**New treatment strategies for Alzheimer’s disease: is there a hope?**

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Alzheimer’s disease (AD) is a progressive and irreversible neurodegenerative disease, and corresponds to the most common cause of dementia worldwide. Although not fully understood, the pathophysiology of AD is largely represented by the neurotoxic events triggered by the beta-amyloid cascade and by cytoskeletal abnormalities subsequent to the hyperphosphorylation of microtubule-associated Tau protein in neurons. These processes lead respectively to the formation of neuritic plaques and neurofibrillary tangles, which are the pathological hallmarks of the disease. Clinical benefits of the available pharmacological treatment for AD with antidementia drugs (namely cholinesterase inhibitors and memantine) are unquestionable, although limited to a temporary, symptomatic support to cognitive and related functions. Over the past decade, substantial funding and research have been dedicated to the search and development of new pharmaceutical compounds with disease-modifying properties. The rationale of such approach is that by tackling key pathological processes in AD it may be possible to attenuate or even change its natural history. In the present review, we summarize the available evidence on the new therapeutic approaches that target amyloid and Tau pathology in AD, focusing on pharmaceutical compounds undergoing phase 2 and phase 3 randomized controlled trials.

**Key words** Alzheimer’s disease - antidementia drug - beta-amyloid - cognitive impairment - Tau - treatment

**Introduction**

Alzheimer’s disease (AD) is the leading cause of dementia worldwide, affecting more than half of the overall number of demented individuals, which has been estimated to be around 24 million across all nations1. The prevalence of dementia is expected to further increase in the forthcoming decades, as a consequence of the steady growth of ageing population in both developed and developing countries. According to World Health Organization projections, about three-quarters of the estimated 1.2 billion elders will be living in low- and middle-income countries by the year 20252. Age-adjusted estimates of dementia prevalence are high (above 5%) in most Asian and Latin American Countries3. However, prevalence rates of dementia seem to be lower (1-3%) in India and sub-Saharan Africa3.

Epidemiological studies conducted in India between 1996 and 2006 indicated that dementia affects 2.7% of the population, AD being the most common cause (1.3%)3. It is noteworthy that these numbers parallel the estimates of dementia and AD in Western societies cut by half; nonetheless the proportion of cases of AD amongst dementia is basically the same.
Lower rates of dementia in India might be interpreted as related to environmental and biological factors such as dietary habits, lifestyle, sociocultural, cardiovascular, and genetic. In a cross-national epidemiological study addressing the association between the apolipoprotein E genotype and the diagnosis of AD, Ganguli et al. indicated that the frequency of the APOE*E4 allele, an important genetic risk factor for late-onset AD, was lower (7%) in northern India as compared to a North-American cohort (11%); interestingly, when the study group was subdivided according to the presence of this risk allele, the strength of association between APOE*E4 and the diagnosis AD was similar both in Indian and US samples. Methodological reasons must also be considered as a possible source of bias in the previous estimates of dementia prevalence in India, such as non-recognition of cases due to limited access of patients to specialized services in remote regions, selective removal of cases due to early mortality, and culturally-related difficulties to ascertain the diagnosis of cognitive impairment. A recent epidemiological study indicated that the prevalence of dementia and AD in south India (6.44 and 3.56%, respectively) was actually higher than previously reported. Additionally, an autopsy-based study demonstrated that the prevalence of AD pathology in India was similar to that observed in developed countries.

Studies have also suggested dissimilarities in the natural course of the disease in India as compared to western countries. As part of the Indo-US study, Chandra et al. reported a lower median survival time after the onset of symptoms of patients with AD in India, as compared to developed countries. Potential causes of this shorter life-expectancy of AD patients are complex and may be secondary to ineffective public health assistance evolving dementia care, but other social, environmental and biological factors may be associated to a more rapid course of the illness. AD patients in India may have significantly more delusions, hallucinations, anxiety and mood symptoms during the course of dementia. With respect to the prodromal stages of AD, a cross-sectional study in India reported a 15 per cent prevalence of mild cognitive impairment (MCI) among individuals aged 50 yr and older, multiple-domain MCI being the most prevalent subtype (8.9%). These findings are similar to those derived from studies performed in developed countries, and were shown to be significantly associated with increasing age, hypertension, and diabetes mellitus. Therefore, the interaction between underlying clinical risk factors and primary degenerative mechanisms in the ageing population is likely to boost the incidence of dementia in India in the near future.

