of the efficacy of AChEIs and memantine indicates that these treatments can result in statistically significant but clinically marginal improvement [Raina *et al.* 2008]. Regarding tolerability, AChEIs are associated with cholinomimetic effects. Nausea (2–8%) and vomiting (1–5%) were reported across all AChEI trials as the most common reasons for trial discontinuation. Dizziness, anorexia and diarrhoea were also commonly experienced; however, improved tolerability has been achieved with transdermal administration of rivastigmine. The most frequently reported adverse events in memantine trials were dizziness, headache and confusion [Alva and Cummings, 2008].

On the basis of recent additional findings on AD pathogenesis, novel treatments under development aim to interfere with the pathogenic steps previously mentioned, in an attempt to block the course of the disease in its early stages (even preclinical). For this reason they have been termed 'disease-modifying' drugs. In this review, new compounds and possible strategies for the development of novel therapies will be discussed (Table 1).

Disease-modifying treatments: modulation of amyloid deposition

The amyloid hypothesis

The APP plays a central role in AD pathogenesis and research as it is the precursor of Aβ, which is the heart of the amyloid cascade hypothesis of AD.

The human *APP* gene was first identified in 1987, independently by several laboratories [Goldgaber *et al*. 1987; Kang *et al*. 1987; Robakis *et al.* 1987; Tanzi *et al.* 1987]. The two *APP* homologues, *APLP1* and *APLP2,* were discovered several years later. APP is a type I membrane protein. Two predicted cleavages, one in the extracellular domain (β-secretase cleavage) and another in the transmembrane region (γ-secretase cleavage) are necessary to release Aβ from the precursor protein. Notably, *APP* is located on chromosome 21 and this provided an immediate connection to the invariant development of AD pathology in people with trisomy 21 (Down's syndrome). The first mutations demonstrated to be causative of inherited forms of familial AD were identified in the *APP* gene, providing evidence that APP plays a central role in AD pathogenesis. Notably, only *APP*, and not its homologues *APLP1* and *APLP*, contains sequences encoding the Aβ domain.

Full-length APP undergoes sequential proteolytic processing. It is first cleaved by α-secretase (non-amyloidogenic pathway) or β-secretase (amyloidogenic pathway) within the luminal domain, resulting in the shedding of nearly the entire ectodomain and the generation of α- or β-C-terminal fragments (CTFs). The major neuronal β-secretase, named BACE1 (β-site APP cleaving enzyme), is a transmembrane aspartyl protease that cleaves APP within the ectodomain, generating the *N*-terminus of Aβ [Vassar, 2004]. Several zinc metalloproteinases, such as TACE/ADAM17, ADAM9 and ADAM10, and the aspartyl protease BACE2, can cleave APP at the α-secretase site [Allinson *et al.* 2003] located within the Aβ domain, thus precluding the generation of intact Aβ.

The second proteolytic event in APP processing involves intramembranous cleavage of α- and

Table 1. Disease-modifying drugs tested in clinical trials in patients with Alzheimer's disease.

	Name	Mode of action	Clinical trial phase	Results
Drugs influencing Aβ deposition				
Anti-amyloid aggregation agents	Tramiprosate (Alzhemed™)	GAG mimetic	III	No effects on cognition (definitive)
	Colostrinin	Inhibits Aβ aggregation	II	Modest improvement in MMSE (not definitive)
	Scyllo-inositol (AZD103)	Inhibits Aβ aggregation	II	Ongoing
Vaccination	AN1792	Aβ removal (active immunisation)	II	Unclear cognitive results – severe adverse events (definitive)
	CAD106		II	Ongoing
	Bapineuzumab	Aβ removal (passive immunisation)	III	Ongoing
	ACC-001		II	Ongoing
	LY2062430		III	Ongoing
	MABT5102A		I	Completed
	PF-04360365		I	Completed
	R1450		I	Completed
	GSK933776A		I	Ongoing
	V950		I	Ongoing
	IVIg		I	Ongoing
SALAs	Tarenflurbil (Flurizan™)	Inhibits γ-secretase	III	No effects on cognition (definitive)
γ-secretase inhibitors	LY450139	Inhibits γ-secretase	III	No effects on cognition (definitive)
	BMS-708163		II	Ongoing
α-secretase potentiation	Etazolate	Increases α-secretase activity	II	Completed
Drugs influencing tau deposition				
	MTC (Rember™)	Interferes with tau aggregation	II	Improvement in cognition (not definitive)
Anti-inflammatory drugs				
	Rofecoxib	NSAID; inhibits COX2	III	No effects on cognition (definitive)
	Naproxen	Nonselective NSAID	III	No effects on cognition (definitive)
	Diclofenac	NSAID	II	No effects on cognition (definitive)
	Celecoxib	NSAID	II	No effects on cognition (definitive)
	Hydroxychloroquine	NSAID	II	No effects on cognition (definitive)
	Nimesulide	NSAID; inhibits COX2	II	No effects on cognition (definitive)
	Indometacin	NSAID	II	No effects on cognition-toxicity (definitive)
Drugs preventing oxidative damage				
	Folate/B_6/B_{12}	Reduction of homocysteine	III	No effects on cognition (definitive)
Drugs interfering with metals				
	PBT2	Metal-protein attenuation	II	Improvement in cognition (not definitive)
	Clioquinol	Inhibits zinc and copper from binding to Aβ	II	Reduction in cognitive decline in more severely affected patients only (definitive)
Statins				
	Simvastatin	Cholesterol reduction	III	Ongoing
	Atorvastatin	Cholesterol reduction	III	No effects on cognition (definitive)

Aβ: amyloid beta, COX: cyclooxygenase, GAG: glycosaminoglycan, MMSE: mini mental state evaluation, SALAs: selective Aβ42-lowering agents.

β-CTFs by γ-secretase, which liberates a 3 kDa protein (p3) and Aβ peptide into the extracellular milieu. The minimal components of γ-secretase include presenilin PS1 or PS2, nicastrin, APH-1 and PEN-2 [Edbauer *et al.* 2003]. Biochemical evidence is consistent with PS1 (or PS2) as the catalytic subunit of the γ-secretase. APH-1 and PEN-2 are thought to stabilize the γ-secretase complex, and nicastrin to mediate the recruitment of APP CTFs to the catalytic site of the γ-secretase. Major sites of γ-secretase cleavage correspond to positions 40 and 42 of Aβ.

Amyloidogenic processing is the favoured pathway of APP metabolism in neurons because of the greater abundance of BACE1, whereas the nonamyloidogenic pathway predominates in other cell types.

It seems that none of the above-mentioned secretases has unique substrate specificity towards APP. Besides APP, a number of other transmembrane proteins undergo ectodomain shedding by enzymes with α-secretase activity. Regarding BACE1, its low affinity for APP led to the hypothesis that APP is not its sole physiological substrate. Similarly, PS1 and PS2 play a crucial role in intramembranous γ-secretase cleavage of several type I membrane proteins other than APP, including Notch1 receptors and their ligands [Koo and Kopan, 2004].

A number of functional domains have been mapped to the extracellular and intracellular region of APP, including metal (copper and zinc) binding, extracellular matrix components (heparin, collagen and laminin), and neurotrophic and adhesion domains. Thus far, a trophic role for APP has been suggested, as it stimulates neurite outgrowth in a variety of experimental settings. The *N*-terminal heparin-binding domain of APP also stimulates neurite outgrowth and promotes synaptogenesis. In addition, an 'RHDS' motif near the extralumenal portion of APP likely promotes cell adhesion, possibly acting in an integrin-like manner [Storey *et al.* 1996].

