



Alzheimer's disease: from pathogenesis to novel therapeutic approaches

The two major neuropathologic hallmarks of Alzheimer's disease are extracellular amyloid β ($A\beta$) plaques and intracellular neurofibrillary tangles. Several additional pathogenic mechanisms have been described, including inflammation, oxidative damage, ion dysregulation and cholesterol metabolism. A number of compounds have been developed, trying to interfere with the above-mentioned mechanisms. These drugs are supposed to modify pathological steps leading to Alzheimer's disease, thus acting on the evolution of the disease. Some of these compounds are under clinical testing, others are in preclinical phases of development. In this review, the main pathogenic steps leading to neurodegeneration will be discussed, together with an update on potentially disease-modifying drugs under testing.

KEYWORDS: Alzheimer's disease ■ amyloid ■ anti-inflammatories ■ disease-modifying drugs ■ inflammation ■ tau

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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 approximately 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 psycho-behavioral disturbances and language impairment. Mutations in genes encoding for amyloid precursors protein or presenilin 1 and presenilin 2 genes (*APP*, *PSEN1* and *PSEN2*, respectively) account for approximately 5% of cases, characterized by an early onset (before 65 years of age). So far, 30 different mutations causing amino acid changes in putative sites for the cleavage of the protein have been described in the *APP* gene in 83 families, together with 172 mutations in *PSEN1* and 14 mutations in *PSEN2* [201].

The two major neuropathologic hallmarks of AD are extracellular amyloid- β ($A\beta$) plaques and intracellular neurofibrillary tangles (NFTs). The production of $A\beta$, which represents a crucial step in AD pathogenesis, is the result of cleavage of APP, which is overexpressed in AD [1]. $A\beta$ forms highly insoluble and proteolysis-resistant fibrils known as senile plaques. In contrast to the low-fibrillar $A\beta$ plaques (diffuse plaques), highly fibrillar forms of $A\beta$ plaques (dense-core plaques, showing all the classical properties of amyloid including β -sheet secondary structure) [2] are associated with glial and neuritic changes of the surrounding tissue (neuritic-plaques) [3]. NFTs

are composed of the tau protein. In healthy subjects, tau is a component of microtubules, which represent the internal support structures for the 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, oxidative damage, iron dysregulation and cholesterol metabolism. In this review, all these mechanisms will be discussed, and treatments under development to interfere with these pathogenic steps presented. Notably, the aim at the basis of the identification of novel compounds is to block the course of the disease in early phases (even preclinical). For this reason they are currently termed 'disease-modifying' drugs. However, this definition is problematic, because whether a medication is disease-modifying is to be proven by the clinical data (i.e., changes of slopes of decline over time) and does not relate to the hypothesized mechanism of action (many pathobiological mechanisms can be claimed to result in disease modification). Therefore, in this review the term 'potentially disease-modifying' will be used. All these compounds are under evaluation, and thus not registered for their use in AD therapy yet.

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Useful tools to both select more homogeneous cohorts of subjects to be included in clinical trials and evidence a disease-modifying effect are biological markers, including cerebrospinal fluid (CSF) A β , tau and phosphorylated (P) tau levels, neuroimaging and positron emission tomography, possibly with the use of amyloid radiotracer Pittsburgh compound-B (see [4] for review). Biomarkers could be used as surrogate outcome measures that will substitute for clinical end points, predicting clinical benefit, or the opposite [5]. Biomarkers are in the process of implementation as primary outcome variables into regulatory guideline documents regarding study design and approval for compounds claiming disease modification [4].

Pathogenesis of Alzheimer's disease

■ The amyloid hypothesis

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

APP gene family

The human *APP* gene was first identified in 1987 by several laboratories independently. 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 A β 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 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 [6], providing an evidence that APP plays a central role in AD pathogenesis. Importantly, only *APP*, but not its homologues *APLP1* and *APLP2*, contain sequences encoding the A β domain.

APP processing

Full-length APP undergoes sequential proteolytic processing. It is first cleaved by α -secretase (nonamyloidogenic pathway) or β -secretase (amyloidogenic pathway) within the luminal domain, resulting in the shedding of nearly the entire ectodomain and generation of α - or β -C-terminal fragments (CTFs). The major neuronal β -secretase, named β -site APP cleaving enzyme (BACE1), is a transmembrane aspartyl

protease that cleaves APP within the ectodomain, generating the N-terminus of A β [7]. Nevertheless, several zinc metalloproteinases such as TACE/ADAM17, ADAM9, ADAM10 and MDC-9, and the aspartyl protease BACE2, can cleave APP at the α -secretase site [8] located within the A β domain, thus precluding the generation of intact A β .

The second proteolytic event in APP processing involves intramembranous cleavage of α - and β -CTFs by γ -secretase, which liberates a 3-kDa protein (p3) and A β peptide into the extracellular milieu. The minimal components of γ -secretase include presenilin (PS)1 or PS2, nicastrin, APH-1 and PEN-2 [9]. Protein subunits of the γ -secretase assemble early during biogenesis and cooperatively mature as they leave the endoplasmic reticulum. 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 A β .

Amyloidogenic processing is the favored pathway of APP metabolism in neurons, due to the greater abundance of BACE1, whereas non-amyloidogenic pathway predominates in other cell types.

It appears that none of the above-mentioned secretases have 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 its ligands [10].

APP role

A number of functional domains have been mapped to the extra- and intra-cellular region of APP, including metal (copper and zinc) binding, extracellular matrix components (heparin, collagen and laminin), 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. Similarly, APP colocalizes with integrins on the surface of axons at sites of adhesion [11,12].

