

## Progress in Alzheimer's disease

Daniela Galimberti · Elio Scarpini

Received: 27 April 2011 / Revised: 8 June 2011 / Accepted: 9 June 2011 / Published online: 25 June 2011  
© Springer-Verlag 2011

**Abstract** After more than one century from Alois Alzheimer and Gaetano Perusini's first report, progress has been made in understanding the pathogenic steps of Alzheimer's disease (AD), as well as in its early diagnosis. This review discusses recent findings leading to the formulation of novel criteria for diagnosis of the disease even in a preclinical phase, by using biological markers. In addition, treatment options will be discussed, with emphasis on new disease-modifying compounds and future trial design suitable to test these drugs in an early phase of the disease.

**Keywords** Alzheimer's disease · Amyloid · Tau · Inflammation · Genetics · Biomarkers · Diagnosis · Disease-modifying drugs

### Alzheimer's disease: clinical aspects and pathogenesis

Alzheimer's disease (AD) is the most common cause of dementia in the elderly, with 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 as well as language impairment.

The two major neuropathological hallmarks of AD are extracellular amyloid beta ( $A\beta$ ) plaques and intracellular

neurofibrillary tangles (NFTs). The production of  $A\beta$ , which is considered a crucial step in AD pathogenesis, is the result of cleavage of a larger peptide, named amyloid precursor protein (APP), which is overexpressed in AD [1].  $A\beta$  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 represent the internal support structures for transport of nutrients, vesicles, mitochondria and chromosomes within the cell. Microtubules also stabilize growing axons, which are necessary for the development and growth of neurites [1]. In AD, tau protein is abnormally hyperphosphorylated and forms insoluble fibrils, originating deposits within the cell.

A number of additional pathogenic mechanisms, possibly overlapping with  $A\beta$  plaques and NFT formation, have been described, including inflammation [2] oxidative damage [3], iron deregulation [4], mitochondrial dysfunction [5] and a number of amyloid-independent hypotheses [6].

In about 95% of cases, the disease is sporadic (caused by the interaction between genetic and environmental factors). Autosomal dominant mutations in APP, presenilin 1 (PSEN1) and presenilin 2 (PSEN2) account for about 5% of cases, often characterized by early onset (before 65 years of age). To date, 32 different mutations, causing amino acid changes at putative sites for cleavage of the protein, have been described in the APP gene in 89 families, together with 182 mutations in PSEN1 and 13 in PSEN2.

### The amyloid hypothesis

The human APP gene was first identified in 1987 by several laboratories independently [7–9]. The two APP homologues, APLP1 and APLP2, were discovered several

D. Galimberti (✉) · E. Scarpini  
Department of Neurological Sciences, "Dino Ferrari" Center,  
Fondazione Cà Granda, IRCCS Ospedale Maggiore Policlinico,  
University of Milan, Via F. Sforza 35, 20122 Milan, Italy  
e-mail: daniela.galimberti@unimi.it

years later. APP is a type I membrane protein. Two predicted cleavages, one in the extracellular domain ( $\beta$ -secretase cleavage) and another in the transmembrane region ( $\gamma$ -secretase cleavage), are necessary to release  $A\beta$  from the precursor protein. APP is located on chromosome 21, and this provided an immediate connection to the invariant development of AD pathology in trisomy 21 (Down's syndrome) individuals. 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. Only APP, but not its homologues APLP1 and APLP2, contains sequences encoding the  $A\beta$  domain.

Full-length APP undergoes sequential proteolytic processing. It is first cleaved by  $\alpha$ -secretase (non-amyloidogenic pathway) or  $\beta$ -secretase (amyloidogenic pathway) within the luminal domain, resulting in the shedding of nearly the entire ectodomain and generation of  $\alpha$ - or  $\beta$ -C-terminal fragments (CTFs). The major neuronal  $\beta$ -secretase is named  $\beta$ -site APP cleaving enzyme (BACE1). It is a transmembrane aspartyl protease which cleaves APP within the ectodomain, generating the N-terminus of  $A\beta$  [10]. In addition, several zinc metalloproteinases, as well as the aspartyl protease BACE2, can cleave APP at the  $\alpha$ -secretase site [11], thus precluding generation of  $A\beta$ . The second proteolytic event in APP processing involves intramembranous cleavage of  $\alpha$ - and  $\beta$ -CTFs by  $\gamma$ -secretase, which liberates a 3-kDa protein (p3) and  $A\beta$  peptide into the extracellular milieu. The minimal components of  $\gamma$ -secretase include PS1 or PS2, nicastrin, APH-1 and PEN-2 [12]. Biochemical evidence is consistent with PS1 (or PS2) as the catalytic subunit of the  $\gamma$ -secretase. APH-1 and PEN-2 are thought to stabilize the  $\gamma$ -secretase complex, and nicastrin to mediate the recruitment of APP CTFs to the catalytic site of the  $\gamma$ -secretase. Major sites of  $\gamma$ -secretase cleavage correspond to positions 40 and 42 of  $A\beta$ .

In the last few years, the concept that amyloid monomers and oligomers are toxic rather than the deposition of amyloid fibrils has emerged. Abnormal accumulation of  $A\beta$  resulting in formation of toxic oligomers is the result of an imbalance between the levels of  $A\beta$  production and clearance.  $A\beta$  oligomers could lead to synaptic damage by forming pore-like structures with channel activity and alterations in glutamate receptors. In addition, they might cause circuitry hyperexcitability, mitochondrial dysfunction, lysosomal failure and alterations in signalling pathways involved in synaptic plasticity, neuronal cells and neurogenesis (see [13] for review).