The so-called “AD-epidemic” will inevitably represent a major public health problem to most nations, because to date there is no effective approach in terms of cure or prevention of dementia. The available pharmacological therapy with antidementia drugs is largely symptomatic, with temporary clinical benefits on cognitive, functional and behavioural manifestations of the disease. This strategy relies on the increment of synaptic availability of acetylcholine, given the cholinergic deficit that arises from neuronal loss in basal forebrain nuclei and cortical projections. This effect is achieved by inhibition of acetyl-cholinesterase by donepezil, galantamine or rivastigmine. Memantine is a voltage-dependent N-methyl D-aspartate (NMDA) receptor antagonist that precludes the excessive release of glutamate as a consequence of nerve cell damage in the cortex. Therefore, the clinical use of cholinesterase inhibitors is indicated for patients with mild, moderate and even severe AD; moderate and severe cases can also be treated with memantine, preferably in combination with the former agents.

In this context, there is an urgent need to develop new drugs with disease-modifying properties for AD. By definition, a disease-modifying drug is a pharmaceutical agent intended to slow the progression of the neurodegenerative process by inhibiting critical events in the pathophysiology of the disease, and therefore, attenuating the pathological load. Allegedly, patients treated with such agents would be expected to have a more benign course of the disease when compared to placebo-treated individuals (Fig. 1). Many candidate drugs with distinct pharmacological mechanisms have been proposed in the recent years and tested in neurobiological models of AD. However, the promising effects that had been attributed to some of these compounds in animal models failed to prove beneficial to humans in early clinical trials. In addition, other drugs that underwent clinical experimentation delivered negative results, either due to limited efficacy or toxicity, when tested by randomized-controlled trials (RCTs). In the present review, we summarize the available results of candidate treatments for AD targeting the pathological cascades related to beta-amyloid and Tau, and focusing on pharmaceutical compounds that have been tested in phase 2 and phase 3 RCTs.
Pathological mechanisms in AD

The “amyloid hypothesis” is a prevailing pathological model of AD, accepted by most researchers. According to this model, the overproduction and accumulation of amyloid-beta (Aβ) peptides in plaques represent an early and central event in the pathophysiology of AD leading to the formation of neuritic plaques, whereas the cytoskeletal changes that arise from the hyperphosphorylation and intracellular aggregation of microtubule-associated protein Tau to form neurofibrillary tangles are regarded as downstream phenomena. These changes evolve in a non-linear and dynamic way, under the effect of other predisposing factors. Both pathological cascades impair neuronal homeostasis and eventually result in cell death and neurodegeneration. These changes manifest clinically with slowly progressive cognitive decline (evolving from mild cognitive impairment to dementia), behavioural symptoms and functional impairment\textsuperscript{12-14}. Amyloid-β is originated by the alternative (possibly abnormal) cleavage of the amyloid precursor protein (APP) into smaller peptides (Aβ\textsubscript{1-40} and Aβ\textsubscript{1-42}), depending on the balance between the activity of major APP cleaving enzymes, \textit{i.e.}, α-secretase (non-amyloidogenic) and β- and γ-secretases (amyloidogenic). In AD, the abnormal regulation of APP secretases, leading to the predominant activation of β- and γ-pathways, is associated with the overproduction of toxic amyloid species. Aβ oligomers (small aggregates of 2 to 12 peptides) and amyloid plaques exert neurotoxic effects; Aβ\textsubscript{1-42} is more prone to form insoluble aggregates (and therefore more toxic) than Aβ\textsubscript{1-40}. The interaction between genetic and environmental factors, along with the homeostatic changes that pertain to the ageing process \textit{per se}, seems to affect the balance between production and clearance of toxic Aβ peptides\textsuperscript{14}.

