Current and future treatments for Alzheimer's disease

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Abstract: Alzheimer's dementia (AD) is increasingly being recognized as one of the most important medical and social problems in older people in industrialized and non-industrialized nations. To date, only symptomatic treatments exist for this disease, all trying to counterbalance the neurotransmitter disturbance. Three cholinesterase inhibitors (CIs) are currently available and have been approved for the treatment of mild to moderate AD. A further therapeutic option available for moderate to severe AD is memantine, an N-methyl-D-aspartate receptor noncompetitive antagonist. Treatments capable of stopping or at least effectively modifying the course of AD, referred to as 'disease-modifying' drugs, are still under extensive research. To block the progression of the disease they have to interfere with the pathogenic steps responsible for the clinical symptoms, including the deposition of extracellular amyloid β plaques and intracellular neurofibrillary tangle formation, inflammation, oxidative damage, iron deregulation and cholesterol metabolism. In this review we discuss current symptomatic treatments and new potential disease-modifying therapies for AD that are currently being studied in phase I–III trials.

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

Introduction

Dementia is increasingly being recognized as one of the most important medical problems in older people with a prevalence rising from 1% at the age of 60 to at least 35% at the age of 90 [Ferri *et al.* 2005]. Within the spectrum of dementias, Alzheimer's disease (AD) is the most prevalent subtype, accounting for about 60% of all dementias. It is characterized clinically by progressive memory and orientation loss and other cognitive deficits, including impaired judgment and decision making, apraxia and language disturbances. These are typically accompanied by various neuropsychiatric symptoms (i.e. depression, apathy, anxiety, agitation, delusions, hallucinations). The continuing expansion of life expectancy, leading to a fast growing number of patients with dementia, particularly AD, has led to an enormous increase in research focused on the discovery of drugs for primary, secondary or tertiary prevention of the disease. Despite all scientific efforts, at the moment there are no effective pharmacotherapeutic options for prevention and treatment of AD.

To date, established treatments are only symptomatic in nature, trying to counterbalance the neurotransmitter disturbance of the disease. Three cholinesterase inhibitors (CIs) are approved for the treatment of mild to moderate AD [Birks, 2006]. A further therapeutic option available for moderate to severe AD is memantine [McShane *et al.* 2006]. At the same time antipsychotic and antidepressant treatments are used for the behavioral symptoms of the disease [Ballard and Corbett, 2010].

Treatments under research include compounds that act on the pathological substrate of the disease: extracellular amyloid β (Aβ) plaques and intracellular neurofibrillary tangles (NFTs).

In this review, current symptomatic treatments and new potential disease-modifying therapies for

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AD that are currently being studied in phase I–III trials are discussed.

Current symptomatic approaches to Alzheimer's disease

Cholinesterase inhibitors

The cholinergic hypothesis of AD concludes that cholinergic systems in the basal forebrain are affected early in the disease process, including loss of acetylcholine neurons, loss of enzymatic function for acetylcholine synthesis and degradation, resulting in memory loss and deterioration of other cognitive and noncognitive functions such as neuropsychiatric symptoms [Bartus *et al.* 1982; Cummings and Back, 1998]. A strategy to enhance the cholinergic transmission by using CIs to delay the degradation of acetylcholine between the synaptic cleft has been proposed. To date, three CIs are approved for the treatment of mild to moderate AD: donepezil (Pfizer, New York, NY, USA), rivastigmine (Novartis, Basel, Switzerland) and galantamine (Janssen, Beerse, Belgium) [Farlow, 2002]. These drugs have been regarded as the standard and first-line treatment for AD. Systemic reviews including many double-blind, randomized, placebo-controlled trials (RCTs) of these three CIs all showed benefit on cognitive functions, activities of daily living (ADL), and global function for patients with mild to moderate AD; there was no significant difference of efficacy between individual CIs [Farlow, 2002; Birks, 2006]. In addition, donepezil is now also approved for the treatment of severe AD in the USA [Cummings *et al.* 2010]. Although tacrine (First Horizon Pharmaceuticals, Alpharetta, Georgia, USA) was the first CI drug approved for AD in 1993, it is no longer used due to hepatotoxicity [Alfirevic *et al.* 2007]. Related systemic reviews showed that the incidence of gastrointestinal adverse effects, such as nausea, vomiting, diarrhea and abdominal cramp, was lower with donepezil than with rivastigmine and galantamine [Alva and Cummings, 2008]. The incidence of adverse effects was associated with higher therapeutic dose. However, it may be that galantamine and rivastigmine may be equal to donepezil in tolerability if a careful and gradual titration routine of more than 3 months is used. The dermal form of rivastigmine provides a lower dose with fewer adverse effects but comparable efficacy, and is was preferred by some caregivers [Blesa *et al.* 2007]. Use of CIs is also reported to be associated with increased rates of syncope, bradycardia and pacemaker insertion. The risk of these adverse events must be weighed carefully against the drugs' benefits [Gill *et al.* 2009].