Despite APP being initially proposed to act as a cell-surface receptor, the evidence supporting this hypothesis has been unconvincing. Only recently, aside from interactions with extracellular matrix proteins, a candidate ligand has been proposed. It was in fact reported that F-spondin, a neuronal secreted signaling glycoprotein that may function in neuronal development and repair, binds to the extracellular domain of APP as well as of APLP1 and APLP2 [13]. This binding reduces β -secretase cleavage of APP, suggesting therefore that F-spondin binding may regulate APP processing.

APP-deficient animals are a useful model to better understand the role of APP. Deficient APP mice did not show major phenotypic abnormalities [14]. However, *APLP2^{-/-}/APLP1^{-/-}* and *APP^{-/-}/APLP2^{-/-}* mutants, but not *APP^{-/-}/APLP1^{-/-}* animals, showed early postnatal lethality, indicating that members of the APP gene family are essential genes, which exhibit partial overlapping functions. Deficiency of all the APP genes leads to death shortly after birth. The majority of animals studied showed cortical dysplasia suggestive of migrational abnormalities of the neuroblasts and partial loss of cortical Cajal Retzius cells [15]. Taken together, these findings presented a convincing picture that members of the APP family play essential roles in the development of the nervous system related to synapse structure and function, as well as in neuronal migration.

Given the trophic properties of APP, it would be natural to predict that overexpression of APP would lead to phenotypes related to the enhanced neurite outgrowth and cell growth, which indeed was demonstrated [16]. However, convincing negative phenotypes, in which APP does not act as trophic factor, have also been reported. For example, overexpression of APP in cells induced to differentiate into neurons led to cell death [17]. Genetic *in vivo* engineering to overexpress APP carrying various familial AD mutations in transgenic mice resulted in the development of A β deposition and A β -associated changes in the brain, including loss of synaptic markers – thus confirming the pathogenic nature of these mutations. A detailed examination also showed axonal swellings and varicosities, which were observed months before any evidence of A β deposition [18].

In this model, tau deposition occurs as a consequence of a deregulation of its phosphorylation induced by A β deposition [19]. Based on this hypothesis, new drugs aimed at blocking tau deposition are under development.

■ Tau & 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 (microtubule associating protein tau [*MAPT*]) consists of 16 exons. In the adult brain, alternative splicing of tau nuclear RNA transcribed on exons 2, 3 and 10 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 two and three. 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 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 comprised of 3R tau [20].

■ Additional pathogenic mechanisms

Role of inflammation in Alzheimer's disease

The fibrillar deposition of extracellular A β is closely associated with a neuroinflammatory response, which includes a local upregulation of acute-phase proteins, complement fragments, cytokines and other inflammatory mediators [21]. So far, epidemiological studies suggested that inflammatory processes play a role in the pathogenesis of AD. Prospective case-cohort studies showed that higher serum levels of certain acute-phase proteins are a risk factor for the development of AD [22–24]. Moreover, epidemiological studies indicate that long-standing use of non-steroidal anti-inflammatory drugs (NSAIDs) can prevent or delay the development of AD [25].

Reports that complement proteins comprising the classical pathway are associated with senile plaques suggest that activation of the classical complement cascade in AD tissue results in bystander cell lysis and may contribute to AD neuropathology. Significantly lower levels of C1q were detected in the CSF of the AD group as compared with controls [26].

Microglial cells are the major producers of inflammatory factors. During the early stages of AD pathogenesis, activated microglia were clustered within classic (dense-cored) plaques in the AD neocortex [27]. These plaques showed strong immunostaining for complement factor C1q and serum amyloid P component (SAP). Plaque-associated factors C1q and SAP may trigger microglia to secrete high levels of proinflammatory cytokines [3].

Activated microglial cells colocalize with A β , and *in vitro* studies demonstrated that A β induces the production of TNF α in such cells [28]. This cytokine is a pleiotropic factor acting as an important mediator of inflammatory responses in a variety of tissues. Levels of TNF α in CSF from AD patients are 25-fold higher than in CSF from age-matched controls [29], suggesting a role for inflammation in neurodegeneration. Nevertheless, other findings demonstrated a protective effect of TNF α , as it likely protects neurons against A β -triggered cytotoxicity [30].

In AD, an increased production of IL-1 has been demonstrated by immunohistochemistry. In particular, it is expressed by microglia localized around amyloid deposits, possibly participating in plaque formation [31].

Conflicting results have been reported with regard to IL-6 levels in serum and CSF of AD patients. However, it has been shown that its mRNA levels are increased in the entorhinal cortex and the superior temporal gyrus of AD patients [31].

Additional cytokines of the IL-6 family are IL-11 and leukemia inhibitory factor (LIF) [32]. IL-11 mean levels were significantly increased in AD and frontotemporal lobar degeneration (FTLD), as compared with controls, whereas CSF LIF levels were not detectable either in patients or controls [33]. In accordance with previous results [34], in AD patients a significantly positive correlation between Mini Mental State Examination (MMSE) scores and IL-11 CSF concentration was observed [33].

In contrast with the previously described cytokines, TGF- β has a mainly anti-inflammatory action. Several data show that its levels are increased in the brain of AD patients, as well as in plasma and CSF. In addition, TGF- β was also found both in amyloid plaques and tangles [31].

As a general comment, microglial-produced 'inflammatory' cytokines have neuropathic as well as neuroprotective actions. For instance, whereas excess levels of TNF α might cause neurotoxicity, low-dose TNF α could, alternatively, trigger the neuroprotective and/or

antiapoptotic genes [35]. The role of glial cells is to support and sustain proper neuronal function, and microglia are no exception to this general principle. In acutely injured CNS, microglia have a neuroprotective and progenerative role [36]. Therefore, the primary mode of action of microglia seems to be the protection of the CNS. Nevertheless, upon excessive or sustained activation, microglia could significantly contribute to chronic neuropathologies, leading to neurotoxicity [3].