Neurofibrillary tangles (NFTs), another pathological hallmark of the disease, are largely constituted by intracellular aggregates of paired helical filaments (PHFs), which arise from the collapse of the neuronal cytoskeleton. The structure and function of microtubules are impaired as a consequence of the abnormal hyperphosphorylation of Tau protein, which precludes its ability to stabilize the monomers of alpha- and beta-tubulin\textsuperscript{14}. Hyperphosphorylated Tau aggregates into oligomers to form PHFs to further originate NFTs\textsuperscript{15}. Several protein kinases are involved in this process, namely glycogen synthase kinase-3 beta (GSK3β), cyclin-dependent kinase-5 (CDK5), and extracellular signal-related kinase-2 (ERK2)\textsuperscript{16}; these enzymes may also be regarded as potential targets for disease-modification, upon their inhibition by specific drugs. GSK-3β, the most important Tau kinase in neurons, is overactive in AD and its overexpression has

\begin{figure}[h]
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\includegraphics[width=\textwidth]{Fig_1.png}
\caption{Natural history of AD with treatment possibilities.}
\end{figure}
been shown to hyperphosphorylate Tau in transgenic mouse models of AD\textsuperscript{17,18}. The inhibition of GSK-3β not only precludes the hyperphosphorylation of Tau, but also yields the dephosphorylation of its abnormally hyperphosphorylated epitopes by the action of protein phosphatases. Therefore, the interruption and reversal of this process may restore microtubular structure and function\textsuperscript{18}. Moreover, GSK-3 inhibition downregulates the amyloidogenic cleavage of APP, which provides further evidence of the cross-talk between these two major pathological cascades in AD\textsuperscript{19}.

**Therapeutic targets for disease modification in AD**

The recognition of core and secondary pathophysiological mechanisms in AD has led to the identification of molecular targets for the development of specific drugs. In fact, more than 200 pharmaceutical compounds are currently undergoing phase 2 and 3 trials\textsuperscript{11}. These compounds can be grossly divided into anti-amyloid agents and drugs that target other pathological pathways. Anti-amyloid compounds can be subdivided into drugs designed to block or inhibit the overproduction or aggregation of Aβ, or to favour its clearance from the brain (Table I; Fig. 2), whereas the latter group can be subdivided according to predominant mechanism of action of the drug, i.e., neurotransmitter and cell-signalling agents, glial cell modulators, neuroprotective agents, and Tau-based therapies (Table II). Studies involving stem-cell and gene therapy are also under way, but at more incipient stages of experimental validation.

In AD, pathophysiological mechanisms change soluble Aβ peptides into fibrillar oligomers and insoluble fibrils, which accumulate extracellularly in the neural tissue and also in the intima of brain and systemic vessels\textsuperscript{34}. Extracellular Aβ oligomers and fibrillar forms cause synaptic dysfunction, affect axons and dendritic spines, and eventually lead to neuronal loss in AD\textsuperscript{35}. Toxic Aβ species also trigger secondary pathological mechanisms (e.g., oxidative stress and inflammation), which hasten neuronal dysfunction and death\textsuperscript{36}. Therefore, pharmacological compounds that favour the clearance of Aβ from the brain, or prevent its aggregation, may represent a strategy to delay the progression of the pathological process in AD. Intracerebral amyloidosis may start in the brain of individuals with AD many years before the onset of clinical symptoms\textsuperscript{37,38}. Evidence of this pathological process can be depicted at prodromal