Reviews and meta-analyses on CIs that have recently been published showed that they delay the decline in cognitive function as measured by the AD Assessment Scale – cognitive subscale (ADAS-cog), global clinical rating, behavior and ADL over 6–12-month periods. These benefits seem to be applicable to mild, moderate and severe AD [Birks, 2006; Hansen *et al* 2008; Qaseem *et al.* 2008]. Compared with those on placebo treatment, patients on CIs generally show an initial mild improvement in cognitive functions over the first 3 months. Thereafter, the mean decline in cognitive functions was also less rapid over the subsequent 3–9 months. At 6 months, the cognitive improvement (*versus* placebo) was 2.7 points over the Mid range of ADAS-cog [Birks, 2006; Hansen *et al.* 2008]. Symptoms that were improved included attention, thinking, memory, praxis, language comprehension and communication [Qaseem *et al.* 2008].

Initiation of CI treatment in the early stages of AD is preferred. A 52-week study of the efficacy of rivastigmine in patients with mild to moderately severe AD reported that patients with AD who started the CI 6 months later achieved lower cognitive performance than those who started the drug immediately after the diagnosis [Farlow *et al.* 2000]. Preserved cognitive function was also observed after 12 months of treatment with rivastigmine in patients with mild AD in comparison to untreated patients who markedly worsened in cognition during the same period [Almkvist *et al.* 2004].

N-methyl-D-aspartate antagonist

A further therapeutic option for moderate to severe AD is memantine (Lundbeck, Valby, Denmark). This drug is an uncompetitive, moderate-affinity N-methyl-D-aspartate (NMDA) antagonist believed to protect neurons from excitotoxicity. A systemic review of double-blind, parallel-group, RCT studies of memantine showed improvement in cognition, ADL and behaviors in people with moderate to severe AD after 6 months of use [McShane *et al.* 2006]. Another systemic review which included six RCT studies indicated that memantine may reduce behavioral and psychological symptoms of dementia [Maidment

et al. 2008]. The most frequently reported adverse events in memantine trials were dizziness, headache and confusion. A small group of patients might develop agitation [Alva and Cummings, 2008].

Combination therapy

RCT studies on parallel groups of patients with moderate to severe AD showed a significant benefit in cognitive function, language, ADL, behaviors and global state from combination use of memantine and donepezil over the placebo group (memantine and placebo) [Tariot *et al.* 2004; Feldman *et al.* 2006; Howard *et al.* 2012]. However, such benefit was not demonstrated in patients with mild to moderate AD [Farlow *et al.* 2010].

Treatment of behavioral and psychological symptoms of dementia in Alzheimer's disease

Noncognitive neuropsychiatric symptoms or behavioral and psychological symptoms of dementia (BPSD) are common in all clinical stages of AD and even in amnestic mild cognitive impairment (MCI) (the predementia stage of AD) with increasing prevalence when dementia progresses. They are the main determining factors for increased caregiver burden and institutionalization of patients. According to a large observational study, BPSD may be grouped into four major symptom clusters with high prevalence: psychosis (38% of the patients, e.g. delusions), affective symptoms (59%, anxiety and depression), hyperactivity (64%, e.g. aggression, disinhibition) and apathy (65%) [Zec and Burkett, 2008].

CIs and memantine may have an effect on behavioral symptoms [Farlow, 2002; Birks, 2006; Maidment *et al.* 2008]. However, when BPSD become more severe, these antidementia drugs may not be as effective and other drugs also need to be given.

Serotonin reuptake inhibitors (SSRIs: fluoxetine, sertraline, paroxetine, citalopram, fluvoxamine) are largely considered to be among the most efficient antidepressants to treat comorbid depression in AD dementia [Zec and Burkett, 2008].

Mirtazapine, venlafaxine and duloxetine, which are combined selective noradrenalin and serotonin inhibitors (SNRIs), and bupropion are other widely used antidepressants in this population. A few RCTs with limited numbers of patients as well as meta-analyses support their efficacy to treat depression in AD dementia [Ballard and Corbett, 2010]. SSRIs may also be taken into consideration for the treatment of agitation and psychosis in AD dementia [Zec and Burkett, 2008]. However, a recent randomized, multicenter, double-blind, placebo-controlled trial of sertraline or mirtazapine for depression in dementia (HTA-SADD) showed absence of benefit compared with placebo and increased risk of adverse events. The trial concluded that the current practice of using these antidepressants, with usual care, for first-line treatment of depression in Alzheimer's disease should be reconsidered [Banerjee *et al.* 2011].

Psychotic symptoms and agitation/aggression are commonly treated with antipsychotics in patients with AD dementia. Atypical agents (olanzapine, risperidone, quetiapine, ziprasidone and aripiprazole) are preferred due to their milder parkinsonian effects [Ballard and Corbett, 2010]. The use of antipsychotics has been discussed controversially, as cerebrovascular morbidity and higher mortality have been found in patients with dementia taking antipsychotics. Furthermore, the use of antipsychotics may be associated with a higher risk of hip fracture and pneumonia, as well as worsening cognitive impairment. The increased mortality may be reduced if antipsychotics are only given over a short period, as stopping the antipsychotic medication may not be associated with a subsequent increase in BPSD [Zec and Burkett, 2008].

Benzodiazepines are used to reduce agitation and anxiety. However, they can also trigger further agitation in older people. An association of greater benzodiazepine use with more rapid cognitive and functional decline has been reported in AD and indeed in older people in general [Zec and Burkett, 2008].