**Tau: role in 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. Tau encoding gene (MAPT: microtubule associated protein tau) consists of 16 exons. In 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 hyperphosphorylated. The profile of alternative splicing differs among pathological phenotypes, such that tau accumulation in AD is a mixture of 3R and 4R tau, Pick disease tends to be 3R tau, corticobasal degeneration and progressive supranuclear palsy tends to be 4R tau, and so-called argyrophilic grain disease accumulates small inclusions comprising 3R tau [14]. In AD, it has been clearly established that tau pathology appears later than  $A\beta$  deposition (see [15] for review).

### Clinical diagnosis: present and future

#### Current criteria

Historically, AD has been considered as a “dual clinicopathological entity”, implying that full confirmation requires both presence of progressive dementia (episodic memory impairment and involvement of at least one additional cognitive domain, with impairment in daily living activities) and demonstration of the presence in the brain of SP and NFTs. Considering that the latter investigations cannot be done during life, AD has evolved primarily into a clinical entity with a probabilistic diagnosis (probable AD). Thus, according to National Institute of Neurological and Communicative Diseases and Stroke (NINCDS)/Alzheimer's Disease and Related Disorders Association (ADRDA) criteria [16], AD could be definite (at autopsy), probable or possible. In the last two decades, however, scientific knowledge regarding the pathogenic events and course of AD has incrementally grown. In particular, research has focussed on the search for biomarkers, which are objective measures showing in vivo biological evidence of AD pathology. The most reliable biomarkers validated in the last few years include: an abnormal cerebrospinal fluid  $A\beta$  and tau profile; the presence of hippocampal atrophy on magnetic resonance imaging (MRI), glucose hypometabolism on positron emission tomography (PET) scan, or presence of a known pathogenic mutation in genes encoding for APP, PSEN1, and PSEN2. In light of this striking evidence, in 2007 new research criteria were proposed [17], intended to move beyond the NINCDS/ADRDA criteria.

## Novel criteria

According to these new criteria, the diagnosis of AD is made when there is both clinical evidence of the disease phenotype and *in vivo* biological evidence of Alzheimer's pathology. The newly reported algorithm proposes that the diagnosis can be made in the presence of episodic memory impairment and a positive biomarker. It has a high level of accuracy even at the stage of earliest clinical manifestations (so-called mild cognitive impairment, MCI; according to Petersen et al. [18]). These new criteria imply that the presence of dementia itself is not necessarily required. Therefore, there is no longer a reason to wait until patients have developed full-blown dementia or to exclude from diagnosis and treatment a large number of patients who lack functional disability although expressing the disease. Within this framework, the designation of probable or possible AD is no longer meaningful. Most importantly, the diagnosis can be uncoupled from a particular threshold of severity, and there is no longer the need to anchor the diagnosis of AD to a clinical dementia syndrome. Nevertheless, biomarkers are only supportive features in the context of a core clinical phenotype.

The new criteria [17] have been reported to capture the prodromal phase of the disease [19–21]. According to evidence reported above, a new lexicon has been proposed [22], in which the term “AD” should refer only to *in vivo* clinico-biological expression of the disease, without the need to confirm the disease by pathologic analysis. The definition of “prodromal AD” refers to the early symptomatic pre-dementia phase, in which memory impairment is present but is not sufficiently severe to affect instrumental activities of daily living, together with a positive biomarker. The term “MCI” should apply to subjects with mild cognitive impairment but negative biomarkers. According to this proposal, and in contrast to the current meaning of MCI as “more at risk for AD”, MCI refers to individuals who will not likely develop AD. Lastly, subjects carrying a causal mutation are defined as having “preclinical AD” even in the absence of clinical symptoms.

In the last few years, the National Institute on Aging (NIA) has sponsored a series of advisory round-table meetings whose purpose was to establish a process for revising diagnostic and research criteria for AD. The recommendation of the advisory board was that three separate workgroups should be formed, with each assigned the task of formulating diagnostic criteria for each of the following phases of the disease: the dementia phase, the symptomatic pre-dementia phase (namely MCI) and the asymptomatic preclinical phase of the disease [23]. The process has been completed and guidelines given. For the dementia state, the core clinical criteria proposed in 1984 [16] are still

considered to be the cornerstone of diagnosis in clinical practice, but biomarker evidence is expected to enhance the pathophysiological specificity of diagnosis of AD dementia [24]. Regarding MCI as expression of AD, the workgroup developed two sets of criteria; the first are based essentially on clinical and neuropsychological analysis and can be used by healthcare providers without access to advanced imaging techniques or CSF analysis; the second are research criteria that include the use of biomarkers (imaging and CSF analysis), which could be used in clinical research settings, including clinical trials [25]. Lastly, concerning asymptomatic pre-dementia, authors give advice, solely intended for research, regarding biomarkers which best predict the risk of progression from “normal” cognition to MCI and dementia. They built a biomarker model for preclinical stage of AD starting from the model proposed by Jack et al. [15], which indicates  $A\beta$  accumulation biomarkers as the first changes before appearance of clinical symptoms. The lag phase between  $A\beta$  accumulation and clinical symptoms remains to be quantified, although current theories suggest that it may be for more than a decade. Later on during AD pathogenesis, biomarkers of synaptic dysfunctions (functional MRI) appear, followed by biomarkers of neuronal loss (structural MRI). Authors acknowledge that the problem in using biomarkers in the preclinical stage is that none of them are static [26].

## Treatment: present and future

### Current treatments

The first drugs developed for AD, anticholinesterase inhibitors (AChEI), were aimed to increase acetylcholine levels, previously demonstrated to be reduced in AD [27]. To date, four acetylcholinesterase inhibitors (AChEI) are approved for treatment of mild to moderate AD: tacrine (First Horizon Pharmaceuticals), donepezil (Pfizer), rivastigmine (Novartis) and galantamine (Janssen) [28]. Donepezil is now approved also for severe AD. Although tacrine was the first drug approved for AD, in 1993, it is rarely used due to hepatotoxicity.