<table>
<thead>
<tr>
<th>Compound</th>
<th>Target/treatment</th>
<th>Current phase</th>
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<tbody>
<tr>
<td>ANI 1792</td>
<td>Vaccine – active immunization</td>
<td>Interrupted at phase I (severe side effects as meningoencephalitis)</td>
</tr>
<tr>
<td>CAD106\textsuperscript{24}</td>
<td>Vaccine – active immunization</td>
<td>Phase I (ongoing)</td>
</tr>
<tr>
<td>Bapineuzumab\textsuperscript{25}</td>
<td>Beta-amyloid monoclonal antibody</td>
<td>Phase III (ongoing)</td>
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<tr>
<td>Solanezumab\textsuperscript{23}</td>
<td>Beta-amyloid monoclonal antibody</td>
<td>Phase III (ongoing)</td>
</tr>
<tr>
<td>Ponezumab\textsuperscript{26}</td>
<td>Beta-amyloid monoclonal antibody</td>
<td>Interrupted at phase II (no efficacy)</td>
</tr>
<tr>
<td>Gantenerzumab\textsuperscript{27}</td>
<td>Beta-amyloid monoclonal antibody</td>
<td>Phase I (ongoing)</td>
</tr>
<tr>
<td>Crenezumab\textsuperscript{27}</td>
<td>Beta-amyloid monoclonal antibody</td>
<td>Phase I (ongoing)</td>
</tr>
<tr>
<td>Semagacestat\textsuperscript{28}</td>
<td>Gamma-secretase inhibitor</td>
<td>Interrupted at phase III (no efficacy and risk for skin cancer)</td>
</tr>
<tr>
<td>Avagacestat\textsuperscript{28}</td>
<td>Gamma-secretase inhibitor</td>
<td>Phase II (ongoing)</td>
</tr>
<tr>
<td>GRL-834\textsuperscript{29}</td>
<td>Beta-secretase inhibitor</td>
<td>Ongoing</td>
</tr>
<tr>
<td>TAK-070\textsuperscript{30}</td>
<td>Beta-secretase inhibitor</td>
<td>Ongoing</td>
</tr>
<tr>
<td>CHF5074\textsuperscript{31}</td>
<td>Nonsteroid antinflammatory agent</td>
<td>Ongoing</td>
</tr>
<tr>
<td>DAPT\textsuperscript{31}</td>
<td>Prototypal gamma-secretase inhibitor</td>
<td>Ongoing</td>
</tr>
<tr>
<td>Curcumin\textsuperscript{22}</td>
<td>Anti-amyloid aggregator</td>
<td>Ongoing</td>
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DAPT, [N-(3, 5-Difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester
or even at preclinical stages of the disease by the analysis of cerebrospinal fluid (CSF) and molecular neuroimaging biomarkers. Concentrations of soluble Aβ in the CSF, which are known to be reduced in patients with MCI who convert to dementia, have been inversely correlated to the rate of cognitive decline and to the severity of neurodegeneration. Although molecular imaging with PET provides measurement of pathological deposition of extracellular fibrillar Aβ, which brings plaques into the brain, there is no definitive neuroimaging confirmation for synaptic dysfunction with currently available tracers.

**Anti-amyloid therapy**

Anti-amyloid strategies comprise pharmaceutical compounds with distinct mechanisms of action, namely drugs that (i) facilitate the clearance; (ii) inhibit the production; or (iii) prevent the aggregation of Aβ. As shown in Table I, many pharmacological compounds have been developed to tackle the “amyloid cascade”, with the prospect of reducing the Aβ burden in the brain of mild to moderately demented AD patients. However, there is evidence that these interventions must be implemented at earlier stages of the disease process, i.e., at the stage of incipient dementia or prior to the conversion from mild cognitive impairment to dementia (in the MCI-AD continuum), or even earlier. It is accepted that two thirds of individuals with MCI may have intracerebral amyloid burden comparable with those with clinically manifested AD, indicating that intracerebral amyloidosis is an early event in the natural history of the disease.

Both active and passive immunization target the reduction of intracerebral Aβ load by eliciting humoral response against the Aβ peptide, facilitating its clearance from the brain by immune-mediated mechanisms. Highly encouraging findings were presented by preclinical studies with transgenic mice with high Aβ load, submitted to active and passive immunization; these strategies proved effective reducing the amount of Aβ in the mouse brain, which was supposedly associated with improvements on behaviour and cognition.
These studies have provided the rationale for the first generation of immunotherapeutic agents for AD, which was based on the active immunization of AD patients with the actual Aβ peptide. Therefore, this strategy induces an IgM response to generate antibodies against pathogenic Aβ, which further mobilize microglia to clean plaques through phagocytosis. Also, the immune response prevents Aβ deposition by removing the excess of soluble Aβ forms from the circulation. Phase 2A studies with the active anti-Aβ vaccine (AN 1792) proved efficacious to remove Aβ plaques from the brain of AD patients; however, the trial was interrupted due to the occurrence of severe adverse events in a substantial proportion of subjects (6%) who received this intervention, which was associated with the induction of a cytotoxic T-cell reaction in brain vessels leading to acute meningoencephalitis. Interim analyses indicated that although the immunization promoted Aβ clearance, it was not associated with clinically relevant benefits, i.e., the immunological response was not associated with the successfully completed clinical trials.