Anticonvulsant drugs like carvamazepine can also reduce BPSD in AD to some degree [Ballard *et al.* 2009].

It is obvious that drugs currently used for the treatment of AD have weak beneficial effects on cognitive function or offer some relief of BPSD. The discovery of new drugs that act during the early stages of AD could be considered a 'medical need' [Mancuso *et al.* 2011]. Early intervention is critical because a delay in treatment is associated with nonreversible symptom progression.

The amyloid hypothesis

The primary histopathologic lesions of Alzheimer's pathology are amyloid plaques, NFTs and neuronal loss. Mature plaques consist of a central amyloid core with surrounding degenerating neurons affected by the toxic effect of the Aβ. NFTs consist of hyperphosphorylated tau protein that has assumed a double helical filament conformation [Cummings, 2008b].

The Aβ derives from the amyloid precursor protein (APP) through sequential proteolysis by β secretase (BACE1) in the extracellular domain and γ secretase in the transmembrane region.

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 the generation of α or β C-terminal fragments (CTFs). The major neuronal β secretase, named BACE1 (β-site APP cleaving enzyme), is a transmembrane aspartyl protease that cleaves APP within the ectodomain, generating the N-terminus of Aβ. The second proteolytic event in APP processing involves intramembranous cleavage of α and β CTFs by γ secretase. Major sites of γ-secretase cleavage correspond to positions 40 and 42 of Aβ. Amyloidogenic processing is the favored pathway of APP metabolism in neurons because of the greater abundance of BACE1, whereas the nonamyloidogenic pathway predominates in other cells [Vassar, 2004].

According to the 'amyloid hypothesis' Aβ production in the brain initiates a cascade of events leading to the clinical syndrome of Alzheimer's dementia [Golde, 2005]. Aβ is a protein consisting of two major forms, Aβ40 and Aβ42. Aβ42 is the most soluble form and has the tendency to aggregate into fibrils that form the major composite of amyloid plaques. It is the predominant form found in the brain parenchyma of patients with AD. Aβ40 is mostly found in the cerebral vasculature as part of 'cerebral amyloid angiopathy'. Aβ has a tendency to cluster into oligomers. Oligomers can form Aβ-fibrils and protofibrils that will eventually form amyloid plaques, which are believed to be nontoxic. It is the forming of amyloid oligomers to which neurotoxicity is attributed and initiates the amyloid cascade. The elements of the cascade include local inflammation, oxidation, excitoxicity (excessive glutamate) and tau hyperphosphorylation. As a result of this process, tau proteins fold into intraneuronic tangles, which results in cell death. Progressive neuronal destruction leads to shortage and imbalance between various neurotransmitters (e.g. acetylcholine, dopamine, serotonin) and to the cognitive deficiencies seen in AD [Cummings, 2008a; Golde, 2005].

On the basis of findings on AD pathogenesis, novel treatments under development aim to interfere with the pathogenic steps previously mentioned in an attempt to block the course of the disease in its early stages [Galimberti and Scarpini, 2011; Golde, 2005]. For this reason they have been termed 'disease-modifying' drugs. In this review, possible strategies for the development of novel disease-modifying therapies will be discussed.

Disease-modifying approaches to Alzheimer's disease

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

Thus, both Aβ and tau are prime targets for disease-modifying therapies in AD. From this point of view, AD could be prevented or effectively treated by decreasing the production of Aβ and tau; preventing aggregation or misfolding of these proteins; neutralizing or removing the toxic aggregate or misfolded forms of these proteins; or a combination of these modalities.

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

Table 1. Disease-modifying treatments: modulation of amyloid deposition.

Drugs interfering with Aβ aggregation	Selective Aβ42-lowering agents	Immunotherapy
Anti-amyloid aggregation agents: Tramiprosate: phase III trial (–) Colostrinin: phase II trial (±) Scyllo-inositol: phase II trial (±)	Inhibition of β-secretase: Nonpeptidic inhibitors: trials in animal models (+) CTS-21166: phase I trials (+)	Active immunization (vaccination): AN-1792: phase II trial (–) CAD-106, V950, ACC-001: phase II trials MABT5102A, PF-04360365, R1450: phase I trials (+) GSK933776A: phase I trials DNA epitope vaccine antibodies against the β-secretase cleavage site mucosal vaccination: trials in animal models (+)
Drugs interfering with metals: PBT2: phase IIa trial (+)	Inhibition of γ-secretase: Semagacestat: phase III trials (–) Tarenflurbil: phase III trials (–) Avagacestat: phase II trial Activation of α-secretase: Etazolate: phase IIa trial (+)	Passive immunization (monoclonal antibodies): Bapineuzumab: phase II trial (+) Solanezumab: phase II trial IVIg: phase III trial

+, encouraging results; –, disappointing results; ±, doubtful results.
Aβ, amyloid β; IVIg, intravenous immunoglobulin.

Disease-modifying treatments: modulation of amyloid deposition

Drugs interfering with amyloid β deposition. Anti-amyloid aggregation agents The hypothesis that aggregation of Aβ leads to toxic oligomeres has driven research into studying compounds that could prevent this aggregation (Table 1) [Cummings, 2008b; Golde, 2005].