APP was initially proposed to act as a cell-surface receptor. Nevertheless, the evidence supporting this hypothesis has been unconvincing. Only recently, aside from interactions with extracellular matrix proteins, has a candidate ligand been proposed. It was in fact reported that F-spondin, a neuronal-secreted signalling glycoprotein that may function in neuronal development and repair, binds to the extracellular domains of APP, APLP1 and APLP2 [Ho and Südhof, 2004]. This binding reduces β-secretase cleavage of APP, suggesting that F-spondin binding may regulate APP processing.

Drugs interfering with Aβ deposition

Anti-amyloid aggregation agents. The most studied anti-amyloid aggregation agent is tramiprosate (Alzhemed™, Neurochem Inc.), a glycosaminoglycan (GAG) mimetic. GAGs binds to soluble Aβ, promoting fibril formation and deposition of amyloid plaques. GAG mimetics compete for GAG-binding sites, thus blocking fibril formation and reducing soluble Aβ [Gervais *et al.* 2001]. A phase I study in healthy adults demonstrated that the drug is well tolerated. A 3-month phase II study was subsequently conducted in 58 patients with mild-to-moderate AD who were randomized to tramiprosate 50 mg, 100 mg or 150 mg twice a day, or placebo. Patients who completed the study were eligible for a 21 month open-label extension with 150 mg twice a day. Baseline CSF Aβ levels declined by up to 70% after 3 months for patients randomly assigned to the 100 mg or 150 mg twice-daily group. However, no differences were observed in cognitive functions between the tramiprosate and placebo groups [Aisen *et al.* 2006]. A phase III study was then carried out in the US in 1052 patients with AD to test the drug's tolerability, efficacy and safety, but it failed to show any significant effect. Multiple factors probably contributed to the failure of the study. Overall, variability among the 67 clinical sites in the trial overwhelmed the observed treatment effects. Unexpected problems arose in the control group, confounding the interpretation of efficacy. In particular, 30% of the control group did not show a decline in cognition over the 18-month trial period, whereas a portion of this group unexpectedly showed a significant improvement in cognition. A similar trial conducted in Europe has been discontinued. In addition, recent data suggest that tramiprosate promotes an abnormal aggregation of the tau protein in neuronal cells [Santa-Maria *et al.* 2007], emphasizing the importance of testing on both types of pathology (amyloid and tau) the potential drugs for the treatment of AD.

Another molecule under testing is colostrinin, a proline-rich polypeptide complex derived from sheep colostrum (O-CLN; ReGen Therapeutics). Colostrinin inhibits Aβ

aggregation and neurotoxicity in cellular assays and improves cognitive performance in animal models. A 3-week phase I study in patients with AD demonstrated it is well tolerated [Leszek *et al.* 1999]. A subsequent phase II trial demonstrated modest improvements in Mini Mental State Evaluation (MMSE) scores for patients with mild AD over a treatment period of 15 months, but this beneficial effect was not sustained during an additional 15 months of continued treatment [Bilikiewicz and Gaus, 2004].

In 2000, McLaurin and colleagues described a compound named *scyllo*-inositol, which is able to stabilize oligomeric aggregates of Aβ and inhibit Aβ toxicity. *Scyllo*-inositol dose-dependently rescued long-term potentiation in mouse hippocampus from the inhibitory effects of soluble oligomers of cell-derived human Aβ [Townsend *et al.* 2006]. As ELND005 (formerly AZD-103), *scyllo*-inositol is being investigated as an orally administered treatment for AD. A 18-month, randomized, double-blind, placebo-controlled, dose-ranging, safety and efficacy study of oral ELND005 in male and female participants aged 50–85 years with mild-to-moderate AD has been carried out by Transition Therapeutics/Elan. A long-term follow-up study in subjects with AD is ongoing (see http://www.clinicaltrials.gov).

Vaccination. In 1999, Schenk and colleagues demonstrated that immunization with Aβ as an antigen attenuated AD-like pathology in transgenic mice overexpressing the *APP* gene by removing amyloid from the central nervous system [Schenk *et al.* 1999]. This transgenic mouse model of AD progressively develops several neuropathological features of the disease in an age-related and brain-region-dependent manner. Immunization of young animals with Aβ prevents the development of plaque formation, neuritic dystrophy and astroglyosis, whereas in older animals, vaccination reduces the extent and progression of AD-like pathologies. Given these preclinical results, a multicentre, randomized, placebo-controlled, phase II double-blind clinical trial using active immunization with Aβ42 plus adjuvant was started in 2001 on 300 patients using the pre-aggregated Aβ peptide AN1792. However, following reports of aseptic meningoencephalitis in 6% of treated patients, the trial was halted after 2–3 injections. Of the 300 patients treated, 60% developed an antibody response. The final results of the trial were published in 2005 [Gilman *et al.* 2005].

Double-blind assessment was maintained for 12 months, demonstrating no significant differences in cognition between antibody responders and the placebo group for the Alzheimer's disease Assessment Scale – Cognitive Subscale (ADAS-Cog), Disability Assessment for Dementia (DAS), Clinical Dementia Rating (CDR), MMSE and Clinical Global Impression of Change (CGIC). In a small subset of patients, cerebrospinal fluid (CSF) tau levels were decreased in antibody responders but Aβ levels were unchanged.

Long-term follow-up of treated patients and further analysis of autopsy data modified and moderated the negative impact of the first results, encouraging additional clinical attempts. Subsequent observations on AN1792-vaccinated patients or transgenic models, and on brain tissue derived from mice and humans using a new tissue amyloid immunoreactivity (TAPIR) method, suggested that antibodies against Aβ-related epitopes are capable of slowing the progression of neuropathology in AD. In a recent 4-year study, Hock and Nitsch followed 30 patients who received a primary and booster immunization in the first year after vaccination, providing further support for continuing the investigation of antibody treatment in AD [Hock and Nitsch, 2005].

In 2008, a paper was published describing the relationship between Aβ42 immune response, degree of plaque removal and long-term clinical outcomes [Holmes *et al.* 2008]. In June 2003, 80 patients (or their caregivers), who had entered the phase I AN1792 trial in 2000, gave their consent for long-term clinical follow-up and post-mortem neuropathological examination. In patients who received immunization, mean Aβ load was lower than in the placebo group. Despite this observation, however, no evidence of improved survival or an improvement in time to severe dementia was observed in such patients. Therefore, plaque removal is not enough to halt progressive neurodegeneration in AD, prompting some intriguing challenges to the amyloid hypothesis.

Although severe adverse events occurred in the first AN1792 trial and cognitive results were unclear, immunization was not abandoned. The treatment was, however, modified from active to passive in order to avoid excessive activation of the T-cell response and thus prevent complications. The humanized monoclonal anti-Aβ antibody bapineuzumab (Wyeth and Elan) has been

tested in a phase II trial in 200 patients with mild-to-moderate AD. The 18-month, multidose, one-to-one randomization trial was conducted at about 30 sites in the US. It was designed to assess safety, tolerability and standard efficacy endpoints (ADAS-Cog, DAS) of multiple ascending doses of bapineuzumab. The 18-month trial included an interim analysis, and data collection on clinical endpoints and biomarkers [Grundman and Black, 2008]. In May 2007, Elan and Wyeth announced plans to start a phase III clinical trial of bapineuzumab. The decision to launch phase III studies before the conclusion of the ongoing phase II was based on the totality of the accumulated clinical data from phase I, phase II and a 4.5-year follow-up study of those patients involved in the original AN1792 trial.