The second-generation of active anti-Aβ immunotherapeutic agents was designed to minimize the risk of eliciting such secondary inflammatory responses or vasogenic oedema, by stimulating soluble Aβ derivative immunogens. These vaccines elicit the immune response to raise antibodies against Aβ monomers and oligomers. Studies with the vaccine CAD106 at phase 1 indicated that it was able to reduce Aβ accumulation in cortical and subcortical brain regions by binding to Aβ aggregates and

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**Table II. Overall pharmacologic treatments other than anti-amyloid therapy under research for Alzheimer’s disease**

<table>
<thead>
<tr>
<th>Neurotransmitter-based</th>
<th>Agents under research</th>
</tr>
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<tbody>
<tr>
<td>Acetylcholine</td>
<td>ST 101, AF 267B, ABT 089, AZD 3480, MEM 3454, EVP-6124, Posiphen, Huperzine</td>
</tr>
<tr>
<td>Serotonin</td>
<td>5-HT4 partial agonists, 5-HT1A agonists/antagonists, 5-HT6 antagonists</td>
</tr>
<tr>
<td>Norepinephrine/Dopamine</td>
<td>MAO A and MAO B inhibitors</td>
</tr>
<tr>
<td>GABA</td>
<td>GABA&lt;sub&gt;γ&lt;/sub&gt; antagonists</td>
</tr>
<tr>
<td>Glutamate</td>
<td>AMPA potentiators</td>
</tr>
<tr>
<td>Glycine</td>
<td>Partial agonists</td>
</tr>
</tbody>
</table>

**Glial modulating drugs**

| Direct glial target | G and GM CSF, Nitrofuribipofen, ONO-2506, Tacrolimus |
| RAGE receptor antagonists | TTP 488 |
| TNF alpha antagonists | Enbrel |

**Neuroprotection**

<table>
<thead>
<tr>
<th>Anti-tau or tau modulators</th>
</tr>
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<tbody>
<tr>
<td>GABA, gamma-aminobutyric acid; RAGE, receptor for advanced glycation endproducts; TNF, tumor necrosis factor; GSK, glycogen synthase kinase; CDK, cyclin-dependent kinase; 5-HT (4, 1A and 6), 5-hydroxytryptamine (receptor subtypes); MAO (A and B), monoamine oxidase (A and B subtypes); AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; PPAR, peroxisome proliferator-activated receptors; SIRT1, sirtuin (silent mating type information regulation 2 homolog)-1; BDNF, brain-derived neurotrophic factor; NGF, nerve growth factor; NAP, neuronal microtubule-interacting agent (a peptide of eight amino acids, NAPVSIPQ)</td>
</tr>
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blocking cellular toxicity, with no evidence of microhaemorrhage, vasogenic oedema, or inflammatory reactions subsequent to activation of T-cells\textsuperscript{28}.

Conversely, passive immunotherapy is based on the intravenous administration of full monoclonal antibodies or antibody fragments from specific exogenous origins, which directly target Aβ\textsubscript{1-42}. This strategy is supposedly not associated with a significant risk of eliciting T-cell inflammatory reactions\textsuperscript{45}. In passive immunization, the binding of antibodies to specific Aβ epitopes induces plaque clearance through microglial activation\textsuperscript{48}. In transgenic mice, peripheral infusion of antibodies raised specifically against the Aβ peptide was shown to reduce brain amyloid load\textsuperscript{27}. As occurred with active immunotherapy, successful preclinical studies with passive immunotherapy were followed by phase 1 and phase 2 trials for AD. Afterward, advantageous results with transgenic animal models encouraged several centers to design clinical trials in order to establish effective treatments for AD patients and to change the disease course. Currently, some passive immunotherapies are being developed. A challengeable aspect concerns the efficacy against pathophysiology of the disease with avoidance of undesired side effects such as microhaemorrhage, vasogenic oedema, encephalitis, or neuroimaging abnormalities\textsuperscript{27}. Across the screening cohorts enrolling placebo-treated patients, rarely occurrences of brain areas of microhaemorrhage have been described\textsuperscript{49,50}. Conversely, another group reported vasogenic oedema with this drug\textsuperscript{28}.