The only Aβ aggregation inhibitor reaching phase III is the synthetic glycosaminoglycan 3-amino-1-propaneosulfonic acid (3APS, tramiprosate) [Gauthier *et al.* 2009]. It is designed to interfere with the binding of glycosaminoglycanes and Aβ. Disappointing results of the North American phase III trial in the year 2007 have led to discontinuation of the European phase III trial. Nevertheless, 3APS will now be commercialized as a branded nutraceutical. However, recent data suggest that tramiprosate promotes an abnormal aggregation of the tau protein in neuronal cells [Santa-Maria *et al.* 2007]. These results emphasize the importance of testing the potential drugs for the treatment of AD on both types of pathology (amyloid and tau).

Another molecule undergoing testing is colostrinin, a proline-rich polypeptide complex derived from sheep colostrum (O-CLN; ReGen Therapeutics, London, UK). Colostrinin inhibits Aβ aggregation and neurotoxicity in cellular assays and improves cognitive performance in animal models. Although a phase II trial demonstrated modest improvements in Mini Mental State Evaluation scores for patients with mild AD over a treatment period of 15 months, this beneficial effect was not sustained during an additional 15 months of continued treatment [Bilikiewicz and Gaus, 2004].

Another compound named scyllo-inositol is able to stabilize oligomeric aggregates of Aβ and inhibit Aβ toxicity in mouse hippocampus. An 18-month, randomized, double-blind, placebo-controlled, dose-ranging, safety and efficacy study of oral scyllo-inositol (ELND005) in participants with mild to moderate AD has been carried out by Transition Therapeutics (Toronto, ON, Canada)/Elan (Dublin, Ireland). A long-term follow-up class II study in subjects with AD provided insufficient evidence to support or refute a benefit of ELND005.

Primary clinical efficacy outcomes were not significant. The safety and cerebrospinal fluid (CSF) biomarker results will guide selection of the optimal dose for future studies, which will target earlier stages of AD [Salloway *et al.* 2011].

Drugs interfering with metals Zinc (Zn) and copper (Cu) are both involved in the aggregation of Aβ42. Several chelators of Zn/Cu have been shown to inhibit Aβ aggregation *in vitro* and in animal studies. PBT2 is a second-generation 8-OH quinoline metal-protein-attenuating compound that affects the $Cu2^{+}$-mediated and $Zn2^{+}$-mediated toxic oligomerization of Aβ. A recent phase IIa study concluded that the safety profile is favorable for the ongoing development of PBT2. The effect on putative biomarkers for AD in CSF but not in plasma suggests a central effect of the drug on Aβ metabolism. Cognitive efficacy was restricted to two measures of executive function. In the *post hoc* analysis, the cognitive, blood marker and CSF neurochemistry outcomes from the trial were subjected to further analysis. Ranking the responses to treatment after 12 weeks with placebo, PBT2 50 mg and PBT2 250 mg revealed that the proportions of patients showing improvement were significantly greater in the PBT2 250 mg group than in the placebo group. These findings further encourage larger-scale testing of PBT2 for AD [Faux *et al.* 2010].

Selective Aβ42-lowering agents. Aβ is generated through proteolytic processing of the transmembrane peptide APP. APP can be cleaved by two competing proteases, α secretase and β secretase. Only cleavage by β secretase, followed by γ-secretase cleavage, which in AD is the dominant pathway, will lead to production of Aβ40 and Aβ42. By inhibiting β secretase and γ secretase or by increasing α-secretase cleavage, Aβ production may be reduced [Cummings, 2008a].

β-site AP- cleaving enzyme inhibition The β-secretase enzyme BACE1 is a promising therapeutic target, although the development of a BACE1 inhibitor therapy is problematic for two reasons. First, BACE1 has been found to have important physiological roles. Therefore, inhibition of the enzyme could have toxic consequences. Second, the active site of BACE1 is relatively large, and many of the bulky compounds that are needed to inhibit BACE1 activity are unlikely to cross the blood–brain barrier. Many of compounds able to inhibit BACE are still in the preclinical phase. Inhibitors based on the peptidomimetic strategy suffer from well known difficulties associated with polypeptides, such as blood–brain barrier crossing, poor oral bioavailability and susceptibility to P-glycoprotein transport. Efforts to overcome these problems led to the design of new nonpeptidomimetic *β*-secretase inhibitors that show high selectivity over BACE2 (BACE1/BACE2 selectivity >100) and other human proteases (cathD, pepsin and renin). Their weak or nonpeptidic character favors CNS penetration and oral bioavailability [Silvestri, 2009]. A ligand-based computational approach is currently used to identify the molecular chemical features required for the inhibition of BACE1 enzyme [John *et al.* 2011].

Only a few β-secretase inhibitors have entered clinical trials to date. The first publicly announced phase I clinical trial on a β-secretase inhibitor CTS-21166 was conducted by CoMentis (South San Francisco, USA) [Hey *et al.* 2008; Albert, 2008; Panza, 2009]. Phase I clinical trials on CTS-21166 have been carried out in healthy young men and evaluated for safety and preliminary Aβ responses. In these clinical trials, β-secretase inhibitor has been shown to reduce human plasma Aβ [Hey *et al.* 2008]. Clearly, the hope for the next step would be to develop inhibitors with better pharmaceutical properties and to carry out well designed efficacy trials to determine if they can rescue cognitive decline in patients with AD [Ghosh *et al.* 2012].

