Alzheimer’s disease: clinical trials and drug development

Francesca Mangialasche, Alina Solomon, Bengt Winblad, Patrizia Mecocci, Miia Kivipelto

Alzheimer’s disease is the most common cause of dementia in elderly people. Research into Alzheimer’s disease therapy has been at least partly successful in terms of developing symptomatic treatments, but has also had several failures in terms of developing disease-modifying therapies. These successes and failures have led to debate about the potential deficiencies in our understanding of the pathogenesis of Alzheimer’s disease and potential pitfalls in diagnosis, choice of therapeutic targets, development of drug candidates, and design of clinical trials. Many clinical and experimental studies are ongoing, but we need to acknowledge that a single cure for Alzheimer’s disease is unlikely to be found and that the approach to drug development for this disorder needs to be reconsidered. Preclinical research is constantly providing us with new information on pieces of the complex Alzheimer’s disease puzzle, and an analysis of this information might reveal patterns of pharmacological interactions instead of single potential drug targets. Several promising randomised controlled trials are ongoing, and the increased collaboration between pharmaceutical companies, basic researchers, and clinical researchers has the potential to bring us closer to developing an optimum pharmaceutical approach for the treatment of Alzheimer’s disease.

Introduction

Alzheimer’s disease mainly affects elderly individuals, and, because of the ageing of populations worldwide, this disorder is reaching epidemic proportions, with a large human, social, and economic burden. Effective treatments are greatly needed. Current drugs for Alzheimer’s disease target cholinergic and glutamatergic neurotransmission, thus improving symptoms, although their neuroprotective activity is still debated (table 1). Much effort is directed towards identifying disease-modifying therapies, with several compounds in different phases of development (figure). In this Review, we provide an update-to-date and comprehensive outline of the status of drug development for Alzheimer’s disease, focusing mainly on compounds being tested in human beings (see webappendix for clinical trial registration details), and citing therapeutic approaches still in preclinical phases (table 2). Drugs are discussed according to their main mechanism of action: those that affect neurotransmission, those that prevent the accumulation of misfolded proteins (amyloid β [Aβ] and tau), and those that rescue mitochondrial function or restore balance of growth factors, as well as other therapeutic approaches. This topic has, for practical reasons, previously often been fragmented into specific debates for different therapeutic strategies. In this paper, we bring together all available clinical results, which are discussed from a clinical and design perspective, and we discuss general problems associated with this topic, including the underlying dominant hypothesis (one protein, one drug, one disease), its consequences, and its need of modification. According to this hypothesis, the aim of drug development is to find a selective compound that acts on a single specific disease target to produce the desired clinical effects. However, such an approach might not be suitable for the complex nature of Alzheimer’s disease.

Cholinergic drugs

The neuropathology of Alzheimer’s disease is characterised by early loss of basal forebrain cholinergic neurons, leading to decreased cholinergic transmission, which can be improved with acetylcholinesterase inhibitors or by modulation of muscarinic and nicotinic acetylcholine receptors. Apart from the acetylcholinesterase inhibitors already approved (table 1), there has been little development of cholinergic drugs.

The (+)-phenserine enantiomer, a derivative of physostigmine, is an acetylcholinesterase inhibitor that can also reduce Aβ precursor protein (APP) and Aβ concentrations by decreasing the translation of APP mRNA. The (+)-phenserine enantiomer, posiphen, has poor acetylcholinesterase inhibitor activity but can also substantially decrease APP production by reducing APP mRNA translation. Phenserine has been tested in randomised controlled trials (RCTs), with good tolerability and some beneficial effects on cognitive functions in patients with mild-to-moderate Alzheimer’s disease. However, results were not clinically significant, as measured by the Alzheimer’s disease assessment scale–cognitive subscale (ADAS-cog) and the clinician’s interview-based impression of change plus caregiver input (CIBIC+), and further RCTs for this drug in Alzheimer’s disease have not been initiated.

Development of muscarinic receptor agonist has had limited success owing to difficulties in obtaining drugs with few adverse effects. Talsaclidine, AF-102B, and AF-267B (NGX-267) are M1 muscarinic receptor agonists that can also affect Aβ production. Talsaclidine and AF-102B decreased CSF Aβ concentrations in patients with Alzheimer’s disease, but these compounds can have potentially undesirable cholinergic receptor-mediated effects, such as increased salivary flow, although for this reason AF-102B and AF-267B have been tested in patients with xerostomia. Enhancement of cholinergic transmission with nicotinic receptor agonists has also been investigated. Ispixonide (AZD-3480) is a selective agonist of the nicotinic receptor α4β2 that has had positive effects on cognition in healthy individuals and in people with age-associated memory impairment. This drug has been studied in patients with mild-to-moderate Alzheimer’s
Aβ1–42:Aβ1–40 ratio, and finally oligomeric Aβ. Results from RCTs have shown that removing plaques will not reverse the damage or stop the Alzheimer’s dementia.52,54 A major concern is the paucity of knowledge on the functions of APP and Aβ.54 Although the amyloid hypothesis has undoubtedly led to the focusing and structuring of the research field for Alzheimer’s disease,54 results from RCTs of anti-amyloid drugs have not yet been translated into clinical practice.