Chemokines are low-molecular-weight chemotactic cytokines that have been shown to play a crucial role in early inflammatory events. Based on the arrangement of cysteine residues, they are divided into two main groups: CXC or α -chemokines, such as interferon- γ -inducible protein-10 (IP-10) and IL-8, responsible for attracting neutrophils, and CC or β -chemokines, such as monocyte chemoattractant protein-1 (MCP-1) and macrophage inflammatory protein-1 α and β (MIP-1 α and β), which act basically on monocytes [37].

Upregulation of a number of chemokines has been associated with AD pathological changes [38]. IP-10 immunoreactivity was markedly increased in reactive astrocytes in AD brains, as well as the level of its expression. Astrocytes positive for IP-10 were found to be associated with senile plaques and showed an apparently coordinated upregulation of MIP-1 β [39,40]. Significant increased IP-10 levels were observed in CSF from patients with mild AD as compared with severe AD. Similarly to mildly impaired AD, IP-10 increased levels were also found in subjects with amnesic mild cognitive impairment (MCI) [34]. Regarding MCP-1 and IL-8, significantly higher levels were found in all AD patients as compared with healthy subjects, and highest peaks were observed in mild AD and MCI [34].

With regard to a possible use of chemokine to easily predict evolution from MCI to AD, so far few investigations in serum have been carried out, despite a growing body of evidence supporting the hypothesis that some peripheral biochemical modifications also occur very early during AD pathogenesis. For instance, serum MCP-1 levels have been demonstrated to be increased in MCI subjects, similarly to findings described in the CSF [41]. Conversely, IP-10 serum levels were not increased in AD patients, but were found to correlate with aging [42].

Oxidative damage

Oxidative stress is supposed to play a remarkable role in the pathogenesis of several

neurodegenerative diseases, including AD. A β and other lesion-associated proteins are a major source of reactive oxygen species (ROS) and other toxic radicals [43]. Increasing evidence supports a role of oxidative stress and impaired energy metabolism in the pathogenesis of the disease: an increase in DNA, lipid and protein oxidation metabolites has been observed in blood as well as post-mortem brain samples from AD patients compared with healthy subjects [44]. Free radicals are produced by mitochondria, as a side product, during the reduction of molecular oxygen. The production of radicals is thought to be higher in cerebral tissue, particularly vulnerable to free radical damage, because of its low content of antioxidants, high content of polyunsaturated fatty acids in neuronal membranes and high oxygen requirements for its metabolic process [45]. Further observations indicate reduced cerebral metabolism in AD [45], as well as reduced activities of specific mitochondrial enzyme complexes, such as cytochrome oxidase [46–48]. Alterations in these key enzymes can favor the aberrant production of ROS. Intracellular oxidative balance is tightly regulated and, therefore, an upregulation of antioxidant compensatory mechanisms would be expected in AD. The induction of Cu/Zn superoxide dismutase, catalase, glutathione peroxidase (GSHPx), glutathione reductase (GSSG-R), peroxiredoxins and a number of heat shock proteins [49] suggests that vulnerable neuronal cells mobilize antioxidant defence in the face of increased oxidative stress [43]. On the other hand, the total antioxidant capacity (TAC; including glutathione, ascorbic acid, uric acid and bilirubin) was shown to be reduced by 24% in plasma samples from AD patients [50]. A link between oxidative stress and hyperhomocysteinemia, which is a known risk factor for the development of AD [51], has been hypothesized, as homocysteine (Hcy) influences DNA repair, promoting the accumulation of DNA damage caused by oxidative stress [52]. Recent *in vitro* studies demonstrate that Hcy increases levels of thiobarbituric acid reactive substances, which represent an index of peroxidation, and decreases levels of total-trapping antioxidant potential in a model of rat hippocampus [53]. High total homocysteine (tHcy) levels are at present considered one of the major risk factors for the development of AD, as a strong, graded association between tHcy levels and the risk of dementia and AD has been demonstrated [51]. In this regard, there is evidence that tHcy levels are increased in late-onset AD (LOAD; disease onset >65 years), but not in early-onset AD

(EOAD; disease onset \leq 65 years), suggesting an influence on this parameter of other pathological conditions, mainly vascular diseases, which often co-occur with LOAD [54].

Similarly to inflammation, emerging evidence indicates that oxidative damage to neuronal RNA and protein is an early event in AD pathogenesis [55]. Moreover, oxidative imbalance is likely to be present in subjects with MCI. Both in MCI and in AD patients, plasma mean levels of nonenzymatic antioxidants and lower activity of antioxidant enzymes appeared to be lower than in controls, with no parallel induction of antioxidant enzymes [56]. In this regard, it has recently been shown that subjects with MCI have plasma, urine and CSF levels of the isoprostane 8,12-iso-iPF2a-VI, which is a marker of *in vivo* lipid peroxidation, higher than healthy subjects [57]. This evidence clearly indicates that oxidative imbalance and subsequent oxidative stress are early events in AD evolution, and are probably secondary to other mechanisms specific to AD but not present in other neurodegenerative diseases [58].