In 2004, the AD2000 Collaborative Group carried out a study aimed at determining whether donepezil produced worthwhile improvements in disability, dependency, behavioural and psychological symptoms, caregivers' psychological wellbeing or delay in institutionalization, in an attempt to clarify the efficacy–cost ratio of such treatment. They concluded that donepezil is not cost effective, and benefits were below minimally relevant thresholds [29].

In 2006, 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 efficacious for mild to moderate AD, although it is not possible to identify patients who will respond to treatment prior 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 appears to be less adverse effects associated with donepezil compared with 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 worthy of consideration [30].

A further therapeutic option available for moderate to severe AD is memantine. This drug is a noncompetitive, moderate-affinity, *N*-methyl-D-aspartate (NMDA) antagonist believed to protect neurons from excitotoxicity. A recent meta-analysis on the efficacy of AChEI and memantine indicated that these treatments can result in statistically significant but clinically marginal improvement [31]. Regarding tolerability, AChEI 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 [32].

#### Disease-modifying drugs

On the basis of recent additional findings on AD pathogenesis, novel treatments under development aim to interfere with pathogenic steps previously mentioned, in an attempt to block the course of the disease in early phases. For this reason they are currently termed “disease-modifying” drugs [33].

#### Modulation of amyloid deposition

##### *Anti-amyloid aggregation agents*

One of the most studied compounds is named tramiprosate (Alzhemed<sup>TM</sup>, Neurochem, Inc.). It is a glycosaminoglycan (GAG) mimetic and binds to soluble A $\beta$ , promoting fibril formation and deposition of amyloid plaques. GAG mimetics compete for GAG-binding sites, thus blocking fibril formation and reducing soluble A $\beta$  [34]. After completing a phase I tolerability study, a 3-month phase II

study was conducted in 58 patients with mild to moderate AD, who were randomized to tramiprosate 50, 100 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 A $\beta$  levels declined by up to 70% after 3 months for patients randomly assigned to the 100 or 50 mg twice-daily group. Nevertheless, no differences were observed in cognitive functions between the tramiprosate and placebo groups [35]. A phase III study was then carried out in the USA in 1,052 patients with AD to test tolerability, efficacy and safety of the drug, but it failed to show significant effects. Another similar trial conducted in Europe has been discontinued (see details in [36]). In addition, recent data suggest that tramiprosate promotes abnormal aggregation of the tau protein in neuronal cells [37], emphasizing the importance of testing on both types of pathology (amyloid and tau) for potential drugs to be used for treatment of AD.

Another molecule under testing is named colostrinin. It is a proline-rich polypeptide complex derived from sheep colostrum (O-CLN; ReGen Therapeutics), which inhibits A $\beta$  aggregation and neurotoxicity in cellular assays and improves cognitive performance in animal models. A 3-week phase I study in patients with AD demonstrated that it is well tolerated [38]. A phase II trial demonstrated modest improvements in Mini-Mental State Examination (MMSE) scores for patients with mild AD over a treatment period of 15 months, but this beneficial effect was not sustained during 15 additional months of continued treatment [39].

Scyllo-inositol (AZD103) is a compound able to stabilize oligomeric aggregates of A $\beta$  and to inhibit A $\beta$  toxicity. It dose-dependently rescued long-term potentiation in mouse hippocampus from the inhibitory effects of soluble oligomers of cell-derived human A $\beta$  [40]. ELND005 (formerly known as AZD-103), scyllo-inositol, is being investigated as an orally administered treatment for AD. The phase II trial completed the treatment of patients receiving 250 mg twice daily dosing. Elan reported that the study's cognitive [Neuropsychological Test Battery (NTB)] and functional [Alzheimer's Disease Cooperative Study-Activities of Daily Living (ADCS-ADL)] co-primary endpoints did not achieve statistical significance. However, Elan reported that 250 mg bid dose “demonstrated a biological effect” on A $\beta$  in CSF, although no details have been provided.

##### *Vaccination*

In 1999 it was demonstrated that immunization with A $\beta$  as an antigen attenuated AD-like pathology in transgenic mice overexpressing the mutant human *APP* gene by removing amyloid from the central nervous system [41]. The tested

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 A $\beta$  prevented development of plaque formation, neuritic dystrophy and astroglyosis, whereas in older animals, vaccination reduced 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 A $\beta$ 42 plus adjuvant was started in 2001 on 300 patients using the pre-aggregated A $\beta$  peptide AN1792. However, following reports of aseptic meningo-encephalitis in 6% of treated patients, the trial was halted after 2–3 injections. Of the 300 patients treated, 60% developed antibody response. The final results of the trial were published in 2005 [42].

Double-blind assessment was maintained for 12 months, demonstrating no significant differences in cognition between antibody responders and 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, CSF tau levels were decreased in antibody responders, but A $\beta$  levels were unchanged.

In 2008, a paper was published describing the relation between A $\beta$ 42 immune response, degree of plaque removal and long-term clinical outcomes [43]. 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 A $\beta$  load was lower than in the placebo group. Despite this observation, however, no evidence of improved survival or improvement of severe dementia with time was observed in such patients. Therefore, it is likely that 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, but the treatment was modified from active to passive to avoid excessive activation of the T-cell response and thus complications. The humanized monoclonal anti-A $\beta$  antibody bapineuzumab (AAB-001, Wyeth and Elan) has been tested in a phase II trial in 200 patients with mild to moderate AD. The 18-month, multi-dose, one-to-one randomization trial was conducted at about 30 sites in the USA. It was designed to assess safety, tolerability and standard efficacy endpoints (ADAS-Cog, DAS) of multiple ascending doses of bapineuzumab in patients. The 18-month trial includes an interim analysis, as well as data collection on clinical endpoints and

biomarkers [44]. On May 21, 2007, Elan and Wyeth announced their plans to start a phase III clinical trial of bapineuzumab. The decision to launch phase III studies prior to 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 analysis carried out, 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, i.e. ApoE non-carriers who received the second lowest of the four doses six times, responded truly well by 78 weeks.