**Table 1: Approved drugs for treatment in Alzheimer’s disease**

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<tr>
<th>Disease stage</th>
<th>Symptomatic activity*</th>
<th>Potential neuroprotective activity</th>
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<tr>
<td>AChEIs: improve cognition, behaviour, and functional and global clinical state</td>
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<tr>
<td>Donepezil</td>
<td>All stages</td>
<td>AChE</td>
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<tr>
<td>Rivastigmine</td>
<td>Mild to moderate</td>
<td>AChE and BChE</td>
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<tr>
<td>Galantamine</td>
<td>Mild to moderate</td>
<td>AChE (nicotinic receptor modulation)</td>
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<tr>
<td>Huperzine-A†</td>
<td>Approved in China for mild-to-moderate stages; dietary supplement in some countries</td>
<td>AChE</td>
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<tr>
<td>NMDA receptor antagonists: improve cognition, behaviour, and functional state</td>
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<tr>
<td>Memantine</td>
<td>Moderate to severe (monotherapy and in combination with AChE)</td>
<td>Uncompetitive, voltage-dependent NMDA receptor antagonist</td>
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The proposed mechanisms of activity for the different drugs and the indicated disease stage for treatment are shown.51–53 Aβ=amyloid β. APP=amyloid precursor protein. AChE=acetylcholinesterase inhibitor. BChE=butyrylcholinesterase inhibitor. *Donepezil, rivastigmine, galantamine, and huperzine-A all increase cholinergic transmission, whereas memantine decreases glutamate excitotoxicity. †Approved for all stages of Alzheimer’s disease in several countries, including the USA, but not in Europe. ‡Published results on randomised controlled trials outside China are not available; optimum therapeutic dosages not yet clear.

**Anti-amyloid therapies**

Alzheimer’s disease drug development is driven mainly by the amyloid hypothesis, and most RCTs are designed to target Aβ (figure). Aβ peptides derive from APP by proteolytic cleavage (by β-secretases and γ-secretases) via the amyloidogenic pathway. Aβ42 is the most frequent form of Aβ, but the Aβ1–42 form has a higher propensity to aggregate and is greatly enriched in amyloid deposits.55 The amyloid hypothesis has undergone several modifications, mainly concerning the type of Aβ thought to cause Alzheimer’s disease: initially this was the amyloid plaque, followed by increased concentrations of Aβ1–42, then increased Aβ1–42:Aβ1–40 ratio, and finally oligomeric Aβ.52 Results from RCTs have shown that removing plaques will not reverse the damage or stop the Alzheimer’s dementia.53 In addition to which Aβ species is toxic, whether Aβ is toxic at all and its role in the pathogenesis of Alzheimer’s disease are unclear.52,53 A major concern is the paucity of knowledge on the functions of APP and Aβ.54 Although the amyloid hypothesis has undoubtedly led to the focusing and structuring of the research field for Alzheimer’s disease,54 results from RCTs of anti-amyloid drugs have not yet been translated into clinical practice.

**Drugs to reduce Aβ production**

**β-secretase inhibitors**

β-secretase (also known as the β-site APP-cleaving enzyme; BACE1) initiates the amyloidogenic pathway. Development of β-secretase inhibitors is challenging, because this enzyme has many substrates (including neuregulin-1, which is involved in myelination) and a wide substrate-binding domain, and drugs to modulate this CNS enzyme must cross the blood–brain barrier. These difficulties are indicated by the scarcity of phase 3 RCTs.

The thiazolidinediones rosiglitazone and pioglitazone are oral drugs for type 2 diabetes that act as β-secretase inhibitors by stimulating the nuclear peroxisome proliferator-activated receptor γ (PPARγ). Activation of these receptors can suppress expression of β-secretase and APP, and promotes APP degradation by increasing its ubiquitination.55 Pioglitazone can cross the blood–brain barrier, although whether rosiglitazone can reach the CNS in human beings is unclear.55 The therapeutic effects of PPARγ agonists in Alzheimer’s disease could be caused by their effect on insulin action, lipid and carbohydrate metabolism, and inflammation. Insulin resistance and peripheral hyperinsulinaemia seem to promote neuropathology of Alzheimer’s disease,56 and both rosiglitazone and pioglitazone increase peripheral insulin sensitivity and reduce concentrations of insulin, which competes with Aβ for degradation by the insulin-degrading enzyme.57
The effects of rosiglitazone on cognition in patients with Alzheimer’s disease or mild cognitive impairment have been studied in large phase 3 RCTs, and pioglitazone has been tested in phase 2 RCTs (NCT00982202, NCT00736996, NCT00550420, NCT00428090, NCT00348309). Only one phase 3 RCT (NCT00428090; A VA105640), in which rosiglitazone was studied as a monotherapy in APOE ε4-stratified individuals with mild-to-moderate Alzheimer’s disease, has so far reported any results: there was no efficacy on cognition or global function.57 The US Food and Drug Administration recently warned about possible cardiac risks associated with the use of rosiglitazone,58 and the development programme for this drug in Alzheimer’s disease has been discontinued, possibly because of negative preliminary results.