Among the analyses, different effects were observed when stratifying patients according to their apolipoprotein E (ApoE) status. Looking at the best result of different groupings, it seemed that a small subset of patients, the ApoE noncarriers who received the second-lowest of the four doses six times, responded truly well by 78 weeks. Therefore the phase III study was started in ApoE4 noncarriers with mild-to-moderate AD (see http://www.clinicaltrials.gov).

Additional antibodies under testing include ACC-001 (Wyeth, two phase II studies ongoing in the US and Japan), LY2062430 (solanezumab, Eli Lilly, a phase III study ongoing), MABT5102A (Genentech, phase I completed), PF-04360365 (Pfizer, phase I completed), R1450 (Hoffman-LaRoche, phase I completed), GSK933776A (GlaxoSmithKline, phase I ongoing) and V950 (Merck, phase I ongoing).

Lastly, natural anti-amyloid antibodies have been found in human intravenous immunoglobulins (IVIg) obtained from the pooled plasma of healthy blood donors. In light of these observations, a phase I trial has been carried out in the US. Eight AD patients were treated with IVIg (Gammagard S/D Immune Globulin Intravenous Human) donated by Baxter Healthcare Corporation. Seven patients completed the study. After 6 months, cognitive function stopped declining in all seven patients and improved in six of them (see http://www.alzforum.org). Additional phase I trials are ongoing [Dodel *et al.* 2010].

Passive vaccination requires repeated infusions, and costs are high. Therefore active vaccination has again been considered, by developing specific antigens designed to generate high Aβ antibody titres without inducing Aβ-reactive T cells. The first compound tested in patients with AD is CAD106 (Novartis). Two small studies have been carried out to evaluate safety, tolerability and antibody response to three subcutaneous injections of CAD106 over 12 months. CAD106 was well tolerated and there was a specific Aβ-IgG response in 16/24 patients in cohort I and 18/22 patients in cohort II. A phase II trial is ongoing.

Selective Aβ42-lowering agents. Tarenflurbil is the first in a new class of drugs, selective Aβ42-lowering agents (SALAs), which modulate γ-secretase activity without interfering with Notch or other γ-secretase substrates [Weggen *et al.* 2001]. It binds to a γ-secretase site other than the active/catalytic centre of relevance to production of Aβ42, thereby altering the conformation of γ-secretase and shifting production away from Aβ42 without interfering with other physiologically essential γ-secretase substrates.

Tarenflurbil (MPC-7869; Myriad Pharmaceuticals; Flurizan™) is the pure *R*-enantiomer of flurbiprofen. It shifts cleavage of APP away from Aβ42, leading to the production of shorter, nontoxic fragments [Beher *et al.* 2004]. By contrast with *S*-flurbiprofen or other nonsteroidal anti-inflammatory drugs (NSAIDs), it does not inhibit cyclooxygenase (COX) 1 or COX 2 and is not associated with gastrointestinal toxicity [Townsend and Praticò, 2005]. In mice, treatment with tarenflurbil reduces amyloid plaque burden and prevents learning and behavioural deterioration [Kukar *et al.* 2007].

A 3-week, placebo-controlled, phase I pharmacokinetic study of tarenflurbil (twice-daily doses of 400, 800 or 1600 mg) in 48 healthy older volunteers showed that the drug is well tolerated, with no evidence of renal or gastrointestinal toxicity. CSF was collected at baseline and after 3 weeks. The compound penetrated the blood–brain barrier in a dose-dependent manner. No significant changes in Aβ42 CSF levels were shown after treatment. However, higher drug concentrations in plasma were related to statistically significantly lower Aβ levels [Galasko *et al.* 2007].

Myriad conducted a 12-month placebo-controlled phase II trial of tarenflurbil in

210 patients with mild-to-moderate AD (MMSE score: 15–26). Patients were randomly assigned to receive tarenflurbil twice daily (400 mg or 800 mg or placebo) for 12 months. Primary outcome measures were the rate of change (slope of decline) of: activities of daily living, quantified by the Alzheimer's Disease Cooperative Study – Activities of Daily Living inventory (ADCS-ADL), global function, measured by the Clinical Dementia Rating scale Sum of Boxes (CDR-SB) and cognitive function, measured by ADAS-Cog. In a 12-month extended treatment phase, patients who had received tarenflurbil continued to receive the same dose, and patients who had received placebo were randomly assigned to tarenflurbil at 800 mg or 400 mg twice daily.

A preliminary analysis revealed that patients with mild AD (MMSE: 20–26) and moderate AD (MMSE: 15–19) responded differently to tarenflurbil according to ADAS-cog and ADCS-ADL; these groups were therefore analysed separately. Patients with mild AD in the 800 mg tarenflurbil group had lower rates of decline than did those in the placebo group in the activities of daily living, whereas slowing of cognitive decline did not differ significantly. In patients with moderate AD, 800 mg tarenflurbil twice daily had no significant effects on ADCS-ADL and ADAS-Cog and had a negative effect on CDR-SB. The most common adverse events included diarrhoea, nausea and dizziness. Patients with mild AD who were in the 800 mg tarenflurbil group for 24 months had lower rates of decline for all three primary outcomes than did patients who were in the placebo group for months 0–12 and a tarenflurbil group for months 12–24 [Wilcock *et al.* 2008].

Given these results, two phase III studies were carried out in the US and Europe. The ActEarliAD trial was started in 2007 throughout Europe. It is an 18-month, multinational, randomized, double-blind, placebo-controlled study in more than 800 patients with AD. Patients enrolled in the trial were treated with tarenflurbil 800 mg twice daily or placebo and attended periodic physician visits for analysis of their performance on memory, cognition and behavioural tests. The two primary clinical endpoints were the change in cognitive decline and function, as measured by ADAS-Cog, and changes in activity of daily living, as measured by the ADCS-ADL. A secondary endpoint of the trial was the change in overall function, measured by CDR-SB. Additional exploratory outcome measures were designed to assess the psychological, physical and financial impact of the disease on caregivers and medical resources. The trial was designed to meet the requirements of the European Agency for the Evaluation of Medicinal Products (EMEA) for the marketing of tarenflurbil in Europe. The global endpoints in the European trial were identical to those in the US trial. As was the case with the phase II trial, all patients in the phase III studies were allowed to take current standard-of -are medicines in addition to tarenflurbil or placebo, provided their dose had been stable for 6 months.

Disappointingly, in July 2008, the sponsor announced that tarenflurbil had failed its definitive phase III trial and would not be developed further (see http://www.alzforum.org). In fact, on both primary efficacy endpoints (the ADAS-cog and ADCS-ADL scales), the treatment and placebo curves overlapped almost completely, and there was no effect whatsoever in the group as a whole. In addition, although the overall side-effect profile was similar between placebo and treatment groups, anaemia, infections and gastrointestinal ulcers appeared more often in the tarenflurbil group than in the placebo group.