In March 2010, imaging analysis of AD patients demonstrated that bapineuzumab reduces cortical PiB retention, a measurement of fibrillar plaque [45].

Additional antibodies under testing include ACC-001 (Wyeth; two phase II studies ongoing in the USA and Japan), LY2062430 (Solanezumab, Ely 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 pooled plasma of healthy blood donors. In light of these observations, a phase I trial has been carried out in the USA. 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 (<http://www.alzforum.org>). Additional phase I trials are ongoing [46].

Passive vaccination implies repeated infusions, and costs are high. Therefore, active vaccination has been considered again, by developing specific antigens designed to generate high A $\beta$  antibody titers without inducing A $\beta$ -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 sub-cutaneous injections of CAD106 over 12 months, demonstrating that it is well tolerated and that there is a specific A $\beta$ -IgG response in 16/24 patients in cohort I and in 18/22 patients in cohort II. A phase II trial is ongoing.

#### *Selective A $\beta$ 42-lowering agents (SALAs)*

Tarenfluril is the first compound in this new class of drugs, which modulate  $\gamma$ -secretase activity without interfering

with Notch or other  $\gamma$ -secretase substrates [47]. It binds to a  $\gamma$ -secretase site different from the active/catalytic centre of relevance to production of A $\beta$ 42, thereby altering the conformation of  $\gamma$ -secretase and shifting production away from A $\beta$ 42 without interfering with other physiologically essential  $\gamma$ -secretase substrates.

Tarenflurbil (MPC-7869; Myriad Pharmaceuticals; Flurizan<sup>TM</sup>) is the pure *R*-enantiomer of flurbiprofen. It shifts cleavage of APP away from A $\beta$ 42; leading to production of shorter, non-toxic fragments [48]. In contrast with *S*-flurbiprofen or other non-steroidal anti-inflammatory drugs (NSAIDs), it does not inhibit cyclo-oxygenase (COX) I or COX 2, and it is not associated with gastrointestinal toxicity [39]. In mice, treatment with tarenflurbil reduces amyloid plaque burden and prevents learning and behavioural deterioration [49].

A 3-week, placebo-controlled, phase I pharmacokinetic study of tarenflurbil (twice-daily doses of 400, 800 or 1,600 mg) in 48 healthy, older volunteers showed that the drug is well tolerated. CSF was collected at baseline and after 3 weeks. The compound penetrated the blood–brain barrier in a dose-dependent manner. No significant changes of A $\beta$ 42 CSF levels were shown after treatment. However, in plasma, higher drug concentrations were related to statistically significantly lower A $\beta$  levels [50].

Myriad conducted a large, placebo-controlled phase II trial for Flurizan of 12-month duration in 210 patients with mild to moderate AD (MMSE score 15–26). Patients were randomly assigned to receive tarenflurbil twice per day (400 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 (ADCS-ADL) inventory; global function, measured by the CDR sum of boxes (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 or 400 mg twice a day.

A preliminary analysis revealed that patients with mild AD (MMSE 20–26) and moderate AD (MMSE 15–19) responded differently to tarenflurbil on the ADAS-Cog and the ADCS-ADL, therefore these groups were analyzed 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 per day 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 [51]. Given these results, two phase III studies were carried out, in the USA and in Europe. The ActEarliAD trial was started in 2007 all over Europe. It is an 18-month, multinational, randomized, double-blind, placebo-controlled study in over 800 patients with AD. The two primary clinical endpoints were changes in cognitive decline and function, as measured by the 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 the CDR-sb. Additional exploratory outcome measures were designed to assess the psychological, physical and financial impact of this disease on caregivers and medical resources. The global endpoints in this trial were identical to those in the US trial (see [35] for review).

Disappointingly, on July 2, 2008, the sponsor of Flurizan announced that this  $\gamma$ -secretase-modulating agent had failed its definitive phase III trial and was no longer a development product (<http://www.alzforum.org>). In fact, for both primary efficacy endpoints, i.e. the ADAS-Cog and the ADCS Activities of Daily Living scales, the treatment and placebo curves overlapped almost completely, and there was no effect whatsoever in the group as a whole. In addition, while the overall side-effect profile was similar between placebo and treatment groups, anemia, infections and gastrointestinal ulcers appeared more often in people on Flurizan than in the placebo group.

#### *$\gamma$ -Secretase inhibition*

Several compounds which inhibit  $\gamma$ -secretase activity in the brain have been identified. Unfortunately,  $\gamma$ -secretase has many biologically essential substrates [52], including the Notch signalling protein, which is involved in differentiation and proliferation of embryonic cells, T-cells and splenic B-cells. Experience with transgenic mice showed that administration of a  $\gamma$ -secretase inhibitor in doses sufficient to remove A $\beta$  concentrations interferes with lymphocyte differentiation [53]. Therefore, safety is a very important consideration for this kind of compounds.

A nonselective  $\gamma$ -secretase inhibitor named 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). A $\beta$  CSF levels were reduced in both 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 [54]. 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 A $\beta$ 40 and A $\beta$ 42 were observed [55]. In light of these results, a phase III trial was started but later discontinued due to lack of efficacy.