On the basis of these studies, suggesting that oxidative imbalance may help in the understanding of whether MCI is a prodromal stage of AD and whether a common pathogenesis between AD and MCI occurs, ROS, tHcy and TAC were evaluated in samples from patients with AD, MCI and vascular dementia (VaD), compared with age-matched healthy subjects. Total Hcy levels were significantly increased in AD as well as in VaD patients compared with controls. Notably, slightly increased tHcy levels were found in MCI patients compared with controls. With regards to ROS levels, no significant differences were shown between patients and controls. TAC was significantly lower in AD patients than in either healthy subjects or VaD patients. No correlation between ROS and TAC levels in each subject was observed [59]. In conclusion, an alteration of some biochemical factors involved in oxidative stress occurs in AD patients. Both tHcy and TAC modifications seem to be early events in the pathogenesis of AD, whereas ROS levels appear to be correlated with age rather than with a specific dementing disorder. This consideration leads to the hypothesis that oxidative imbalance observed in AD is mainly due to a decreased TAC rather than to an increased production of ROS [59].

The metallobiology hypothesis

It was first observed in 1994 that A β becomes amyloidogenic upon reaction with stoichiometric amounts of Zn²⁺ and Cu²⁺ [60]. A β is rapidly

precipitated by Zn^{2+} , Cu^{2+} and Fe^{3+} also induce marked $A\beta$ aggregation, but only under mildly acidic conditions [61], such as those believed to occur in AD brain. The precipitation of $A\beta$ by these ions is reversible with chelation [62], in contrast with 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 the Cu-mediated generation of the toxic lipid oxidation product 4-hydroxynoneal (see [61] for review).

Cholesterol and vascular-related risk factors

It has been repeatedly shown that apolipoprotein (Apo)E $\epsilon 4$ carriers have a higher risk of developing AD. Since ApoE is the major cholesterol transporter in the CNS, a link between cholesterol and AD is suggested. The brain is the most cholesterol-rich organ of the body, which is synthesized by 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 (see [63] for review).

Hypertension is the strongest risk factor for AD and VaD when these conditions are considered together [64]. The penetrating arteries in the circle of Willis are particularly sensitive to the effects of hypertension and suffer early and selective damage during chronic hypertension [65]. Hypertension is closely associated with atherosclerosis and vascular function, and in the brain this results in hypoperfusion and ischemic conditions of the nucleus basalis of Meynert. Targeting molecular mechanisms and using dietary methods and therapies are grounded in reducing free radicals and associated oxidative stress-related damage initiating hypertension [66].

Drugs for Alzheimer's disease treatment

■ Symptomatic treatments

To date, four acetylcholinesterase inhibitors (AChEIs) are approved for the treatment of mild-to-moderate AD: tacrine (First Horizon Pharmaceutical, GA, USA), donepezil (Pfizer, NY, USA), rivastigmine (Novartis, Basel, Switzerland) and galantamine (Janssen, NJ, USA) [67]. Donepezil is now approved for severe AD as well. Although tacrine was the first drug approved for AD in 1993, it is rarely used due to

hepatotoxicity [68]. Although there have been a few clinical trials aimed at comparing these AChEIs, there is no general consensus for differentiation among these therapies [68]. Besides symptomatic treatments, potentially disease-modifying drugs are under development (TABLE 1) and will be discussed in the next part of this review.

A recent article described the results of a meta-analysis obtained through the Cochrane Dementia and Cognitive Improvement Group's Specialized Register [69]. A total of 13 randomized, double-blind, placebo-controlled trials with donepezil, rivastigmine and galantamine were considered. It was concluded that the three AChEIs are efficacious for mild-to-moderate AD, although it is not possible to identify patients who will respond to treatment prior to initiation. There is no evidence that treatment with a AChEI is not cost-effective. Despite the slight variations in the mode of action of the three AChEIs, 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 worth consideration [69].

A further therapeutic option available for moderate-to-severe AD is memantine. This drug is an uncompetitive, moderate-affinity, NMDA antagonist believed to protect neurons from excitotoxicity. A recent meta-analysis on the efficacy of ChEIs and memantine indicates that these treatments can result in statistically significant but clinically marginal improvement [70].

A novel AChEI named dimebon has been recently tested in a randomized, double-blind, placebo-controlled study, demonstrating it is safe, well-tolerated and significantly able to improve the clinical course of patients with mild-to-moderate AD [71].

A number of additional compounds acting on cognition are under testing, including phenserine, muscarinic M1 agonists, M2 antagonists, nicotinic agonists and huperizine A (see [72] for review).

■ Drugs interfering with $A\beta$ deposition

Anti-amyloid aggregation agents

Several anti- $A\beta$ aggregation agents are currently in clinical testing. Despite their biological mechanisms of action being incompletely understood

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

Drug class	Name	Number of patients	Duration (months unless otherwise stated)	Proposed modes of action	Clinical trial phase	Results	Ref.
Drugs influencing Aβ deposition							
Antiaggregants	Tramiprosate (Alzhemed™)	1052	18	GAG mimetic	Phase III	No efficacy (definitive)	–
	Colostrin	105	15 + 15 extension	Inhibits A β aggregation	Phase II	Modest improvement in MMSE (not definitive)	[79]
	Scyllo-inositol (AZD103)	340*	18	Inhibits A β aggregation	Phase II	Ongoing	–
Vaccination	AN1792	300	Halted after approximately 12 months	A β removal (active immunization)	Phase II	Unclear cognitive results – severe adverse events (definitive)	[82,83]
	Bapineuzumab	200	18	A β removal (passive immunization)	Phase III	Ongoing	[87]
γ -secretase inhibitors	LY450139	51	3	Inhibits γ -secretase	Phase II	No changes in cognitive measures Phase III trial ongoing	[92]
SALAs	Tarenflurbil (Flurizan™)	210	12 + 12 extension 18	Inhibits γ -secretase	Phase II	No effect on cognition	[103,202]
	MTC (Rember™)	>800	6	Interferes with tau aggregation	Phase II	Improvement in cognition (not definitive)	[105]
Anti-inflammatory drugs							
	Rofecoxib	351	12	NSAID, inhibits COX2	Phase III	No efficacy (definitive)	[109,110]
	Naproxene	351	12	Nonselective NSAID	Phase III	No efficacy (definitive)	[110]
	Diclofenac	41	25 weeks	NSAID	Phase II	No efficacy (definitive)	[111]
	Celecoxib	–	52 weeks	NSAID	Phase II	No efficacy (definitive)	[112]
	Hydroxychloroquine	20	3	NSAID	Phase II	No efficacy (definitive)	[114]