γ-secretase inhibition. Several compounds that inhibit γ-secretase activity in the brain have been identified. Nevertheless, γ-secretase has many biologically essential substrates [Pollack and Lewis, 2005]. One of the most physiologically important γ-secretase substrate is the Notch signalling protein, which is involved in the differentiation and proliferation of embryonic cells, T cells, gastrointestinal goblet cells and splenic B cells. Experience with transgenic mice has shown that the administration of a γ-secretase inhibitor in doses sufficient to lower Aβ concentrations interferes with lymphocyte differentiation [Wong *et al.* 2004]. Safety is therefore a very important consideration for this class of compound.

A nonselective γ-secretase inhibitor, LY450139 (Eli Lilly), has been evaluated in a phase I placebo-controlled study in 37 healthy adults (at doses ranging from 5 to 50 mg). Aβ CSF levels were reduced in both the active treatment and placebo groups, but differences were not statistically significant. Transient gastrointestinal adverse effects (bleeding, abdominal pain) were reported by two subjects treated with 50 mg [Siemers *et al.* 2005]. A subsequent phase II randomized, controlled

trial was carried out in 70 patients with AD. Patients were given 30 mg for 1 week followed by 40 mg for 5 weeks. Treatment was well tolerated. No significant changes in plasma and CSF Aβ40 and Aβ42 were observed [Siemers *et al.* 2006].

Subsequently, a multicentre, randomized, double-blind, dose-escalation, placebo-controlled trial was carried out. Fifty-one patients with mild-to-moderate AD were randomized to receive placebo or LY450139 (100 mg or 140 mg). The LY450139 groups received 60 mg/day for 2 weeks, then 100 mg/day for 6 weeks, then either 100 or 140 mg/day for an additional 6 weeks. Primary outcomes included safety, tolerability and CSF/plasma Aβ levels; secondary outcome was neuropsychological testing. LY450139 was well tolerated at doses up to 140 mg/day for 14 weeks. Plasma Aβ, but not CSF, levels were reduced in treated patients, consistent with inhibition of γ-secretase. No differences were seen in cognitive or functional measures [Fleisher *et al.* 2008].

A potent γ-secretase inhibitor, BMS-708163 (Bristol-Myers Squibb), was tested in a phase I clinical trial. After 18 days, BMS-708163 decreased CSF Aβ40 and Aβ42 by 30% following a daily dose of 100 mg and by 60% at daily dose of 150 mg. A phase II study is ongoing (see http://www.alzforum.org).

α-secretase potentiation. Etazolate (EHT 0202, ExonHit Therapeutics) stimulates the neurotrophic α-secretase (nonamyloidogenic) pathway and inhibits Aβ-induced neuronal death, providing symptomatic relief and modifying disease progression. *In vitro*, it is neuroprotective against Aβ42, and neuroprotection is associated with sAPPα induction [Marcade *et al.* 2008]. After a phase I study in healthy volunteers, a phase II clinical trial has been recently completed that assessed safety, tolerability and preliminary efficacy on cognition and behaviour in AD patients, as well as quantification of sAPPα in blood (see http://www.alzforum.org).

β-site APP cleaving enzyme inhibition. Research into compounds able to inhibit β-site APP cleaving enzyme (BACE) is still in the preclinical phase. The pharmacophore model of arylpiperazine amide derivatives was built using the Discovery Studio 2.0 software package and the best pharmacophore model was validated by enrichment and the receiver operating characteristic (ROC) method. According to the best model, 11 *N*-phenyl-1-arylamide, *N*-phenylbenzenesulfonamide derivatives, compounds 26–28, and 33a–33g, were designed to be synthesized and their BACE 1 inhibitory activities were determined experimentally. The theoretical results were in good agreement with the experimental values. Compound 33d, which displayed the highest BACE 1 activity among the two series, was chosen to study the protein binding pattern and the result showed that it was in close contact with two essential catalytic aspartates (Asp32 and Asp228) of the BACE 1 [Huang *et al.* 2008]. Other compounds with a potential BACE1 inhibitory effect include neocorylin [Choi *et al.* 2008], epigallocatechin-3-gallate and curcumin [Shimmyo *et al.* 2008] and N (4)-substituted piperazine naphthamide derivatives [Laras *et al.* 2009].

Disease-modifying treatments: modulation of tau deposition

Tau and Alzheimer's disease

Tau is relatively abundant in neurons but is present in all nucleated cells and functions physiologically to bind microtubules and stabilize microtubule assembly for polymerization. The tau-encoding gene (*MAPT;* Microtubule Associating Protein Tau) consists of 16 exons. In the adult brain, alternative splicing of tau nuclear RNA results in six tau isoforms having either three or four peptide repeats of 31 or 32 residues in the *C*-terminal region encoded on exon 10, comprising the microtubule binding domain, or differing in the expression of zero, one or two inserts encoded on exon 2 and 3. During neurodegeneration, tau is abnormally phosphorylated. The profile of alternative splicing differs among pathological phenotypes, such that tau accumulation in AD is a mixture of 3R and 4R tau, Pick's disease tends to be 3R tau, corticobasal degeneration and progressive supranuclear palsy tend to be 4R tau, and so-called argyrophilic grain disease accumulates small inclusions of 3R tau [Castellani *et al.* 2008].

Drugs interfering with tau deposition

A phase II trial of a tau-blocking compound named methylthioninium chloride (MTC) is ongoing (TauRx Therapeutics, Rember™). This is a reducing agent better known as methylene blue, a deep blue dye used in analytical chemistry, as a tissue stain in biology, and in various industrial products. MTC interferes with tau aggregation by acting on self-aggregating

truncated tau fragments [Wischik *et al.* 1996]. The company conducted a phase II trial randomizing 321 patients with mild or moderate AD to treatment with either placebo or one of three oral doses of MTC: 30 mg, 60 mg or 100 mg three times daily. Patients were not taking AChEI or memantine. Primary outcomes were to compare the effect of MTC with that of placebo on cognitive abilities measured by the ADAS-Cog at 24 weeks. Preliminary results were presented at the 2008 International Conference on Alzheimer's Disease. The 100 mg dose was found to have a formulation defect limiting release of the therapeutic form of MTC, therefore this arm was discontinued. In CDR-moderate subjects at the 60 mg dose, a significant improvement was shown relative to placebo of −5.4 ADAS-Cog units. There was no placebo decline in CDR-mild AD over the first 24 weeks, preventing initial efficacy analysis. A problem with the use of this drug is that urine becomes blue, resulting in a lack of blinding. These preliminary results need to be considered cautiously until definitive data are published.

An interesting approach to block tau deposition is to inhibit kinases responsible for tau hyperphosporylation. Despite the large number of tau phosphorylation sites and the ability of multiple kinases to phosporylate individual sites, glycogen synthase kinase 3 (GSK3β) has emerged as potential therapeutic target [Balaraman *et al.* 2006]. The most studied compound able to inhibit GSK3 is lithium, but several other compounds are under development, including pyrazolopyrazines, pyrazolopyridines, the aminothiazole AR-A014418, and sodium valproate [Martinez and Perez, 2008; Schneider and Mandelkow, 2008].

Similarly to AD, vaccination approaches have been considered, but the development of a successful therapy is complicated by the fact that tau protein is intracellular.