A potent  $\gamma$ -secretase inhibitor named BMS-708163 (Bristol-Myers Squibb) was tested in a phase I clinical trial, showing it decreased CSF A $\beta$ 40 and A $\beta$ 42 by 30% with daily dose of 100 mg after 18 days, and by 60% at daily dose of 150 mg. A phase II study has recently been completed.

#### *$\alpha$ -Secretase potentiation*

Etazolate (EHT 0202, Exonhit Therapeutics) stimulates the neurotrophic  $\alpha$ -secretase (non-amyloidogenic) pathway and inhibits A $\beta$ -induced neuronal death, providing symptomatic relief and modifying disease progression. In vitro, it is neuroprotective against A $\beta$ 42, and neuroprotection is associated with secretory amyloid precursor protein  $\alpha$  (sAPP $\alpha$ ) induction [56]. After a phase I study in healthy volunteers, a phase II clinical trial to assess safety, tolerability and preliminary efficacy on cognition and behaviour in AD patients, as well as quantification of sAPP $\alpha$  in blood, is ongoing (<http://www.alzforum.org>).

#### Modulation of tau deposition

A phase II trial of a tau-blocking compound named methyl thioninium chloride (MTC) is ongoing (TauRx Therapeutics, Rember<sup>TM</sup>). This is a reducing agent better known as methylene blue, a deep-blue dye used in analytical chemistry and as a tissue stain in biology. MTC interferes with tau aggregation by acting on self-aggregating truncated tau fragments [57]. A phase II trial was carried out, randomizing 321 patients with mild or moderate AD to treatment with either placebo or one of three oral doses of MTC (30, 60 or 100 mg) three times a day. Patients were not taking AChEI or memantine. Primary outcomes included the effect of MTC versus 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. A significant improvement relative to placebo of  $-5.4$  ADAS-Cog units in CDR-moderate subjects at the 60 mg dose was shown. 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 hyperphosphorylation. Despite the large number of tau phosphorylation sites and the ability of multiple kinases to phosphorylate individual sites, glycogen synthase kinase 3 $\beta$  (GSK3 $\beta$ ) has emerged as a potential therapeutic target [58]. 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 valproate [59, 60].

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.

#### **Additional therapeutic approaches**

Several additional therapeutic approaches have been proposed in the last few years (see [36] for review). Here, we will mention only the most studied compounds.

##### Anti-inflammatory drugs

Epidemiologic evidence suggests that long-term use of NSAIDs protects against development of AD [61]. Despite this premise, in prospective studies, rofecoxib [62], naproxen [63], diclofenac [64], celecoxib [65], dapsone [66], hydroxychloroquine [67] and nimesulide [68] failed to slow progression of cognitive decline in patients with mild to moderate AD. In contrast, indomethacin may delay cognitive decline in this subset of patients, but gastrointestinal toxicity is treatment limiting [69]. Given the considerations reported above, NSAIDs are no longer considered to be viable treatment options for patients with AD.

##### Molecules addressing oxidative damage

Whether reduction of homocysteine levels with high-dose folate, vitamin B6 and vitamin B12 supplementation can slow the rate of cognitive decline in subjects with AD has been investigated in a multicentre, randomized, controlled clinical trial named VITAL (High Dose Supplements to Reduce Homocysteine and Slow the Rate of Cognitive Decline in Alzheimer's Disease). Four hundred nine individuals with mild to moderate AD (MMSE 14–26) and normal folic acid, vitamin B12 and homocysteine (Hcy) levels were included. Participants were randomly assigned to two groups of unequal size (60% treated with high-dose supplements—5 mg/day folate, 25 mg/day vitamin B6, and 1 mg/day vitamin B12—and 40% treated with identical placebo) for 18 months. The main outcome measure was the change in the cognitive subscale of the ADAS-Cog.

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 of rate of change in ADAS-Cog score during 18 months, or any secondary measures [70].

Additional potential antioxidants include mitoquinone (Antipodian 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 [71].

#### Drugs interfering with metals

In 1994 it was observed that  $A\beta$  becomes amyloidogenic upon reaction with stoichiometric amounts of  $Zn^{2+}$  and  $Cu^{2+}$  [72].  $A\beta$  is rapidly precipitated by  $Zn^{2+}$ .  $Cu^{2+}$  and  $Fe^{3+}$  also induce marked  $A\beta$  aggregation, but only under mildly acidic conditions [73], such as those believed to occur in AD brain. The precipitation of  $A\beta$  by these ions is reversible with chelation, in contrast to fibrillization, which is irreversible. Cu, Fe and Zn play more of a role than merely assembling  $A\beta$ . When binding  $Cu^{2+}$  or  $Fe^{3+}$ ,  $A\beta$  reduces the metal ions and produces  $H_2O_2$  by double electron transfer to  $O_2$ . In addition,  $A\beta$  promotes Cu-mediated generation of toxic lipid oxidation product 4-hydroxynoneal [73].

The compound named PBT2 was designed to modify the course of AD by preventing metal-dependent aggregation, deposition and toxicity of  $A\beta$ . PBT2 acts at three levels of the “amyloid cascade”: inhibiting the redox-dependent formation of toxic soluble oligomers, preventing deposition of  $A\beta$  as amyloid plaques and promoting clearance by mobilizing and neutralizing  $A\beta$  from existing deposits [74]. PBT2 has recently been 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  $A\beta_{42}$  concentration compared with those treated with placebo [75]. 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, showing a reduction in the rate of cognitive decline in the subgroup of more severely affected patients only [76]. According to Cochrane collaborative study, it was not clear from this trial that 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 [77].

#### Final remarks: impact of biomarkers on disease-modifying trial design

From data presented in this review, a few considerations emerge, which should be taken into account for planning future clinical trials.