γ-*Secretase inhibition* γ Secretase is a nucleoprotein complex with at least four different proteins from which preseniline PS-1 and PS-2 seem to be responsible for the enzymatic action on APP. Unfortunately, besides APP, γ secretase has many other substrates and cleaves several other transmembrane proteins, including the Notch receptor 1, which is necessary for growth and development. Notch-related side effects of γ-secretase inhibition (severe gastrointestinal and hemopoetic side effects) have been hampering the development of clinically useful γ-secretase inhibitors so far [Wong *et al.* 2004].

The most studied γ-secretase inhibitor, which is semagacestat (LY-450139), was shown to dose-dependently decrease the generation of Aβ in the CSF of healthy people [Siemers *et al.* 2005]. Unfortunately, two large phase III clinical trials of semagacestat in patients with mild to moderate AD were prematurely interrupted because of the observation of detrimental effects on cognition and functionality in patients receiving the drug compared with those receiving placebo. These detrimental effects were mainly ascribed to the inhibition of Notch processing and to the accumulation of the neurotoxic precursor of Aβ (the C-terminal fragment of APP or CTFβ) resulting

from the block of the γ-secretase cleavage activity on APP [Imbimbo and Giardina, 2011]. Two large phase III studies in patients with mild AD with tarenflurbil (or R-flurbiprofen), which is a putative γ-secretase modulator, were also completely negative. The failure of tarenflurbil was ascribed to low potency and brain penetration. New Notch-sparing γ-secretase inhibitors and more potent and brain penetrant γ-secretase modulators are being developed with the hope of overcoming the previous setbacks [Imbimbo and Giardina, 2011].

A potent γ-secretase inhibitor, BMS-708163 (avagacestat; Bristol-Myers Squibb, New York, NY, USA), was tested in a phase I clinical trial. After 18 days, BMS-708163 caused a decrease in CSF Aβ40 and Aβ42 of 30% following a daily dose of 100mg as well as a decrease of 60% at a daily dose of 150mg. A phase II study is ongoing [Tong *et al* 2012].

α-Secretase potentiation Etazolate (EHT 0202; ExonHit Therapeutics, Paris, France) stimulates the neurotrophic α-secretase (nonamyloidogenic) pathway and inhibits Aβ-induced neuronal death, providing symptomatic relief and modifying disease progression. The recent pilot, randomized, double-blind, placebo-controlled, parallel group, multicentre, phase IIA study was conducted in 159 randomized patients with mild to moderate AD. EHT0202 was shown to be safe and generally well tolerated. These first encouraging safe results support further development of EHT0202 to assess its clinical efficacy and to confirm its tolerability in a larger cohort of patients with AD and for a longer period of time [Vella *et al.* 2011].

Immunotherapy. Immunotherapy is one of the strategies being studied by most pharmaceutical companies. The mechanism behind amyloid clearance by immunotherapy has not been fully elucidated. At least six mechanisms that are not mutually exclusive are considered to elicit a humoral response: First, by direct disassembly of plaques by conformation-selective antibodies; second, by antibody-induced activation of microglial cells and phagocytosis of pathological protein deposits; third, by noncomplement-mediated phagocytosis activation of microglial cells; fourth, by neutralization of toxic soluble oligomers; fifth, by a shift in equilibrium toward efflux of specific proteins from the brain, creating a peripheral sink by clearance of circulating Aβ cell-mediated immune responses; and finally, immunoglobulin M (IgM)-mediated hydrolysis. All these mechanisms may play roles depending on the specific immunotherapeutic scenario [Wisniewski and Konietzko, 2008] (Figure 2).

Both active immunization (vaccination) and passive immunization (monoclonal antibodies) are being studied. After promising preclinical results in animal studies, one of the first active vaccination trials was initiated using human Aβ1-42 (AN-1792) in conjunction with a T-helper adjuvant (QS-21). Unfortunately, in 2002, the phase II vaccination trial was discontinued because of the occurrence of meningoencephalitis (6%) [Gilman *et al.* 2005]. Additionally, only 19.7% of the AN-1792-treated patients developed the predetermined antibody response.

Double-blind assessment was maintained for 12 months, demonstrating no significant differences in cognition between antibody responders and the placebo group. In a small subset of patients, CSF tau levels were decreased in antibody responders but Aβ levels were unchanged [Gilman *et al.* 2005]. 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 of AN1792-vaccinated patients or transgenic models, and of brain tissue taken from mice and humans using a new tissue amyloid immunoreactive method suggested that antibodies against Aβ-related epitopes are capable of slowing down the progression of neuropathology in AD. In a recent 4-year study, Hock and Nitsch followed 30 patients who received a primary and booster immunization in the first year after vaccination, providing further support for continuation of the investigation of antibody treatment in AD [Hock and Nitsch, 2005].