Disease-modifying treatments: modulation of inflammation and oxidative damage

Anti-inflammatory drugs

Epidemiological evidence suggests that long-term use of NSAIDs protects against the development of AD [McGeer *et al.* 1996]. Despite this premise, prospective studies of rofecoxib [Reines *et al.* 2004], naproxen [Aisen *et al.* 2003], diclofenac [Scharf *et al.* 1999], celecoxib [Soininen *et al.* 2007], dapsone [Eriksen *et al.* 2003], hydroxychloroquine [Aisen *et al.* 2001] and nimesulide [Aisen *et al.* 2002] failed to slow progression of cognitive decline in patients with mild-to-moderate AD. By contrast, indometacin may delay cognitive decline in this subset of patients, but gastrointestinal toxicity is treatment-limiting [Rogers *et al.* 1993]. Because of general concerns about lack of efficacy, gastrointestinal toxicity, myocardial infarction and stroke, NSAIDs are not considered to be viable treatment options for patients with AD.

Molecules addressing oxidative damage

A trial to determine whether the reduction of homocysteine levels with high-dose folate, vitamin B_6 and vitamin B_{12} supplementation can slow the rate of cognitive decline in subjects with AD has been tried in a multicentre, randomized, controlled clinical trial named VITAL (VITamins to slow ALzheimer's disease). The study included 409 individuals with mild-to-moderate AD (MMSE between 14 and 26) and normal folic acid, vitamin B_{12} and homocysteine (Hcy) levels. Participants were randomly assigned to two groups of unequal size (60% treated with high-dose supplements: 5 mg/day folate, 25 mg/day vitamin B_6 and 1 mg/day vitamin B_{12}; and 40% treated with identical placebo); duration of treatment was 18 months. The main outcome measure was the change in the ADAS-Cog score. A total of 340 participants completed the trial. Although the vitamin supplement regimen was effective in reducing Hcy levels, it had no beneficial effect on the primary cognitive measure, the rate of change in ADAS-Cog score over 18 months, or on any secondary measures [Aisen *et al.* 2008].

Additional potential antioxidants include mitoquinone (Antipodean Pharmaceuticals), vitamin E, *Ginkgo biloba* and natural polyphenols such as green tea, wine, blueberries and curcumin. Clinical trial with vitamin E and omega-3 fatty acids did not show beneficial effects in AD patients [Barten and Albright, 2008].

Disease-modifying treatments: additional approaches

Drugs interfering with metals

In 1994 it was observed that Aβ becomes amyloidogenic upon reaction with stoichiometric amounts of Zn^{2+} and Cu^{2+} [Bush *et al.* 1994]. Aβ is rapidly precipitated by Zn^{2+}. Cu^{2+} and Fe^{3+} also induce marked Aβ aggregation, but only under mildly acidic conditions [Bush,

2008], such as those believed to occur in the brains of people with AD. The precipitation of Aβ by these ions is reversible with chelation, by contrast with fibrillization, which is irreversible. Cu, Fe and Zn play a greater role than merely assembling Aβ. When binding Cu^{2+} or Fe^{3+}, Aβ reduces the metal ions and produces H_2O_2 by double electron transfer to O_2. In addition, Aβ promotes the Cu-mediated generation of the toxic lipid oxidation product 4-hydroxynonenal [Bush, 2008].

PBT2, an analogue of clioquinol, was designed to modify the course of AD by preventing metal-dependent aggregation, deposition and toxicity of Aβ. PBT2 acts at three levels of the 'amyloid cascade': it inhibits the redox-dependent formation of toxic soluble oligomers, prevents deposition of Aβ as amyloid plaques, and promotes clearance by mobilizing and neutralizing Aβ from existing deposits [Cherny *et al.* 2001]. PBT2 has been recently tested in a phase II trial. Seventy-eight patients with mild AD were randomly assigned to PBT2 50 mg, PBT2 250 mg or placebo (in addition to AChEI) for 12 weeks. No serious adverse events were reported by patients on PBT2. Patients treated with PBT2 250 mg had a dose-dependent and significant reduction in CSF Aβ42 concentration, compared with those treated with placebo [Lannfelt *et al.* 2008]. Cognitive efficacy was, however, restricted to two measures only, therefore future larger and longer trials are needed to test the efficacy of this drug on cognition.

The parent compound, clioquinol (PBT1, Prana Biotechnology), was tested in a clinical trial for AD that showed a reduction in the rate of cognitive decline in the more severely affected subgroup of patients only [Ritchie *et al.* 2003]. According to a Cochrane Collaboration study, it was not clear from this trial whether clioquinol showed any positive clinical result. The two statistically significant positive results were seen for the more severely affected subgroup of patients; however, this effect was not maintained at the 36-week endpoint, and this group was small (eight treated subjects). The sample size was small. Details of randomization procedure or blinding were not reported [Jenagaratnam and McShane, 2006].

Modulation of cholesterol and vascular-related risk factors

It has repeatedly been shown that ApoE carriers have a higher risk of developing AD. Because ApoE is the major cholesterol transporter in the central nervous system, a link between cholesterol and AD is suggested. The brain is the most cholesterol-rich organ of the body, and most of the cholesterol is synthesized in astrocytes. A link between hypercholesterolemia, cardiovascular diseases and AD has also been suggested. Additional vascular-related risk factors for AD include hypertension, atrial fibrillation, hyperhomocysteinemia, atherosclerosis and stroke [Hooijmans *et al.* 2008].

Hypertension is the strongest risk factor for AD and vascular dementia when these conditions are considered together [Skoog *et al.* 1996]. It is closely associated with atherosclerosis and vascular function, and in the brain results in hypoperfusion and ischemic conditions of the nucleus basalis of Meynert. The use of targeted molecular mechanisms and dietary methods and therapies is grounded in reducing free radicals and associated oxidative stress-related damage initiating hypertension [Vasdev *et al.* 2007].

Epidemiological studies have indicated that patients treated for cardiovascular disease with cholesterol-lowering therapy (statins) showed a decreased prevalence of AD [Jick *et al.* 2000]. Simvastatin is a prodrug, hydrolyzed *in vivo* to generate mevinolinic acid, an active metabolite that is structurally similar to HMG-CoA. This metabolite competes with HMG-CoA for binding HMG-CoA reductase, a hepatic microsomal enzyme. Simvastatin metabolites are high-affinity HMG-CoA reductase inhibitors, reducing the quantity of mevalonic acid, a precursor of cholesterol.

CLASP is an ongoing randomized, double-blind, placebo-controlled, parallel-assignment phase III trial to investigate the safety and effectiveness of simvastatin to slow the progression of AD. The clinical trial involves 400 participants with mild-to-moderate AD, and the objective is to evaluate the safety and efficacy of simvastatin to slow the progression of AD, as measured by ADAS-Cog. Measures of clinical global change (ADCS-CGIC; Alzheimer's Disease Cooperative Study – Clinical Global Impression of Change), mental status, functional ability, behavioural disturbances, quality of life and economic indicators will also be made. The study medication will be as follows: 20 mg simvastatin or matching placebo to be given for 6 weeks, followed by 40 mg simvastatin or matching placebo for the remainder of the 18-month study period (see http://www.clinicaltrials.gov).