*Estimated
 COX2: Cyclo-oxygenase 2; GAG: Glycosaminoglycan; Hcy: Homocysteine; MMSE: Mini Mental State Examination; NSAID: Nonsteroidal anti-inflammatory drug; SALA: Selective amyloid-lowering agent; TNF- α : Tumor necrosis factor- α .

Table 1. Potentially disease-modifying drugs tested in clinical trials in patients with Alzheimer's disease (cont.).

Drug class	Name	Number of patients	Duration (months unless otherwise stated)	Proposed modes of action	Clinical trial phase	Results	Ref.
Anti-inflammatory drugs (cont.)							
	Nimesulide	40	6	NSAID, inhibits COX2	Phase II	No efficacy (definitive)	[115]
	Indomethacin	–	6	NSAID	Phase II	No efficacy–toxicity (definitive)	[116]
	Rosiglitazone (Avandia™)	511	6	Antidiabetic and anti-inflammatory	Phase II	No efficacy Phase III ongoing (not definitive)	[118]
	Etanercept	12	6	Inhibits TNF- α	Open study	Improvement in cognition (not definitive)	[119]
Drugs preventing oxidative damage							
	Folate/B6/B12	340	18	Reduction of Hcy	Phase III	No effects	[122]
Drugs interfering with metals							
	PBT2	78	3	Metal–protein attenuation	Phase II	Improvement in cognition (not definitive)	[124]
	Clioquinol	36	9	Inhibits zinc and copper from binding to A β	Phase II	Reduction in cognitive decline in more severely affected patients only (definitive)	[125]
Statins							
	Simvastatin	400*	18	Cholesterol reduction	Phase III	Ongoing	[203]
	Atorvastatin	641	20	Cholesterol reduction	Phase III	Ongoing	[128]

*Estimated.
COX2: Cyclo-oxygenase 2; GAG: Glycosaminoglycan; Hcy: Homocysteine; MMSE: Mini Mental State Examination; NSAID: Nonsteroidal anti-inflammatory drug; SALA: Selective amyloid-lowering agent; TNF- α : Tumor necrosis factor- α .

they are believed to prevent fibril formation and to facilitate soluble A β clearance. The most studied is tramiprosate (AlzhemedTM, Neurochem, Inc., Québec, Canada), a glycosaminoglycan (GAG) mimetic. GAGs binds to soluble A β , promoting fibril formation and deposition of amyloid plaques. GAG mimetics compete for GAG-binding sites, thus blocking fibril formation and reducing soluble A β [73]. In transgenic mice, tramiprosate reduces plaque burden and decreases CSF A β levels, but cognitive and behavioral outcomes in this animal model have not been reported [74]. 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 50, 100 or 150 mg tramiprosate twice a day or placebo. Patients who completed the study were eligible for a 21-month open-label extension with 150 mg twice daily. Baseline CSF A β levels declined by up to 70% after 3 months for patients randomly assigned to the 100 or 150 mg twice-daily group. However, no differences were observed in cognitive functions between the tramiprosate and placebo groups [75]. A Phase III study was then carried out in the USA in 1052 patients with AD to test tolerability, efficacy and safety of the drug. The study failed to show any significant effect. Multiple factors likely contributed to the failure of the study. Overall, variability among the 67 clinical sites in the trial overwhelmed the observed treatment effects. In particular, changes in people's concomitant treatment with cognitive-enhancing drugs including cholinesterase inhibitors, memantine and antidepressants affected the results for the primary cognitive end points based on neuropsychological testing. Unexpected problems also arose in the control group, confounding the interpretation of the efficacy of tramiprosate. A total of 30% of the control group showed no decline in cognition over the 18-month trial period, whereas a portion of this group unexpectedly showed a significant improvement in cognition. Another 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 [76], emphasizing the importance of testing on both types of pathology (amyloid and tau) the potential drugs to be used for the 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, London, UK) that inhibits

A β aggregation and neurotoxicity in cellular assays and improves cognitive performance in animal models [77]. A 3-week Phase I study in patients with AD demonstrated it is well tolerated [78]. A subsequent Phase II trial demonstrated modest improvements in 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 [79].

In 2000, McLaurin *et al.* [80] described a compound, named *scyllo*-inositol, which is able to stabilize oligomeric aggregates of A β and to inhibit A β toxicity. *Scyllo*-inositol (AZD103) dose-dependently rescued long-term potentiation in mouse hippocampus from the inhibitory effects of soluble oligomers of cell-derived human A β [81]. This compound is in Phase II clinical trials (Transition Therapeutics, Ontario, Canada/Elan, Dublin, Ireland).