First is the concept that the term “AD” should encompass the underlying pathology and not only refer to clinical stages of the disease. To disambiguate this term, we should speak of “AD-P” when there is evidence of the underlying brain disease pathophysiological process, and to “AD-C” when clinical symptoms emerge (including not only AD dementia but also MCI). According to evidence presented in this review, AD-P is thought to start before AD-C. Despite this concept being theoretically extremely useful, in practice there are still a number of open questions, including:

1. In the presence of AD-P, how can the manifestation of AD-C be predicted?
2. How can biomarkers be used to this aim? It is possible that  $A\beta$  accumulation itself would be necessary but not sufficient to produce clinical manifestations of the disease.
3. Are disease-modifying drugs useful at the stage of mild dementia or MCI, or are they efficacious in the earliest phase of AD-P (before onset of symptoms)?
4. In the latter case, considering that biologically active treatments may be associated with risks of adverse side-effects, we should predict the emergence of AD-C with sufficient certainty to weigh the risk–benefit ratios to begin a treatment in asymptomatic individuals.

Although resolution of these open questions requires future efforts, it is clear that future clinical trials should be carried out in early phases of the disease. A recent report of a taskforce, aimed at giving consensus criteria for the design of clinical trials for AD, suggests that the optimal stage for efficacy trial of disease-modifying drugs should be prior to dementia onset [78]. Selection of AD cases should be extended to prodromal AD (MCI plus a positive biomarker), and outcomes should be modified in order to demonstrate both the clinical improvement and the modification effect on the pathogenic steps underlying the disease. In this regard, indicators useful as surrogate outcome measures (surrogate biomarkers) should be identified in order to: (1) provide substitutes for clinical endpoints (i.e. neuropsychological testing), (2) provide tools able to predict clinical benefit, or the opposite, and (3) demonstrate



whether there are disease-modifying properties. So far, none of the proposed biomarkers for early diagnosis have been validated as a surrogate marker for monitoring treatments.

**Conflict of interest** None.

## References

- Griffin WS (2006) Inflammation and neurodegenerative diseases. *Am J Clin Nutr* 3(suppl):470S–474S
- Galimberti D, Fenoglio C, Scarpini E (2008) Inflammation in neurodegenerative disorders: friend or foe? *Curr Aging Sci* 1(1):30–41
- Reddy VP, Zhu X, Perry G, Smith MA (2009) Oxidative stress in diabetes and Alzheimer's disease. *J Alzheimers Dis* 16(4):763–774
- Adlard PA, Bush AI (2006) Metals and Alzheimer's disease. *J Alzheimers Dis* 10(2–3):145–163
- Santos RX, Correia SC, Wang X, Perry G, Smith MA, Moreira PI, Zhu X (2010) Alzheimer's disease: diverse aspects of mitochondrial malfunctioning. *Int J Clin Exp Pathol* 3(6):570–581
- Pimplikar SW, Nixon RA, Robakis NK, Shen J, Tsai LH (2010) Amyloid-independent mechanisms in Alzheimer's disease pathogenesis. *J Neurosci* 30(45):14946–14954
- Goldgaber D, Lerman MI, McBride OW, Saffiotti U, Gajdusek DC (1987) Characterization and chromosomal localization of a cDNA encoding brain amyloid of Alzheimer's disease. *Science* 235(4791):877–880
- Robakis NK, Ramakrishna N, Wolfe G, Wisniewski HM (1987) Molecular cloning and characterization of a cDNA encoding the cerebrovascular and the neuritic plaque amyloid peptides. *Proc Natl Acad Sci USA* 84(12):4190–4194
- Tanzi RE, Gusella JF, Watkins PC, Bruns GA, St George-Hyslop P, Van Keuren ML et al (1987) Amyloid beta protein gene: cDNA, mRNA distribution, and genetic linkage near the Alzheimer locus. *Science* 235(4791):880–884
- Vassar R (2004) BACE1: the beta-secretase enzyme in Alzheimer's disease. *J Mol Neurosci* 23(1–2):105–114
- Allinson TM, Parkin ET, Turner AJ, Hooper NM (2003) ADAMs family members as amyloid precursor protein alpha-secretases. *J Neurosci Res* 74(3):342–352
- Edbauer D, Winkler E, Regula JT, Pesold B, Steiner H, Haass C (2003) Reconstitution of gamma-secretase activity. *Nat Cell Biol* 5(5):486–488
- Crews L, Masliah E (2010) Molecular mechanisms of neurodegeneration in Alzheimer's disease. *Hum Mol Genet* 19(1):R12–R20
- Castellani RJ, Nunomura A, Lee H, Perry G, Smith MA (2008) Phosphorylated tau: toxic, protective, or none of the above. *J Alzheimers Dis* 14:377–383
- Jack CRJ Jr, Knopman DS, Jagust WJ, Shaw LM, Aisen PS, Weiner MW et al (2010) Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. *Lancet Neurol* 9:119–128
- McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM (1984) Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology* 34:939–944
- Dubois B, Feldman HH, Jacova C, Dekosky ST, 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
- Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos EG, Kokmen E (1999) Mild cognitive impairment: clinical characterization and outcome. *Arch Neurol* 56(3):303–308
- Bouwman FH, Verwey NA, Klein M, Kok A, Blankenstein MA, Sluimer JD et al (2010) New research criteria for the diagnosis of Alzheimer's disease applied in a memory clinic population. *Dement Geriatr Cogn Disord* 30(1):1–7
- Jager CA, Honey TE, Birks J, Wilcock GK (2010) Retrospective evaluation of revised criteria for the diagnosis of Alzheimer's disease using a cohort with post-mortem diagnosis. *Int J Geriatr Psychiatry* 25(10):988–997
- Frisoni GB, Galluzzi S, Signorini M, Garibotto V, Paghera B, Binetti G et al (2010) Preliminary evidence of validity of the revised criteria for Alzheimer disease diagnosis: report of 2 cases. *Alzheimer Dis Assoc Disord* 24(1):108–114
- Dubois B, Feldman HH, Jacova C, Cummings JL, Dekosky ST, Barberger-Gateau P et al (2010) Revising the definition of Alzheimer's disease: a new lexicon. *Lancet Neurol* 9(11):1118–1127
- Jack RJ Jr, Albert MS, Knopman DS, McKhann GM, Sperling RA, Carrillo MC et al (2011) Introduction to the recommendations from the National Institute on Aging and the Alzheimer's Association workgroup on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* 7(3):257–262
- McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR Jr, Kawas CH et al (2011) The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging and the Alzheimer's Association workgroup. *Alzheimers Dement* 7(3):263–269
- Albert MS, DeKosky ST, Dickson D, Dubois B, Feldman HH, Fox NC et al (2011) The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging and Alzheimer's Association workgroup. *Alzheimers Dement* 7(3):270–279
- Sperling RA, Aisen PS, Beckett LA, Bennett DA, Craft S, Fagan AM et al (2011) Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging and the Alzheimer's Association workgroup. *Alzheimers Dement* 7(3):280–292
- Lawrence AD, Sahakian BJ (1998) The cognitive psychopharmacology of Alzheimer's disease: focus on cholinergic systems. *Neurochem Res* 23(5):787–794
- Farlow M (2002) A clinical overview of cholinesterase inhibitors in Alzheimer's disease. *Int Psychogeriatr/IPA* 14(Suppl 1):93–126
- Courtney C, Farrell D, Gray R, Hills R, Lynch L, Sellwood E et al (2004) Long-term donepezil treatment in 565 patients with Alzheimer's disease (AD2000): randomised double-blind trial. *Lancet* 363(9427):2105–2115
- Birks J (2006) Cholinesterase inhibitors for Alzheimer's disease. *Cochrane Database Syst Rev* 1:CD005593
- 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(5):379–397
- Alva G, Cummings JL (2008) Relative tolerability of Alzheimer's disease treatments. *Psychiatry (Edgmont)* 5(11):27–36
- Scarpini E, Scheltens P, Feldman H (2003) Treatment of Alzheimer's disease: current status and new perspectives. *Lancet Neurol* 2(9):539–547
- 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