The Lipitor's Effect in Alzheimer's Dementia (LEADe) study tests the hypothesis that a statin (atorvastatin 80 mg daily) will provide a benefit on the course of mild-to-moderate AD in patients receiving background therapy of donepezil 10 mg daily. An international, multicentre, double-blind, randomized, parallel-group study with a double-blind randomized withdrawal phase in patients with mild-to-moderate AD (MMSE = 13–25) was carried out. Inclusion criteria included age 50–90 years, receiving donepezil 10 mg for at least 3 months before randomization, and low-density lipoprotein cholesterol levels (LDL-C) 2.5 to 3.5 mmol/l (95–195 mg/dl). Co-primary endpoints were changes in ADAS-Cog and ADCS-CGIC scores. A confirmatory endpoint was rate of change in whole brain and hippocampal volumes in patients who were enrolled in the magnetic resonance imaging substudy [Jones *et al.* 2008]. Despite a promising premise, there were no significant differences in the co-primary endpoints of ADAS-Cog or ADCS-CGIC, or the secondary endpoints, although atorvastatin was generally well tolerated [Feldman *et al.* 2010].

Final remarks

From data presented in this review, three main considerations have emerged that should be taken into account when planning future clinical trials.

First, the mechanisms underlying the pathogenesis of AD need to be thoroughly investigated before starting the development of novel compounds claimed to be disease-modifying. Despite promising premises related to the so-called amyloid hypothesis and other pathogenic mechanisms, large phase III trials with potentially disease-modifying properties have failed to demonstrate any effect on cognition. A good lesson comes from the neuropathological analysis of brains from patients who received immunization, which demonstrated that although mean Aβ load was lower than in the placebo group, there was no evidence of improved survival or improvement in time to severe dementia. Plaque removal therefore seems not to be sufficient to halt progressive neurodegeneration in AD, prompting some intriguing challenges to the amyloid hypothesis. In light of these considerations, it is of crucial importance to better understand the relationship between tau, Aβ and other factors for developing novel potentially disease-modifying drugs.

The second point to be addressed is that treatments for AD appear effective only in certain phases of the disease. A few disease-modifying compounds showed some benefits in mild but not moderate AD. The same was observed for anti-inflammatory drugs, for which recent studies have demonstrated a high degree of inflammation in very mild but not in severe AD. Therapeutic trials should therefore be carried out as early as possible during the course of the disease, which requires the identification of more accurate tools for early diagnosis. In this regard, new research diagnostic criteria for early and specific diagnosis were proposed in 2007 [Dubois *et al.* 2007], introducing the use of CSF analysis, structural and functional imaging and genetics, together with classical neuropsychological testing. Large-scale international controlled multicentre trials are engaged in phase III development of the feasible core imaging and CSF biomarker candidates in AD (US, European, Australian and Japanese AD Neuroimaging Initiative, and the German Dementia Network). If validation of these new criteria is to be achieved, they should be considered in the setting of future clinical trials to identify more homogeneous study groups.

Last, indicators useful as surrogate outcome measures (surrogate biomarkers) should be identified in order to have substitutes for clinical endpoints (i.e. neuropsychological testing), tools able to predict clinical benefit or the opposite, and to demonstrate whether the drug has disease-modifying properties. So far, no biomarker proposed for early diagnosis has been validated as a surrogate marker for monitoring treatments.

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References

Adlard, P.A. and Bush, A.I. (2006) Metals and Alzheimer's disease. *J Alzheimers Dis* 10: 145–163.

Aisen, P.S., Marin, D.B., Brickman, A.M., Santoro, J. and Fusco, M. (2001) Pilot tolerability studies of hydroxychloroquine and colchicine in Alzheimer disease. *Alzheimer Dis Assoc Disord* 15: 96–101.

Aisen, P.S., Saumier, D., Briand, R., Laurin, J., Gervais, F., Tremblay, P. *et al.* (2006) A phase II study

targeting amyloid-beta with 3APS in mild-to-moderate Alzheimer disease. *Neurology* 67: 1757–1763.

Aisen, P.S., Schafer, K.A., Grundman, M., Pfeiffer, E., Sano, M., Davis, K.L. *et al.* (2003) Effects of rofecoxib or naproxen vs placebo on Alzheimer's disease progression: a randomized controlled trial. *JAMA* 289: 2819–2826.

Aisen, P.S., Schmeidler, J. and Pasinetti, G.M. (2002) Randomized pilot study of nimesulide treatment in Alzheimer's disease. *Neurology* 58: 1050–1054.

Aisen, P.S., Schneider, L.S., Sano, M., Diaz-Arrastia, R., van Dyck, C.H., Weiner, M.F. *et al.* (2008) High-dose B vitamin supplementation and cognitive decline in Alzheimer disease: a randomized controlled trial. *JAMA* 300: 1774–1783.

Allinson, T.M., Parkin, E.T., Turner, A.J. and Hooper, N.M. (2003) ADAMs family members as amyloid precursor protein alpha-secretases. *J Neurosci Res* 74: 342–352.

Alva, G. and Cummings, J.L. (2008) Relative tolerability of Alzheimer's disease treatments. *Psychiatry (Edgmont)* 5: 27–36.

Balaraman, Y., Limaye, A.R., Levey, A.I. and Srinivasan, S. (2006) Glycogen synthase kinase 3β and Alzheimer's disease: pathophysiological and therapeutic significance. *Cell Mol Life Sci* 63: 1226–1235.

Barten, D.M. and Albright, C.F. (2008) Therapeutic strategies for Alzheimer's disease. *Mol Neurobiol* 37: 171–186.

Beher, D., Clarke, E.E., Wrigley, J.D., Martin, A.C., Nadin, A., Churcher, I. *et al.* (2004) Selected nonsteroidal anti-inflammatory drugs and their derivatives target γ-secretase at a novel site: evidence for an allosteric mechanism. *J Biol Chem* 279: 43419–43426.

Bilikiewicz, A. and Gaus, W. (2004) Colostrinin (a naturally occurring, proline-rich, polypeptide mixture) in the treatment of Alzheimer's disease. *J Alzheimers Dis* 6: 17–26.

Birks, J. (2006) Cholinesterase inhibitors for Alzheimer's disease. *Cochrane Database Syst Rev* 1, CD005593.

Bush, A.I. (2008) Drug development based on the metals hypothesis of Alzheimer's disease. *J Alzheimers Dis* 15: 223–240.

Bush, A.I., Pettingell, W.H., Multhaup, G., Paradis, M., Vonsattel, J.P., Gusella, J.F. *et al.* (1994) Rapid induction of Alzheimer Aβ amyloid formation by zinc. *Science* 265: 1464–1467.

Castellani, R.J., Nunomura, A., Lee, H., Perry, G. and Smith, M.A. (2008) Phosphorylated tau: toxic, protective, or none of the above. *J Alzheimers Dis* 14: 377–383.

Cherny, R.A., Atwood, C.S., Xilinas, M.E., Gray, D.N., Jones, W.D., McLean, C.A. *et al.* (2001) Treatment with a copper-zinc chelator markedly and rapidly inhibits β-amyloid accumulation in Alzheimer's disease transgenic mice. *Neuron* 30: 665–676.

Choi, Y.H., Yon, G.H., Hong, K.S., Yoo, D.S., Choi, C.W., Park, W.K. *et al.* (2008) In vitro BACE-1 inhibitory phenolic components from the seeds of *Psoralea corylifolia*. *Planta Med* 74: 1405–1408.

Dodel, R., Neff, F., Noelker, C., Pul, R., Du, Y., Bacher, M. *et al.* (2010) Intravenous immunoglobulins as a treatment for Alzheimer's disease: rationale and current evidence. *Drugs* 70: 513–528.