Amyloid removal: vaccination

In 1999, Schenk *et al.* demonstrated that immunization with A β as an antigen attenuated AD-like pathology in transgenic mice overexpressing the *APP* gene by removing amyloid from the CNS [82]. 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 A β prevents the development of plaque formation, neuritic dystrophy and astroglyosis, whereas in older animals, vaccination reduces extent and progression of AD-like pathologies. Given these preclinical results, a multicenter, randomized, placebo-controlled, Phase II double-blind clinical trial using active immunization with A β 42 plus adjuvant was started in 2001 on 300 patients using the preaggregated A β 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 [83].

Double-blind assessment was maintained for 12 months, demonstrating no significant differences in cognition between antibody responders and the placebo group for the AD assessment scale-cognitive subscale (ADAS-cog), Disability Assessment for Dementia (DAS), Clinical Dementia Rating, MMSE and Clinical Global Impression of Change. In a small subset of patients, CSF tau levels were decreased in antibody responders, but A β levels were unchanged.

A quite disappointing observation was the finding of greater brain volume decrease and greater ventricular enlargement in responders than in placebo patients, as seen with MRI [84]. Nevertheless, this brain atrophy was not associated with worsening of cognitive performances. A possible explanation is that the brain volume changes observed may result from an association between amyloid removal and intracerebral fluid shifts.

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 A β -related epitopes are capable of slowing the progression of neuropathology in AD. Hock and Nitsch [85] followed 30 patients who received a prime and booster immunization over the first year after vaccination for 4 years, providing further support to continue investigation of antibody treatment in AD.

In 2008, a paper was published describing the relation between A β 42 immune response, degree of plaque removal and long-term clinical outcomes [86]. 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 β load was lower than in the placebo group. However, despite this observation, 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, but the treatment was modified from active into passive in order to avoid excessive activation of the T-cell response and thus prevent complications. The humanized monoclonal anti-A β antibody bapineuzumab (Wyeth, NJ, USA 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 approximately 30 sites in the USA. It was designed to assess safety, tolerability and standard efficacy end points (ADAS-Cog,

Neuropsychological Test Battery and DAS) of multiple ascending doses of bapineuzumab in patients. The 18-month trial includes an interim analysis, as well as data collection on clinical end points and biomarkers [87]. 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. Nevertheless, equivocal results of the Phase II study have been presented at the 2008 International Conference on AD (ICAD), held in IL, USA [87].

γ -secretase inhibition

Several compounds that inhibit γ -secretase activity in the brain have been identified. Nevertheless, γ -secretase has many biologically essential substrates [88]. One of the most physiologically important γ -secretase substrate is the Notch signaling 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 demonstrated that the administration of a γ -secretase inhibitor in doses sufficient to remove A β concentrations interferes with lymphocyte differentiation and alters the structure of intestinal goblet cells [89]. Therefore, safety is a very important consideration for this kind of compound.

A nonselective γ -secretase inhibitor named LY450139 (Eli Lilly, IN, USA) has been evaluated in a Phase I placebo-controlled study in 37 healthy adults (at doses ranging from 5 to 50 mg). A β CSF levels were reduced in both active treatment and placebo groups, but differences were not statistically significant. Transient gastrointestinal adverse effects (bleeding and abdominal pain) were reported by two subjects treated with 50 mg [90]. A subsequent Phase II randomized, controlled trial was carried out in 70 patients with AD. Patients were administered 30 mg LY450139 for 1 week followed by 40 mg for 5 weeks. Treatment was well tolerated. No significant changes in plasma and CSF A β 40 and A β 42 were observed [91].

Subsequently, a multicenter, randomized, double-blind, dose-escalation, placebo-controlled trial was carried out. A total of 51 patients with mild-to-moderate AD were randomized to receive placebo or LY450139 (100 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 6 additional weeks. Primary outcomes included safety, tolerability and CSF/plasma A β levels; secondary outcome was neuropsychological testing. LY450139 was generally well-tolerated at doses of up to 140 mg/day for 14 weeks. However, adverse events were seen, including three possible drug rashes, three reports of hair color change and three cases of adverse event-related discontinuation; therefore, close clinical monitoring will be needed in future studies. Plasma A β , but not CSF, levels were reduced in treated patients, consistent with inhibition of γ -secretase. No differences were seen in cognitive or functional measures [92].

BACE inhibition

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 ROC method. According to the best pharmacophore model, 11 *N*-phenyl-1-arylamide, *N*-phenylbenzenesulfonamide derivatives, compounds 26–28, and 33 a–g, were designed to be synthesized and their BACE1 inhibitory activities were determined experimentally. Their theoretical results were in good agreement with the experimental values. Compound 33d, which displayed the highest BACE1 activity among these 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 BACE1 [93]. Other compounds with a potential BACE1 inhibitory effect include neocorylin [94], (-)-epigallocatechin-3-gallate and curcumin [95] and *N*(4)-substituted piperazine naphthamide derivatives [96].

Selective A β 42-lowering agents

Tarenflurbil is the first compound in this new class of drugs, which modulate γ -secretase activity without interfering with Notch or other γ -secretase substrates [97]. It binds to a γ -secretase site other than the active/catalytic center of relevance to production of A β 42, thereby altering the conformation of γ -secretase and shifting production away from A β 42 without interfering with other physiologically essential γ -secretase substrates.

Tarenflurbil (MPC-7869, FlurizanTM; Myriad Pharmaceuticals, UT, USA) is the pure *R*-enantiomer of flurbiprofen. It shifts cleavage of APP away from A β 42, leading to the production

of shorter nontoxic fragments [98,99]. In contrast with *S*-flurbiprofen or other NSAIDs, it does not inhibit cyclo-oxygenase (COX) I or COX 2, and it is not associated with gastrointestinal toxicity [100]. In mice, treatment with tarenflurbil reduces amyloid plaque burden and prevents learning and behavioral deterioration [101].