The occurrence of encephalitis led to the development of new vaccines, which lack the amino acid parts thought to be responsible for the T-cell response mediated encephalitis, but retain the residues (4–10) required for antibodies to bind to Aβ. Most of these vaccines are now being tested in phase I and II trials: the CAD-106 trial led by Novartis/Cytos (Basel, Switzerland) and the V950 trial initiated by Merck (Whitehouse Station, NJ, USA) [Brody and Holtzman, 2008]. Additional antibodies under testing include ACC-001 (Wyeth, New Jersey, USA, two phase II studies ongoing in the USA and Japan), MABT5102A (Genentech, San Francisco,

California, USA, phase I completed), PF-04360365 (Pfizer, phase I completed), R1450 (Hoffman-LaRoche, Basel, Switzerland, phase I completed), GSK933776A (GlaxoSmithKline, London, UK, phase I completed) [ClinicalTrials.gov; Galimberti and Scarpini, 2011].

Given the adverse reactions of the active immunization and the variable antibody response to vaccines in older individuals, passive immunization directed against various domains of Aβ emerged as an alternative immunotherapeutic strategy. A point of concern in these therapies is the occurrence of cerebral microhemorrhages. The underlying mechanism is probably related to vascular amyloid deposits (congophilic amyloid angiopathy), present in nearly all patients with AD. The need for vascular repair and regeneration during Aβ immunotherapy is another argument for early treatment and subtle clearance over a long period of time [Wilcock *et al.* 2007].

More advanced is the Elan/Wyeth trial of AAB-001 monoclonal antibody (bapineuzumab), which entered phase III testing in 2007. This approach involves passive immunization with an Aβ N-terminal directed, humanized monoclonal antibody. The murine version of this antibody binds to both soluble and aggregated Aβ. The multiple-dose phase II trial including 240 participants did not attain statistical significance on the primary efficacy endpoints in the whole study population. Some patients in the treatment group had a vasogenic edema, which is a serious side effect. However, in the subgroup of participants who did not have the apolipoprotein E (ApeE) ε4 allele, clinically significant benefits were recorded in several scales and magnetic resonance imaging showed smaller loss of brain volume. Looking at the best result of different groupings, it seemed that a small subset of patients, the ApoE noncarriers who received the second lowest of the four doses six times, responded really well in 78 weeks. Therefore the phase III study was initiated in ApoE4 noncarriers with mild to moderate AD [Wisniewski and Konietzko, 2008].

The next most advanced trial to our knowledge is the Eli Lilly and Co. (Indianapolis, IN, USA) phase II trial of LY2062430 (solanezumab), which involves passive vaccination with an Aβ central domain directed, humanized monoclonal antibody. Systemic administration of the closely related central domain mouse monoclonal antibody m266 rapidly improved behavioral performance and decreased plaque formation in preclinical studies. However, this antibody did not worsen intracerebral hemorrhage or vascular pathology in older APP transgenic mice. The phase II results have been reported and no safety concerns were raised; a phase III study is being conducted [Brody and Holtzman, 2008].

Finally, natural antiamyloid antibodies have been found in human intravenous immunoglobulins (IVIgs) obtained from the pooled plasma of healthy blood donors. In light of these observations, a phase I trial has been carried out in the USA. Eight patients with AD were treated with IVIg (Gammagard S/D immune globulin intravenous human) donated by Baxter Healthcare Corporation (Deerfield, IL, USA). Seven patients completed the study. After 6 months, cognitive function stopped declining in all seven patients and improved in six. In 2009, a phase III clinical trial involving more than 360 patients with AD was initiated and may provide conclusive evidence for the effect of IVIg as a treatment option for AD [Dodel *et al.* 2010].

Passive vaccination requires repeated infusions, which have a high cost. Therefore active vaccination is always taken into consideration.

Data from preclinical studies regarding mice suggest that novel immunotherapeutic strategies like DNA epitope vaccine [Qu *et al.* 2010], antibodies against the β-secretase cleavage site of the APP [Rakover *et al.* 2007] and mucosal vaccination [Hara *et al.* 2011] could be used as safe and effective methods for AD therapy. DNA epitope vaccines have received substantial interest because of the ease of selectively designing them to elicit specific immune responses. Mucosal vaccination is an alternative way to achieve humoral response. Its mechanism is based on the presence of lymphocytes in the mucosa of the nasal cavity and gastrointestinal tract. It produces primarily secretory IgA antibodies, but when the antigen is coadministered with adjuvants such as cholera toxin subunit B and heat labile *Escherichia coli* enterotoxin, substantial serum IgM titers can be achieved. It has a more limited humoral response with little or no cell-mediated immunity. The last developed mucosal immunotherapy for AD by nasal administration used a recombinant Sendai virus vector carrying Aβ1-43 and mouse interleukin-10 cDNA. It induced good antibody responses to Aβ. When APP transgenic mice (Tg2576) received this vaccine once nasally, the Aβ plaque

Table 2. Disease-modifying treatments: modulation of tau deposition.

Interfering with tau deposition	Interfering with tau phosphorylation	Immunotherapy
Methylene blue: phase II trial (+)	Tau kinase inhibitors (lithium: phase I trial (–) in AD (+) in MCI)	Vaccination: preclinical trials

+, encouraging results; –, disappointing results.
AD, Alzheimer's disease; MCI, mild cognitive impairment.1

burden was significantly decreased 8 weeks later, without inducing inflammation in the brain. Tg2576 mice showed significant improvement in cognitive functions when examined 3 months after the vaccination [Hara *et al.* 2011].