Dubois, B., Feldman, H.H., Jacova, C., Dekosky, S.T., Barberger-Gateau, P., Cummings, J. *et al.* (2007) Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS-ADRDA criteria. *Lancet Neurol* 6(8): 734–746.

Edbauer, D., Winkler, E., Regula, J.T., Pesold, B., Steiner, H. and Haass, C. (2003) Reconstitution of gamma-secretase activity. *Nat Cell Biol* 5: 486–488.

Eriksen, J.L., Sagi, S.A., Smith, T.E., Weggen, S., Das, P., McLendon, D.C. *et al.* (2003) NSAIDs and enantiomers of flurbiprofen target gamma-secretase and lower Abeta 42 in vivo. *J Clin Invest* 112: 440–449.

Farlow, M. (2002) A clinical overview of cholinesterase inhibitors in Alzheimer's disease. *Int Psychogeriatr* 14(Suppl 1): 93–126.

Feldman, H.H., Doody, R.S., Kivipelto, M., Sparks, D.L., Waters, D.D., Jones, R.W. *et al.* (2010) Randomized controlled trial of atorvastatin in mild to moderate Alzheimer disease: LEADe. *Neurology* 74: 956–964.

Fleisher, A.S., Raman, R., Siemers, E.R., Becerra, L., Clark, C.M., Dean, R.A. *et al.* (2008) Phase 2 safety trial targeting amyloid beta production with a gamma-secretase inhibitor in Alzheimer disease. *Arch Neurol* 65: 1031–1038.

Galasko, D.R., Graff-Radford, N., May, S., Hendrix, S., Cottrell, B.A., Sagi, S.A. *et al.* (2007) Safety, tolerability, pharmacokinetics, and Aβ levels after short-term administration of R-flurbiprofen in healthy elderly individuals. *Alzheimer Dis Assoc Disord* 21: 292–299.

Galimberti, D., Fenoglio, C. and Scarpini, E. (2008) Inflammation in neurodegenerative disorders: friend or foe? *Curr Aging Sci* 1: 30–41.

Gervais, F., Chalifour, R., Garceau, D., Kong, X., Laurin, J., Mclaughlin, R. *et al.* (2001) Glycosaminoglycan mimetics: a therapeutic approach to cerebral amyloid angiopathy. *Amyloid* 8(Suppl 1): 28–35.

Gilman, S., Koller, M. and Black, R.S. (2005) Clinical effects of Aβ immunization (AN1792) in patients with AD in an interrupted trial. *Neurology* 64: 1553–1562.

Goldgaber, D., Lerman, M.I., McBride, O.W., Saffiotti, U. and Gajdusek, D.C. (1987) Characterization and chromosomal localization of a cDNA encoding brain amyloid of Alzheimer's disease. *Science* 235: 877–880.

Griffin, W.S. (2006) Inflammation and neurodegenerative diseases. *Am J Clin Nutr* 3(Suppl): 470S–474S.

Grundman, M. and Black, R. (2008) Clinical trials of bapineuzumab, a beta-amyloid targeted immunotherapy in patients with mild to moderate Alzheimer's disease. *Alzheimers Dement* 4(Suppl 2): T166.

Ho, A. and Südhof, T.C. (2004) Binding of F-spondin to amyloid-beta precursor protein: a candidate amyloid-beta precursor protein ligand that modulates amyloid-beta precursor protein cleavage. *Proc Natl Acad Sci USA* 101: 2548–2553.

Hock, C. and Nitsch, R. (2005) Clinical observations with AN1792 using TAPIR analyses. *Neurodegener Dis* 2: 273–276.

Holmes, C., Boche, D., Wilkinson, D., Yadegarfar, G., Hopkins, V., Bayer, A. *et al.* (2008) Long-term effect of $A\beta_{42}$ immunisation in Alzheimer's disease: follow-up of a randomised, placebo-controlled phase I trial. *Lancet* 372: 216–223.

Hooijmans, C.R. and Kiliaan, A.J. (2008) Fatty acids, lipid metabolism and Alzheimer pathology. *Eur J Pharmacol* 585: 176–196.

Huang, W., Yu, H., Sheng, R., Li, J. and Hu, Y. (2008) Identification of pharmacophore model, synthesis and biological evaluation of N-phenyl-1-arylamide and N-phenylbenzenesulfonamide derivatives as BACE 1 inhibitors. *Bioorg Med Chem* 16: 10190–10197.

Jenagaratnam, L. and McShane, R. (2006) Clioquinol for the treatment of Alzheimer's Disease. *Cochrane Database Syst Rev* 25, CD005380.

Jick, H., Zornberg, G.L., Jick, S.S., Seshadri, S. and Drachman, D.A. (2000) Statins and the risk of dementia. *Lancet* 356: 1627–1631.

Jones, R.W., Kivipelto, M., Feldman, H., Sparks, L., Doody, R., Waters, D.D. *et al.* (2008) The Atorvastatin/Donepezil in Alzheimer's Disease Study (LEADe): design and baseline characteristics. *Alzheimers Dement* 4: 145–153.

Kang, J., Lemaire, H.G., Unterbeck, A., Salbaum, J.M., Masters, C.L., Grzeschik, K.H. *et al.* (1987) The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. *Nature* 325: 733–736.

Koo, E.H. and Kopan, R. (2004) Potential role of presenilin-regulated signaling pathways in sporadic neurodegeneration. *Nat Med* 10(Suppl): S26–S33.

Kukar, T., Prescott, S., Eriksen, J.L., Holloway, V., Murphy, M.P., Koo, E.H. *et al.* (2007) Chronic administration of R-flurbiprofen attenuates learning impairments in transgenic amyloid precursor protein mice. *BMC Neurosci* 8: 54.

Lannfelt, L., Blennow, K., Zetterberg, H., Batsman, S., Ames, D., Harrison, J. *et al.* (2008) Safety, efficacy, and biomarker findings of PBT2 in targeting Abeta as a modifying therapy for Alzheimer's disease: a phase IIa, double-blind, randomized, placebo-controlled trial. *Lancet Neurol* 7: 779–786.

Laras, Y., Garino, C., Dessolin, J., Weck, C., Moret, V., Rolland, A. *et al.* (2009) New N(4)-substituted piperazine naphthamide derivatives as BACE-1 inhibitors. *J Enzyme Inhib Med Chem* 24: 181–187.

Lawrence, A.D. and Sahakian, B.J. (1998) The cognitive psychopharmacology of Alzheimer's disease: focus on cholinergic systems. *Neurochem Res* 23: 787–794.

Leszek, J., Inglot, A.D., Janusz, M., Lisowski, J., Krukowska, K. and Georgiades, J.A. (1999) Colostrinin: a proline-rich polypeptide (PRP) complex isolated from ovine colostrum for treatment of Alzheimer's disease. A double-blind, placebo-controlled study. *Arch Immunol Ther Exp (Warsz)* 47: 377–385.

Marcade, M., Bourdin, J., Loiseau, N., Peillon, H., Rayer, A., Drouin, D. *et al.* (2008) Etazolate, a neuroprotective drug linking GABA(A) receptor pharmacology to amyloid precursor protein processing. *J Neurochem* 106: 392–404.

Martinez, A. and Perez, D.I. (2008) GSK-3 inhibitors: a ray of hope for the treatment of Alzheimer's disease? *J Alzheimers Dis* 15: 181–191.