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 of A β 42 CSF levels were shown after treatment. However, in plasma, higher drug concentrations were related to statistically significant lower A β levels [102].

Myriad Pharmaceuticals conducted a large, placebo-controlled Phase II trial for tarenflurbil 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 mg or 800 mg or placebo) for 12 months. Primary outcome measures for the trial 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-sum of boxes (CDR-sb); and cognitive function, measured by the 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 800 or 400 mg tarenflurbil 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 in 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 diarrhea, 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 [103].

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. Patients enrolled in the trial were treated with 800 mg twice a day of either tarenflurbil or placebo and attended periodic physician visits for analysis of their performance on memory, cognition and behavioral tests. The two primary clinical end points of the trial were the change 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 end point 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 trial was designed to meet the requirements of the European Agency for the Evaluation of Medicinal Products (EMA) for marketing of tarenflurbil in Europe. The global end points in this 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 are allowed to take currently standard-of-care medicines in addition to tarenflurbil or placebo, provided their dose has been stable for 6 months.

Disappointingly, on July 2, 2008, the sponsor of tarenflurbil announced that this γ -secretase-modulating agent had fallen flat in its definitive Phase III trial and was finished as a development product [202]. In fact, on both primary efficacy end points, 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 those patients on tarenflurbil than in the placebo group.

■ Drugs interfering with tau deposition

A Phase II trial of a tau-blocking compound named methyl thioninium chloride (MTC) is ongoing (Rember™; TauRx Therapeutics, Singapore). 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 [104]. 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, 60 or 100 mg three times a day. Patients were not taking acetylcholinesterase inhibitors or memantine. Primary outcomes were to compare the effect of MTC to placebo on cognitive abilities measured by the ADAS-Cog at 24 weeks. Preliminary results were presented at the 2008 ICAD [105]. 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. Significant efficacy was demonstrated separately in mild and moderate subgroups [105]. A problem with the use of this drug is that urine becomes blue, resulting in a lack of blinding. In addition, results described have been presented at the ICAD conference [105], but the study is not completed yet. Thus, these preliminary results need to be considered cautiously until definitive data will be published.

An interesting approach to blocking 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 (GSK3 β) has emerged as a potential therapeutic target (see [106] for review). The most-studied compound able to inhibit GSK3 is lithium, but several other compounds are under development (reviewed in [107]).

■ Anti-inflammatory drugs

A large body of epidemiologic evidence suggested that long-term use of NSAIDs protects against the development of AD [25,108]. Nevertheless, prospective studies of rofecoxib, naproxen or diclofenac failed to slow progression of cognitive decline in patients with mild-to-moderate AD [109–111], as did celecoxib [112], dapsone [113], hydroxychloroquine [114] and nimesulide [115]. In contrast, indomethacin may delay cognitive decline in this subset of patients, but gastrointestinal toxicity is treatment-limiting [116,117]. Due to 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.

A promising compound is rosiglitazone (AVANDIA®), an antidiabetic agent with anti-inflammatory properties, which was tested in two small clinical trials. Rosiglitazone treatment for 24 weeks resulted in a modest but significant improvement in cognition in non-ApoE $\epsilon 4$ carriers, but no improvement and rather a decline in cognition in $\epsilon 4$ carriers was demonstrated [118]. A Phase III trial is currently ongoing.

Lastly, a rapid improvement in verbal fluency and aphasia following perispinal etanercept administration was described [119]. Etanercept is a TNF α inhibitor, which acts by blocking the binding of this cytokine to its receptor. It was tested in 12 patients with mild-to-severe AD, at a dose of 25–50 mg weekly for 6 months, showing improvement in a number of neuropsychological tests, particularly in verbal fluency [119].

■ Drugs preventing oxidative damage

Ginkgo biloba extracts have antioxidant activity, and counteract the aggregation or deposition of A β *in vitro* and in animal models. These promising preclinical hints lead to the development of a large trial. The GEM study is a randomized, double-blind, placebo-controlled trial designed to test whether EGb 761, a commercial extract contained in many over-the-counter ginkgo preparations, at a dose of 120 mg twice a day, could delay the onset of AD in older adults. Participants included 2587 cognitively normal elderly volunteers, and 482 with MCI (mean age for all: 79.1 years). Half of the subjects received the treatment and half the placebo. All were followed for an average of 6.1 years. The measured end points were onset of dementia of any cause or AD. No differences were found in the incidence of all-cause dementia, or AD in particular, between those receiving ginkgo and the placebo group [120].

Additional potential antioxidants include mitoquinone (Antipodean Pharmaceuticals, Auckland, New Zealand), vitamin E and natural polyphenols such as green tea, wine, blueberries and curcumin. Clinical trials with vitamin E and omega-3 fatty acids did not show beneficial effects in AD patients (see [121] for review).

A trial to determine whether 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 multicenter, randomized, controlled clinical trial named VITAL (High

Dose Supplements to Reduce Homocysteine and Slow the Rate of Cognitive Decline in AD). A total of 409 individuals with mild-to-moderate AD (MMSE between 14 and 26, inclusive) and normal folic acid, vitamin B(12) and Hcy levels were included. Participants were randomly assigned to two groups of unequal size (60% treated with high-dose supplements [5 mg/day of folate, 25 mg/day of vitamin B(6), 1 mg/day of vitamin B(12)] and 40% treated with identical placebo); duration of treatment was 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, rate of change in ADAS-cog score during 18 months or on any secondary measures [122].