35. Aisen PS, 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(10):1757–1763
36. Galimberti D, Scarpini E (2011) Alzheimer's disease: from pathogenesis to disease-modifying approaches. *CNS Neurol Disord Drug Targets* 10(2):163–174
37. Santa-Maria I, Hernández F, Del Rio J, Moreno FJ, 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(1):17
38. Leszek J, Inglot AD, Janusz M, Lisowski J, Krukowska K, Georgiades JA (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(6):377–385
39. Bilikiewicz A, Gaus W (2004) Colostrinin (a naturally occurring, proline-rich, polypeptide mixture) in the treatment of Alzheimer's disease. *J Alzheimer's Dis* 6:17–26
40. Townsend KP, Praticò D (2005) Novel therapeutic opportunities for Alzheimer's disease: focus on nonsteroidal anti-inflammatory drugs. *FASEB J* 19(12):1592–1601
41. Schenk D, Barbour R, Dunn W (1999) Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in PDAPP mouse. *Nature* 400:173–177
42. Gilman S, Koller M, Black RS (2005) Clinical effects of A $\beta$  immunization (AN1792) in patients with AD in an interrupted trial. *Neurology* 64:1553–1562
43. Holmes C, Boche D, Wilkinson D, Yadegarfar G, Hopkins V, Bayer A et al (2008) Long-term effect of A $\beta$ <sub>42</sub> immunisation in Alzheimer's disease: follow-up of a randomised, placebo-controlled phase I trial. *Lancet* 372:216–223
44. Grundman M, 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
45. Rinne JO, Brooks DJ, Rossor MN, Fox NC, Bullock R, Klunk WE et al (2010) 11C-PiB PET assessment of change in fibrillar amyloid-beta load in patients with Alzheimer's disease treated with bapineuzumab: a phase 2, double-blind, placebo-controlled, ascending-dose study. *Lancet Neurol* 9(4):363–372
46. 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(5):513–528
47. Weggen S, Eriksen JL, Das P, Sagi SA, Wang R, Pietrzik CU et al (2001) A subset of NSAIDs lower amyloidogenic Abeta42 independently of cyclooxygenase activity. *Nature* 414(6860):212–216
48. Behr D, Clarke EE, Wrigley JD, Martin AC, Nadin A, Churcher I et al (2004) Selected nonsteroidal anti-inflammatory drugs and their derivatives target  $\gamma$ -secretase at a novel site: evidence for an allosteric mechanism. *J Biol Chem* 279:43419–43426
49. Kukar T, Prescott S, Eriksen JL, Holloway V, Murphy MP, Koo EH et al (2007) Chronic administration of R-flurbiprofen attenuates learning impairments in transgenic amyloid precursor protein mice. *BMC Neurosci* 8:54
50. Galasko DR, Graff-Radford N, May S, Hendrix S, Cottrell BA, Sagi SA et al (2007) Safety, tolerability, pharmacokinetics, and A $\beta$  levels after short-term administration of R-flurbiprofen in healthy elderly individuals. *Alzheimer Dis Assoc Disord* 21(4):292–299
51. Wilcock GK, Black SE, Hendrix SB, Zavitz KH, Swabb EA, Laughlin MA et al (2008) Efficacy and safety of tarenfluril in mild to moderate Alzheimer's disease: a randomised phase II trial. *Lancet Neurol* 7(6):483–493
52. Pollack SJ, Lewis H (2005) Secretase inhibitors for Alzheimer's disease: challenges of promiscuous protease. *Curr Opin Investig Drugs* 6:35–47
53. Wong GT, Manfra D, Poulet FM, Zhang Q, Josien H, Bara T et al (2004) Chronic treatment with the  $\gamma$ -secretase inhibitor LY-411,575 inhibit  $\beta$ -amyloid peptide production and alters lymphopoiesis and intestinal cell differentiation. *J Biol Chem* 279:12876–12882
54. Siemers E, Skinner M, Dean RA, Gonzales C, Satterwhite J, Farlow M et al (2005) Safety, tolerability, and changes in amyloid  $\beta$  concentrations after administration of a  $\gamma$ -secretase inhibitor in volunteers. *Clin Neuropharmacol* 28:126–132
55. Siemers ER, Quinn JF, Kaye J, Farlow MR, Porsteinsson A, Tariot P et al (2006) Effects of a  $\gamma$ -secretase inhibitor in a randomized study of patients with Alzheimer disease. *Neurology* 66:602–604
56. 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(1):392–404
57. Wischik CM, Edwards PC, Lai RY, Roth M, Harrington CR (1996) Selective inhibition of Alzheimer disease-like tau aggregation by phenothiazines. *Proc Natl Acad Sci USA* 93(20):11213–11218
58. Balaraman Y, Limaye AR, Levey AI, Srinivasan S (2006) Glycogen synthase kinase 3 $\beta$  and Alzheimer's disease: pathophysiological and therapeutic significance. *Cell Mol Life Sci* 63:1226–1235
59. Martinez A, Perez DI (2008) GSK-3 inhibitors: a ray of hope for the treatment of Alzheimer's disease? *J Alzheimers Dis* 15:181–191
60. Schneider A, Mandelkow E (2008) Tau-based treatment strategies in neurodegenerative diseases. *Neurotherapeutics* 5:443–457
61. McGeer PL, Schulzer M, Mc Geer EG (1996) Arthritis and anti-inflammatory agents as possible protective factors for Alzheimer's disease: a review of 17 epidemiologic studies. *Neurology* 47:425–432
62. Reines SA, Block GA, Morris JC, Liu G, Nessly ML, Lines CR 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
63. Aisen PS, Schafer KA, Grundman M, Pfeiffer E, Sano M, Davis KL et al (2003) Effects of rofecoxib or naproxen vs placebo on Alzheimer's disease progression: a randomized controlled trial. *JAMA* 289:2819–2826
64. Scharf S, Mander A, Ugoni A, Vajda F, Christophidis N (1999) A double-blind, placebo controlled trial of diclofenac/misoprostol in Alzheimer's disease. *Neurology* 53:197–201
65. Soininen H, West C, Robbins J, Niculescu L (2007) Long-term efficacy and safety of celecoxib in Alzheimer's disease. *Dement Geriatr Cogn Disord* 23(1):8–21
66. Eriksen JL, Sagi SA, Smith TE, Weggen S, Das P, McLendon DC et al (2003) NSAIDs and enantiomers of flurbiprofen target gamma-secretase and low Abeta 42 in vivo. *J Clin Invest* 112(3):440–449
67. Aisen PS, Marin DB, Brickman AM, Santoro J, Fusco M (2001) Pilot tolerability studies of hydroxychloroquine and colchicine in Alzheimer disease. *Alz Dis Assoc Disord* 15(2):96–101
68. Aisen PS, Schmeidler J, Pasinetti GM (2002) Randomized pilot study of nimesulide treatment in Alzheimer's disease. *Neurology* 58(7):1050–1054
69. Rogers J, Kirby LC, Hempelman SR, Berry DL, McGeer PL, Kaszniak AW et al (1993) Clinical trial of indomethacin in Alzheimer's disease. *Neurology* 43:1609–1611
70. Aisen PS, Schneider LS, Sano M, Diaz-Arrastia R, van Dyck CH, Weiner MF et al (2008) High-dose B vitamin supplementation