McGeer, P.L., Schulzer, M. and Mc Geer, E.G. (1996) Arthritis and anti-inflammatory agents as possible protective factors for Alzheimer's disease: a review of 17 epidemiologic studies. *Neurology* 47: 425–432.

McLaurin, J., Golomb, R., Jurewicz, A., Antel, J.P. and Fraser, P.E. (2000) Inositol stereoisomers stabilize an oligomeric aggregate of Alzheimer amyloid beta peptide and inhibit Abeta-induced toxicity. *J Biol Chem* 275: 18495–18502.

Pollack, S.J. and Lewis, H. (2005) Secretase inhibitors for Alzheimer's disease: challenges of promiscuous protease. *Curr Opin Investig Drugs* 6: 35–47.

Raina, P., Santaguida, P., Ismaila, A., Patterson, C., Cowan, D., Levine, M. *et al.* (2008) Effectiveness of cholinesterase inhibitors and memantine for treating dementia: evidence review for a clinical practice guideline. *Ann Intern Med* 148: 379–397.

Reddy, V.P., Zhu, X., Perry, G. and Smith, M.A. (2009) Oxidative stress in diabetes and Alzheimer's disease. *J Alzheimers Dis* 16: 763–774.

Reines, S.A., Block, G.A., Morris, J.C., Liu, G., Nessly, M.L., Lines, C.R. *et al.* (2004) Rofecoxib Protocol 091 Study Group. Rofecoxib: no effect on Alzheimer's disease in a 1-year, randomized, blinded, controlled study. *Neurology* 62: 66–71.

Ritchie, C.W., Bush, A.I., Mackinnon, A., Macfarlane, S., Mastwyk, M., MacGregor, L. *et al.* (2003) Metal-protein attenuation with iodochlorhydroxyquin (Clioquinol) targeting Aβ amyloid deposition and toxicity in Alzheimer disease. *Arch Neurol* 60: 1685–1691.

Robakis, N.K., Ramakrishna, N., Wolfe, G. and Wisniewski, H.M. (1987) Molecular cloning and characterization of a cDNA encoding the cerebrovascular and the neuritic plaque amyloid peptides. *Proc Natl Acad Sci USA* 84: 4190–4194.

Rogers, J., Kirby, L.C., Hempelman, S.R., Berry, D.L., McGeer, P.L., Kaszniak, A.W. *et al.* (1993) Clinical trial of indomethacin in Alzheimer's disease. *Neurology* 43: 1609–1611.

Santa-Maria, I., Hernández, F., Del Rio, J., Moreno, F.J. and Avila, J. (2007) Tramiprosate, a drug of potential interest for the treatment of Alzheimer's disease, promotes an abnormal aggregation of tau. *Mol Neurodegener* 2: 17.

Scharf, S., Mander, A., Ugoni, A., Vajda, F. and Christophidis, N. (1999) A double-blind, placebo controlled trial of diclofenac/misoprostol in Alzheimer's disease. *Neurology* 53: 197–201.

Schenk, D., Barbour, R. and Dunn, W. (1999) Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in PDAPP mouse. *Nature* 400: 173–177.

Schneider, A. and Mandelkow, E. (2008) Tau-based treatment strategies in neurodegenerative diseases. *Neurotherapeutics* 5: 443–457.

Shimmyo, Y., Kihara, T., Akaike, A., Niidome, T. and Sugimoto, H. (2008) Epigallocatechin-3-gallate and curcumin suppress amyloid beta-induced beta-site APP cleaving enzyme-1 upregulation. *Neuroreport* 19: 1329–1333.

Siemers, E.R., Quinn, J.F., Kaye, J., Farlow, M.R., Porsteinsson, A., Tariot, P. *et al.* (2006) Effects of a γ-secretase inhibitor in a randomized study of patients with Alzheimer disease. *Neurology* 66: 602–604.

Siemers, E., Skinner, M., Dean, R.A., Gonzales, C., Satterwhite, J., Farlow, M. *et al.* (2005) Safety, tolerability, and changes in amyloid β concentrations after administration of a γ-secretase inhibitor in volunteers. *Clin Neuropharmacol* 28: 126–132.

Skoog, I., Lernfelt, B., Landahl, S., Palmertz, B., Andreasson, L.A., Nilsson, L. *et al.* (1996) 15-year longitudinal study of blood pressure and dementia. *Lancet* 347: 1141–1145.

Soininen, H., West, C., Robbins, J. and Niculescu, L. (2007) Long-term efficacy and safety of celecoxib in Alzheimer's disease. *Dement Geriatr Cogn Disord* 23: 8–21.

Stefani, M. and Liguri, G. (2009) Cholesterol in Alzheimer's disease: unresolved questions. *Curr Alzheimer Res* 6: 15–29.

Storey, E., Spurck, T., Pickett-Heaps, J., Beyreuther, K. and Masters, C.L. (1996) The amyloid precursor protein of Alzheimer's disease is found on the surface of static but not activity motile portions of neurites. *Brain Res* 735: 59–66.

Tanzi, R.E., Gusella, J.F., Watkins, P.C., Bruns, G.A., St George-Hyslop, P., Van Keuren, M.L. *et al.* (1987) Amyloid beta protein gene: cDNA, mRNA distribution, and genetic linkage near the Alzheimer locus. *Science* 235: 880–884.

Townsend, M., Cleary, J.P., Mehta, T., Hofmeister, J., Lesne, S., O'Hare, E. *et al.* (2006) Orally available compound prevents deficits in memory caused by the Alzheimer Amyloid-β oligomers. *Ann Neurol* 60: 668–676.

Townsend, K.P. and Praticò, D. (2005) Novel therapeutic opportunities for Alzheimer's disease: focus on nonsteroidal anti-inflammatory drugs. *FASEB J* 19: 1592–1601.

Vasdev, S., Gill, V. and Singal, P. (2007) Role of advanced glycation end products in hypertension and atherosclerosis: therapeutic implications. *Cell Biochem Biophys* 49: 48–63.

Vassar, R. (2004) BACE1: the beta-secretase enzyme in Alzheimer's disease. *J Mol Neurosci* 23: 105–114.

Weggen, S., Eriksen, J.L., Das, P., Sagi, S.A., Wang, R., Pietrzik, C.U. *et al.* (2001) A subset of NSAIDs lower amyloidogenic Abeta42 independently of cyclooxygenase activity. *Nature* 414: 212–216.

Wilcock, G.K., Black, S.E., Hendrix, S.B., Zavitz, K.H., Swabb, E.A., Laughlin, M.A. *et al.* (2008) Efficacy and safety of tarenflurbil in mild to moderate Alzheimer's disease: a randomised phase II trial. *Lancet Neurol* 7: 468–469.

Wischik, C.M., Edwards, P.C., Lai, R.Y., Roth, M. and Harrington, C.R. (1996) Selective inhibition of Alzheimer disease-like tau aggregation by phenothiazines. *Proc Natl Acad Sci USA* 93: 11213–11218.

Wong, G.T., Manfra, D., Poulet, F.M., Zhang, Q., Josien, H., Bara, T. *et al.* (2004) Chronic treatment with the γ-secretase inhibitor LY-411,575 inhibit β-amyloid peptide production and alters lymphopoiesis and intestinal cell differentiation. *J Biol Chem* 279: 12876–12882.