Several passive immunotherapeutic agents have been evaluated by RCTs over the past years, namely bapineuzumab, solanezumab, gantenerumab, ponezumab, and crenezumab. These monoclonal antibodies have high affinity to antigenic determinant epitopes of Aβ, binding either to soluble forms or in plaques, being further recognized by B- and T-cells to promote its clearance from the brain\textsuperscript{27,48}. In addition, monoclonal antibodies may delay amyloid-β burden or stop its accumulation in the brain\textsuperscript{27,48}. Bapinezumab represents the humanized Aβ monoclonal antibody that has been most tested as a candidate drug for the treatment of AD, and is currently undergoing phase 3 studies. In phase 2 studies, this compound was examined in a randomized placebo controlled trial to determine dose, safety, and efficacy in 234 patients with mild to moderate AD. Patients were given six infusions, in 13 distinct weeks, with last evaluation at week 78\textsuperscript{25}. Ascending doses of bapinezumab were shown to reduce intracerebral Aβ load through increased clearance from the brain; however, this effect was not associated with a significant benefit in clinical outcomes. Reversible and asymptomatic or transient vasogenic oedema was detected in 9.7 per cent of immunized patients, being more frequent in those receiving higher doses and in APOE*4 carriers\textsuperscript{25}. Recently, during The European Federation of Neurological Societies annual meeting, in Stockholm, Sweden (2012), researchers reported that bapinezumab failed to protect against cognitive and functional decline of AD patients undergoing a phase 3 trial\textsuperscript{23}.

Solanezumab is another humanized monoclonal antibody that binds to the mid domain of soluble forms of Aβ peptide. Preliminary results indicated that it was effective promoting Aβ clearance from the brain; however, two placebo-controlled phase 3 trials failed to demonstrate clinical benefits\textsuperscript{25}. Rare occurrences of micro-haemorrhage and vasogenic oedema have also been described with this drug\textsuperscript{28,49}. In a study conducted in Japan with AD patients with mild to moderate dementia, treatment with solanezumab was associated with a significant increase in plasma concentrations of the Aβ peptide, reflecting the increased clearance of Aβ from the brain, in the absence of relevant side effects\textsuperscript{50}.

The monoclonal antibody ponezumab targets the amino-terminal portion of Aβ\textsubscript{1-40}. In animal models this antibody significantly reduced cerebral Aβ burden and cerebral amyloid angiopathy, along with improvements in behaviour\textsuperscript{27}. In a phase 1 study, a single intravenous dose of ponezumab was shown to be safe and well tolerated, and associated with increments in plasma and CSF concentrations of the Aβ peptide, suggesting that the drug may alter central Aβ levels. However, subsequent phase 2 studies did not confirm clinical efficacy, and development of ponezumab for mild to moderate AD was interrupted\textsuperscript{26}. There are other ongoing trials with humanized monoclonal antibodies raised against the Aβ peptide, such as gantenerumab and crenezumab. Gantenerumab seems to reduce the cerebral Aβ load in AD patients in a dose-dependent fashion\textsuperscript{27}. However, it is yet to be determined whether treatment with this antibody can slow disease progression and improve clinical outcomes\textsuperscript{49}. Data from a phase 1 clinical trial testing another humanized anti-Aβ monoclonal antibody, known as MABT5102A, to protect against Aβ\textsubscript{1-42} oligomer-induced cytotoxicity, showed no vasogenic oedema, even among APOE*4 carriers\textsuperscript{50}. 
Other anti-amyloid strategies have been addressed by clinical trials. Preliminary studies support that the production and accumulation of Aβ can be downregulated by the specific γ-secretase inhibitors avagacestat and semagacestat\(^{56,28}\).

Recently, semagacestat, a non-selective gamma-secretase inhibitor, has been examined as a potential treatment for AD patients\(^{51}\). Unfortunately, preliminary results from two ongoing long-term phase III trials found no efficacy. The studies were interrupted because researchers verified it did not slow disease progression and, in addition, was associated with increasing cognitive impairment and worsening daily living activities, as well with increasing risk for skin cancer with semagacestat\(^{51}\). Although vasogenic oedema has been a rare vascular event, a report described exacerbation of psoriatic skin lesions with this drug\(^{21,52}\).

Avagacestat has been considered as a potently inhibitor of Ab40 and Ab42 formation, with selectivity for effects on APP relative to Notch proteins which interfere with cell proliferation, differentiation, and apoptosis. In a study enrolling healthy subjects, this compound exerted a potent selective gamma-secretase inhibition with decreased CSF Ab levels, as well as the inhibition of the human Notch proteins\(^{53}\). The authors reported a good tolerability profile and no changes in histopathology of skin or in lymphocytes following 28 days of dosing, although this period was not enough to confirm such clinical alterations in humans\(^{52}\). A phase II study with AD outpatients reported a dose-dependent of avagacestat on CSF amyloid isoforms and Tau protein. However, the authors demonstrated no signicant reduction in these biomarkers, although they reported an acceptable safety and tolerability with this drug\(^{54}\). Another study was designed to compare chronic treatment of transgenic mice models of AD between the nonsteroidal anti-inflammatory CHF5074 and a prototypal gamma-secretase inhibitor (DAPT)\(^{31}\). The authors observed that the CHF5074 compound has effectively prevented amyloid accumulation in the brain tissue and behavioural impairment; however, no significant effects were found with DAPT treatment.