■ Drugs interfering with metals

PBT2 was designed to modify the course of AD by preventing metal-dependent aggregation, deposition and toxicity of A β . PBT2 acts at three levels of the 'amyloid cascade': it inhibits the redox-dependent formation of toxic soluble oligomers, prevents deposition of A β as amyloid plaques and promotes clearance by mobilizing and 'neutralizing' A β from existing deposits [123]. PBT2 has been recently tested in a Phase II trial. A total of 78 patients with mild AD were randomly assigned to PBT2 50 mg, 250 mg or placebo (in addition to acetylcholinesterase inhibitors) 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 β 42 concentration compared with those treated with placebo [124]. However, cognitive efficacy was 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, Victoria, Australia) 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 [125]. According to the 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 end point, and this group was small (eight treated subjects). The sample size was small and details of randomization procedure or blinding were not reported [126].

■ Statins

Epidemiological studies indicated that patients treated for cardiovascular disease with cholesterol-lowering therapy (statins) showed a decreased prevalence of AD [127].

Simvastatin is a pro-drug, 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.

Cholesterol Lowering Agent to Slow Progression (CLASP) of Alzheimer's disease 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 includes the treatment of patients 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), mental status, functional ability, behavioral disturbances, quality of life and economic indicators will also be made.

The sample size will include 400 participants enrolled from approximately 40 sites. Study medication will be as follows: 20 mg of simvastatin or matching placebo to be given for 6 weeks, followed by 40 mg of simvastatin or matching placebo for the remainder of the 18-month study period [203].

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 a cholinesterase inhibitor (donepezil 10 mg daily). An international, multicenter, double-blind, randomized, parallel-group study with a double-blind randomized withdrawal phase of patients with mild-to-moderate AD (MMSE score: 13–25) was started. Inclusion criteria included age 50–90 years, receiving 10 mg donepezil for at least 3 months before randomization and low-density lipoprotein cholesterol levels (LDL-C) of 2.5–3.5 mmol/l (95 to 195 mg/dl). Co-primary end points are changes in ADAS-cog and ADCS-CGIC scale scores. A confirmatory end point is the rate of change in whole brain and hippocampal volumes in patients who were enrolled in the MRI substudy. Enrollment of 641 subjects is complete [128].

Future perspective

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

First, mechanisms at the basis of the pathogenesis of AD need to be deeply investigated before developing novel potentially disease-modifying compounds. Despite promising premises related to the so-called 'amyloid hypothesis', as well as to other pathogenic mechanisms, large Phase III trials with potentially disease-modifying properties 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 A β load was lower than in the placebo group, there was no evidence of improved survival or improvement in time to severe dementia. Therefore, plaque removal does not seem to be sufficient to halt progressive neurodegeneration in AD, prompting some intriguing challenges to the amyloid hypothesis. In light of these results, it is of crucial importance to better understand the relationship between tau, A β and other factors for developing novel potentially disease-modifying drugs.

The second point to be addressed is that treatments for AD could be 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, according to recent studies demonstrating a high degree of inflammation in very mild but not severe AD. Therefore, therapeutic trials should be carried out as early as possible during the course of the disease, implying the need to identify more accurate tools for early diagnosis. In this regard, new research diagnostic criteria have been proposed in 2007 [129] introducing the use of CSF analysis, structural (CT scan, MR) and functional (positron emission tomography, single photon emission computed tomography) imaging and genetics, together with classical neuropsychological testing, for early and specific diagnosis. Large-scale international controlled multicenter trials are engaged in Phase III development of the core feasible imaging and CSF biomarkers candidates in AD (USA, European, Australian and Japanese AD Neuroimaging Initiative, and the German Dementia Network) [4]. If the validation of these new criteria will be achieved, they should be considered in the setting of future clinical trials to identify more homogeneous study groups.

Third, indicators useful as surrogate outcome measures (surrogate biomarkers) should be identified in order to:

- Have substitutes for clinical end points (i.e., neuropsychological testing)
- Have tools able to predict clinical benefit, or the opposite
- Demonstrate whether there are disease-modifying properties.

So far, none of the biomarkers proposed for early diagnosis has been validated as a surrogate marker for monitoring treatments.

The contribution of experts working in the field of basic science such as biochemistry, molecular biology and immunology will

be mandatory to generate new pathogenic hypotheses essential for the development of truly innovative therapeutic approaches.

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Executive summary

Key events in the pathogenesis of AD

- Formation of senile plaques, made of extracellular accumulation of fibrillar amyloid- β ($A\beta$) peptide. This event is possibly influenced by Zn^{2+} and Cu^{2+} .
- Intracellular formation of neurofibrillary tangles, made of hyperphosphorylated tau protein.
- Microglial activation, production of cytokines and chemokines.
- Oxidative damage.
- Influence of cholesterol and vascular risk factors.

Potentially disease-modifying drugs

- Drugs interfering with $A\beta$ deposition.
- Drugs interfering with tau deposition.
- Anti-inflammatories.
- Antioxidants.
- Drugs interfering with metals.
- Statins.

Results of clinical trials

- Phase III studies with drugs interfering with $A\beta$ deposition and some anti-inflammatories failed to demonstrate significant benefits.
- Possible reasons for this included: a poor knowledge about the relationships among $A\beta$, tau and other pathogenic steps; nonhomogeneous study populations; too advanced phase of the disease; and a need for surrogate biomarkers to demonstrate whether there is a modifying effect.

Future perspective

- Cerebrospinal fluid and imaging biomarkers could be used to select more homogeneous study groups and to identify surrogate biomarkers that could substitute for clinical end points.

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- 203 CLASP – a multi-center, randomized, double-blind, placebo-controlled trial of simvastatin to slow the progression of Alzheimer's disease
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