Disease-modifying treatments: modulation of tau deposition

Drugs interfering with tau deposition. Multiple compounds have been identified through cell culture or *in vitro* screens as tau aggregation inhibitors (Table 2). A phenothiazine, methylene blue (MB) or methylthioninium chloride, has previously been used in humans and is currently being evaluated in AD trials. The problem with this drug is that urine is colored blue, resulting in a lack of blinding. However, promising results have emerged from a phase II clinical trial testing MB as a potential therapy for AD, as improvements in cognitive function of patients with AD after 6 months of MB administration have been reported [Gura, 2008].

Drugs interfering with tau phosphorylation. The intriguing link between phosphorylation and tau pathology has provided the boost to examine the role of kinase inhibitors as potential therapeutics targeting tau. Kinases induce the hyperphosphorylation of tau [Yiannopoulou *et al.* 2009]. Despite the large number of tau phosphorylation sites and the ability of multiple kinases to phosporylate individual sites, glycogen synthase kinase 3 (GSK3β) has emerged as a potential therapeutic target. The most studied compound able to inhibit GSK3 is lithium, but several other compounds are under development, including pyrazolopyrazines, pyrazolopyridines, the aminothiazole AR-A014418, and sodium valproate [Martinez and Perez, 2008]. In recent studies, the effect of short-term treatment on cognitive and biological outcomes in people with amnestic MCI was shown and supports the notion that lithium has disease-modifying elements with potential clinical implications in the prevention of AD [Forlenza *et al.* 2011].

Immunotherapy. Vaccination approaches targeting tau have been considered, but the development of a successful therapy is complicated because tau protein is intracellular [Galimberti and Scarpini, 2011].

Disease-modifying treatments: modulation of inflammation and oxidative damage

Anti-inflammatory drugs. Epidemiological evidence suggests that long-term use of NSAIDs protects against the development of AD. Despite this premise, prospective studies showed lack of efficacy [Aisen *et al.* 2002, 2003] or treatment-limiting gastrointestinal toxicity [Rogers *et al.* 1993].

Molecules addressing oxidative damage. Potential antioxidants include mitoquinone, vitamin E, Ginkgo biloba, natural polyphenols such as green tea, wine, blueberries and curcumin, ω3 fatty acids, folate, vitamin B6 and vitamin B12 supplementation. A trial to determine whether the 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 had no beneficial effect on the primary cognitive measure, the rate of change in ADAS-cog score over 18 months, or on any secondary measures, although the vitamin supplement regimen was effective in reducing homocysteine levels [Aisen *et al.* 2006]. Clinical trials with vitamin E and ω3 fatty acids did not show beneficial effects in patients with AD [Barten and Albright, 2008].

More recent data have revealed that tumor necrosis factor (TNF), one of the few gliotransmitters, has strikingly acute effects on synaptic physiology. These complex influences on neural health suggest that manipulation of this cytokine might have

important impacts on diseases characterized by glial activation, cytokine-mediated neuroinflammation and synaptic dysfunction. Toward such manipulation in AD, a 6-month study was conducted with 15 patients with probable AD who were treated weekly with perispinal injection of etanercept, an FDA-approved TNF inhibitor that is now widely used for the treatment of rheumatoid arthritis and other systemic diseases associated with inflammation. The results demonstrated that perispinal administration of etanercept could provide sustained improvement in cognitive function for patients with AD. Additionally, the authors were impressed by the striking rapidity with which these improvements occurred in the study patients. Nevertheless, etanercept merits further study in RCTs [Griffin, 2008].

Disease-modifying treatments: additional approaches

Modulation of cholesterol and vascular-related risk factors. A link between hypercholesterolemia, cardiovascular diseases and AD has also been suggested. Additional vascular-related risk factors for AD include hypertension, atrial fibrillation, hyperhomocysteinemia, atherosclerosis and stroke [Hooijmans and Kiliaan, 2008]. Epidemiological studies have indicated that patients treated for cardiovascular disease with cholesterol-lowering therapy (statins) showed a decreased prevalence of AD [Jick *et al.* 2000]. The Lipitor's Effect in Alzheimer's Dementia (LEADe) study tested the hypothesis that a statin (atorvastatin 80mg daily) is beneficial to patients with mild to moderate AD receiving background therapy of donepezil 10mg daily. Despite a promising premise, there were no significant differences in the coprimary or secondary endpoints, although atorvastatin was generally well tolerated [Feldman *et al.* 2010].

Simvastatin metabolites are high-affinity 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase inhibitors, reducing the quantity of mevalonic acid, a precursor of cholesterol. Cholesterol Lowering Agent (simvastatin) to Slow Progression (CLASP) of Alzheimer's Disease Study is an ongoing randomized, double-blind, placebo-controlled, parallel-assignment phase III trial that investigates the safety and effectiveness of simvastatin in slowing down the progression of AD. It has not yet published its results [McGuinness *et al.* 2010]. However, a randomized, double-blind, placebo-controlled recent trial of simvastatin was conducted in individuals with mild to moderate AD and normal lipid levels. Simvastatin had no benefit on the progression of symptoms in individuals with mild to moderate AD despite significant lowering of cholesterol [Sano *et al.* 2011].