The inhibition of beta-secretase is another potential mechanism of disease modification in AD, given the major role of this enzyme in the amyloidogenic cleavage of the amyloid precursor protein (APP). BACE-1 (β-site amyloid precursor protein cleaving enzyme 1) produces two peptides (Ab40 and Ab42), and its inhibition with specific compounds precludes the excess of amyloid and its accumulation into plaques\(^{55}\).

An inhibitor of β-secretase (GRL-8234) was recently investigated in young transgenic mice with decreased soluble amyloid-beta in the brain tissue and with rescued behaviour performance\(^{29}\). These findings indicated that β-secretase inhibitors play a role in reduction of amyloid-beta plaque load at an early stage of AD pathogenesis, as well as in older mice, suggesting possible benefits for treatment of AD patients at a later stage of the disease\(^{29}\).

TAK-070 is a non-petpidic agent that inhibits BACE-1 in a dose-dependent and non-competitive maner. However, the inhibitory mechanism of this compound are not clear, and the reduction of amyloid-beta has been considered modest with low selectivity over other enzymes\(^{30}\). Using an AD transgenic mouse model, the immunogenicity and efficacy of the compoundAF20513 was tested and it was found that this active vaccine induced cellular and humoral immune responses against beta-amyloid pathophysiology in the brain tissue of the animals, without inducing microglial activation\(^{56}\). In addition, the amyloid-β anti-aggregator curcumin could be a future therapeutic strategy\(^{22}\). However, large clinical trials are needed to test this and other promising drugs.

The occurrence of vasogenic oedema and multiple cortical and subcortical vascular lesions represent adverse events that must be considered in Aβ immunotherapy with monoclonal antibodies. These radiological findings are associated with cerebral amyloid angiopathy-related inflammation (CAA-ri), and can be asymptomatic or present clinically with acute or sub-acute manifestations such as headache, seizures, focal neurological deficits, and behavioural disturbances\(^{57,58}\). This reaction was observed with bapineuzumab and other monoclonal antibodies but, interestingly, it was also reported in patients treated with the γ-secretase inhibitor avagacestat\(^{26,59}\).

**Tau-oriented strategies**

Given its critical role in pathogenesis of AD, drug development may also target the production, processing (phosphorylation) and aggregation of Tau protein\(^{33}\). Three ‘anti-tau’ strategies have been addressed by clinical trials. Agents like methylene-blue (Rember) and NAP (AL-108) favour the stabilization of microtubules, while lithium salts may prevent Tau
Two widely used mood stabilizers, lithium and valproate, are also inhibitors of this enzyme, reducing tau phosphorylation in animal models\textsuperscript{61}. Proteomic studies in AD patients treated with valproate indicated ten differentially expressed proteins related to functional classes implicated in the neurobiology of the disease and to the therapeutic action of drug\textsuperscript{62}. However, in a multicenter clinical trial conducted by the Alzheimer’s Disease Cooperative Study (ADCS), an accelerated decrease in total brain and hippocampal volume was observed after 1 year of followup among patients treated with divalproex sodium, which was accompanied by greater cognitive impairment\textsuperscript{63}. In addition, valproate treatment of moderately demented AD patients did not delay the emergence of neuropsychiatric symptoms of agitation or psychosis, and was not associated with reduction of cognitive or functional decline; rather, it was associated with adverse effects such as somnolence, gait disturbance, tremor, diarrhoea and weakness\textsuperscript{64}.