- and cognitive decline in Alzheimer disease: a randomized controlled trial. *JAMA* 300(15):1774–1783
71. Barten DM, Albright CF (2008) Therapeutic strategies for Alzheimer's disease. *Mol Neurobiol* 37:171–186
  72. Bush AI, Pettingell WH, Multhaup G, Paradis M, Vonsattel JP, Gusella JF et al (1994) Rapid induction of Alzheimer A $\beta$  amyloid formation by zinc. *Science* 265:1464–1467
  73. Bush AI (2008) Drug development based on the metals hypothesis of Alzheimer's disease. *J Alzheimers Dis* 15:223–240
  74. Cherny RA, Atwood CS, Xilinas ME, Gray DN, Jones WD, McLean CA et al (2001) Treatment with a copper-zinc chelator markedly and rapidly inhibits  $\beta$ -amyloid accumulation in Alzheimer's disease transgenic mice. *Neuron* 30:665–676
  75. 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(9):779–786
  76. Ritchie CW, Bush AI, Mackinnon, Macfarlane S, Mastwyk M, MacGregor L et al (2003) Metal-protein attenuation with iodochlorhydroxyquin (Clioquinol) targeting A $\beta$  amyloid deposition and toxicity in Alzheimer disease. *Arch Neurol* 60:1685–1691
  77. Jenagaratnam L, McShane R (2006) Clioquinol for the treatment of Alzheimer's disease. *Cochrane Database Syst Rev* 25(1): CD005380
  78. Aisen PS, Andrieu S, Sampaio C, Carrillo M, Khachaturian ZS, Dubois B et al (2011) Report of the task force on designing clinical trials in early (predementia) AD. *Neurology* 76(3):280–286