Final remarks

Currently available treatments for AD (donepezil, rivastigmine, galantamine and memantine) are symptomatic and do not decelerate or prevent the progression of the disease. However, these therapies demonstrate modest, but particularly consistent, benefit for cognition, global status and functional ability [Herrmann *et al.* 2011].

The search for disease-modifying interventions has focused largely on compounds targeting the Aβ pathway. To date, many treatments targeting this pathway, such as tarenflurbil, tramiprosate and semagacestat, have been unsuccessful in demonstrating efficacy in the final clinical stages of testing [Gauthier *et al.* 2009; Imbimbo and Giardina, 2011].

However, colostrinin, scyllo-inositol, PBT2, avagacestat, etazolate and active and passive immunization methods, treatments also targeting the Aβ pathway, are being tested in advanced clinical trials.

At the same time, other possible neuronal mechanisms that seem to play important roles in the pathophysiology of this multifactorial disorder, such as tau deposition and hyperphosphorylation, neuroinflammation and oxidative stress, are being researched as promising therapeutic targets. Clinical trials with drugs interfering with tau deposition or phosphorylation (lithium) are ongoing [Martinez and Perez, 2008]. Clinical trials of potential antioxidants such as vitamin E and ω3 fatty acids did not show beneficial effects in patients with AD [Barten and Albright, 2008].

Etanercept, a TNF inhibitor that is now widely used for the treatment of systemic diseases associated with inflammation, provided sustained improvement in cognitive function in patients with mild to severe AD after perispinal administration in a 6-month, open-label pilot study. However, etanercept merits further study in RCTs [Griffin, 2008].

Modulation of cholesterol and vascular-related risk factors is an additional possible disease-modifying approach. CLASP is an ongoing phase III trial

investigating the effectiveness of simvastatin in slowing down the progression of AD [McGuinness *et al.* 2010].

The development of disease-modifying drugs for AD is recognized as a worldwide necessity. These must presumably be drugs that will modify, either by stabilizing or slowing, the molecular pathological steps leading to neurodegeneration and finally dementia.

The required design of clinical trials to test this concept raises many questions regarding the study populations, the duration of trials, the necessary primary and secondary endpoints, including biomarkers [Vellas *et al*. 2007]. It has been recognized that, to modify AD, which has recently been redefined to have presymptomatic and symptomatic phases, one must attempt to treat patients when neuronal dysfunction is far from full blown and largely irreversible [Dubois *et al*. 2010; Sperling *et al*. 2011].

The following considerations have emerged that should be taken into account when planning future clinical trials:

(1) The mechanisms underlying the pathogenesis of AD need to be thoroughly investigated before focusing on the development of novel disease-modifying compounds. Despite promising premises related to different pathogenic mechanisms, large phase III trials with potentially disease-modifying properties have failed to demonstrate any effect on cognition. It is of crucial importance to better understand the relationship between tau, Aβ and other factors to develop successful disease-modifying drugs [Galimberti and Scarpini, 2011].

(2) Treatments of AD appear effective only in certain phases of the disease. A few disease-modifying compounds have shown some benefits in mild but not moderate AD or even in MCI. Therapeutic trials should therefore be carried out as early as possible during the course of the disease, which requires the identification of more accurate tools for early diagnosis. New criteria for the diagnosis of AD have enlarged the window for the detection of the early stages of the disease and include biomarkers mechanistically related to AD pathology. Adoption of these early biomarkers in implementing design of future studies is highly desirable [Galimberti and Scarpini, 2011; Salomone *et al*. 2011].

(3) AD is heterogeneous in clinical presentation, underlying neuropathology and mixed causes (especially in late-onset AD). This fact is one more reason to improve our tools for detecting patients with amnestic MCI at high risk of converting to AD before the different full-blown clinical features of the disease appear. A major challenge will also be to identify subgroups with homogeneous biomarkers. At present, the focus in AD drug development is shifting from treatment to prevention [Salomone *et al*. 2011; Vellas *et al*. 2011].

(4) Indicators useful as surrogate outcome measures (surrogate biomarkers like magnetic resonance imaging, CSF tau and Aβ, and amyloid positron emission tomography) should be identified to have substitutes for clinical endpoints (i.e. neuropsychological testing), tools able to predict clinical benefit or the opposite, and to demonstrate whether the drug has disease-modifying properties [Galimberti and Scarpini, 2011].

(5) Prolonged development times delay effective therapies from reaching patients in need. Several strategies are promising for answering the crucial question of, 'How much information is sufficient to proceed to phase III without excessive risk for failure?' Phase II proof of concept (POC) (IIa) and dose-finding (IIb) studies represent major challenges in drug development. Biomarkers, population enrichment with risk factors, clinical measures with greater sensitivity than standard trial instruments and adaptive dose–response designs might represent other strategies applicable to POC studies. All of these strategies are being considered as means of shortening phase IIb studies and creating a seamless interface with phase III. None of these strategies have been validated in a successful drug development program [Cummings, 2008b].

In conclusion, the new strategies seem to focus on examining the potential neuroprotective activity of disease-modifying drugs in the presymptomatic stages of AD, with the help of biomarkers that predict disease progression before development of overt dementia.

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