Lithium, a potent GSK-3 inhibitor, has shown neuroprotective action such as reducing Tau concentration and phosphorylation, via the inhibition of GSK-3\textbeta\textsuperscript{65}. This agent also exhibited a significant reduction of Aβ production, axonal degeneration and releasing the transforming growing factor beta 1 (TGF-β1) in experimental studies of cultured neurons and transgenic mice\textsuperscript{65-70}. Epidemiologic studies showed a reduced risk of developing dementia among patients with bipolar disorder treated chronically with lithium\textsuperscript{71,72}, but there is limited evidence of the clinical benefits of lithium treatment for patients with or at risk for AD\textsuperscript{73-76}. In a recent single-centre, placebo-controlled trial conducted in a sample of patients with amnestic MCI, long-term lithium treatment was associated with stabilization of cognitive and functional parameters, in addition to a reduction in CSF concentrations of phosphorylated Tau\textsuperscript{76}. In a previous phase 2 study in patients with mild AD treated with lithium for a shorter (10 wk) period, no significant differences were observed in biological (GSK-3 activity and CSF biomarkers) or clinical (cognitive and functional) outcomes\textsuperscript{74}. In addition to that, the safety limits of chronic lithium treatment for older adults need to be determined, given the risk of renal impairment, hypothyroidism and other metabolic adverse effects\textsuperscript{77}. Other pharmaceutical compounds targeting GSK-3\textbeta, such as the specific inhibitors AR-A014418, SRN-003-55, CHIR-98014 and SB216763, have so far shown neuroprotective effects in preclinical models of AD\textsuperscript{78}.

Methyllithioninium chloride (known as Rember), also known as methylene-blue, is a phenothiazine widely used as a histological stain. Currently, it is a promising compound being evaluated in AD trials. The drug has anti-oxidative properties and has been shown to reduce Aβ oligomerization, given its ability to bind to the Tau domain that is required for its aggregation, therefore, preventing the formation of PHFs\textsuperscript{79}. In animal models, it has been shown to decrease Aβ levels and prevent cognitive deficits in triple transgenic mice\textsuperscript{80}. A phase 2b randomized trial of Rember monotherapy in mild and moderate AD patients suggested cognitive benefits in volunteers\textsuperscript{81}. Results of a phase 3 trial are soon expected to assure its safety profile and efficacy as a disease-modifying drug for AD\textsuperscript{82}.

**Conclusions and future directions**

To date, treatment of AD relies on the symptomatic effects of cholinesterase inhibitors and NMDA-receptor antagonists. Many promising compounds have been validated by experimental models as candidate disease-modifying drugs for AD; however, only a few of these appear in the pipeline of drug development, or have been clinically tested by RCTs. Overall results from these trials have so far been negative. Most phase 3 trials with candidate drugs for AD in the last decade failed to present unequivocal clinical benefits, or were suspended due to severe adverse events. Likewise, results of ongoing phase 2 trials are discrete at most. This rather pessimistic scenario must be weighed against the huge financial costs of the transition from phase 2 to phase 3 trials, and further translation of this knowledge into clinical practice. Several issues must be addressed in future studies with candidate drugs in order to yield clinically relevant results, and support their generalization for the treatment of the massive population of patients with (and at risk for) AD. One critical issue refers to the stage of the disease in which one given treatment is prone to deliver clinically relevant benefits. The majority of studies with candidate disease-modifying drugs for AD recruited patients with clinically manifested dementia, when it may be too late to grant clinical benefits from the modification of the pathological process. In other words, by treating demented patients with such drugs it may be possible to prove the concept of disease modification, but this effect may not be translated into significant changes to the natural course of the disease. Forthcoming trials should rather target disease modification in patients with very mild clinical symptoms (such as incipient dementia or amnestic MCI), i.e., at prodromal or even pre-clinical stages of AD\textsuperscript{83}. A recent consensus paper published by the National Institute of Aging/
Alzheimer’s Association working group proposed a three-stage stratification of patients that could be used to guide for the recruitment populations for secondary prevention or interventional trials. With respect to trials in MCI, it is essential to improve the definition of cases, i.e., to recruit amnestic MCI patients with true AD pathology and, therefore, higher risk of conversion to dementia. For this purpose, recruitment of patients for future studies should be rationally based on new diagnostic technologies including biochemical and imaging biomarkers (e.g., CSF concentrations of Aβ42, total Tau and phosphor-Tau, molecular imaging of amyloid with PET). Another important point refers to the duration of trials, and the best moment to start the intervention, given the long period of time prior to the onset of symptoms in which the pathological changes are evolving within the brain. In other words, it is imperative to develop a better understanding of the disease process and the possibilities of modification of the AD pathogenesis, which requires evidence-based answers for the following three questions: who should be treated; when to start; and for how long? Finally, one should better define the outcomes of these trials, with biological measures to prove that the drug is efficacious in disease-modification even in the absence of significant clinical improvement. Therefore, the notion of prevention of dementia in at-risk individuals must be reinforced.

References


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