No phase 3 RCTs in new β-secretase inhibitors are ongoing, but several new β-secretase inhibitors are under investigation. CTS-21166, an orally administered compound, was well tolerated and reduced plasma Aβ concentrations in a phase 1 RCT in healthy volunteers.59

γ-secretase inhibitors and modulators
Development of γ-secretase inhibitors presents challenges similar to those for β-secretase inhibitors: γ-secretase, the enzyme responsible for the final step in Aβ generation, is one of the main complexes involved in intramembranous cleavage of several proteins, including APP, Notch receptor, and various neuronal substrates (e.g., ErbB4, p75NTR neurotrophin receptor, N-cadherin, and the sodium channel β4 subunit).60 Collateral effects of γ-secretase inhibitors include haematological and gastrointestinal toxicity, skin reactions, and changes to hair colour, mainly caused by inhibition of the Notch signalling pathway, which is involved in cell differentiation.

At least seven γ-secretase inhibitors have reached clinical testing: semagacestat (LY-450139), MK-0752, E-2012, PRX-03140, PUFAs, SGX-130, and AF-267B. The effects of these agents on cognition and global function in Alzheimer’s disease have not been reported to date.
BMS-708163, PF-3084014, begacestat (GSI-953), and NIC3-15. Semagacestat reduces Aβ concentrations in the plasma and Aβ production in the CNS. Plasma Aβ concentrations have a biphasic pharmacokinetic response: first, they decrease, concomitant to increasing semagacestat concentrations, then there is a rebound

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<td>Ladostigil (TV-226)</td>
<td>Increased cholinergic transmission, increased dopamine, serotonin, and adrenaline levels in the brain; decreased Aβ levels and neuroprotection in cellular and animal models of AD.</td>
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<td>Decrease in plasma Aβ concentrations, then there is a rebound response: first, they decrease, concomitant to increasing plasma Aβ concentrations, then there is a rebound response.</td>
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<tr>
<td>M-30</td>
<td>Increased dopamine, serotonin, and adrenaline levels; decreased Aβ levels in the brain and neuroprotection in cellular and animal models of AD.</td>
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<tr>
<td>Derivative of rasagiline and rivastigmine, acts as an AChEI and monoamine oxidase inhibitor; also has antioxidant properties and can modulate APP processing and cellular signalling pathways.</td>
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<tr>
<td>Increased cholinergic transmission and decreased Aβ levels in cellular and animal models of AD.</td>
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<tr>
<td>Derivative of the polyamineamide caproctamine and the synthetic derivative of coenzyme Q, idebenone; acts as an AChEI, β-secretase inhibitor, and Aβ anti-aggregator; has antioxidant properties and decreases tau hyperphosphorylation.</td>
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<td>Decreased brain Aβ1-40 and Aβ1-42, including vascular fibrillar Aβ deposits; increased cognition and good safety profile (no inflammatory or autoimmune response) in animal models of AD.</td>
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<tr>
<td>Anti-β-site antibodies (IBIS1): mask the β-secretase cleavage site on APP, thus limiting APP processing by β-secretase.</td>
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<td>Anti-β-secretase antibodies: inhibit processing of APP by β-secretase.</td>
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<td>Increased Aβ production in PrPC knockout mice; anti-PrPC antibodies rescue synaptic plasticity in mouse hippocampal neurons.</td>
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<td>Modulators of PrP (normal form of the prion protein): PrP can inhibit β-secretase activity and can bind to Aβ oligomers, thus mediating their synaptotoxicity; no specific drugs have been proposed.</td>
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<td>γ-secretase modulation</td>
<td>Shifted γ-secretase cleavage from Aβ1-42 to shorter and less toxic variants in vitro.</td>
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<td>NGX series compounds: act as GSM</td>
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<td>NSAIDs: target the γ-secretase cleavage site of APP</td>
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<td>Anti-Aβ antibodies: promote Aβ removal from the brain; several are in preclinical testing.</td>
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<td>Target specific antibodies, including site-directed humanised monoclonal antibodies (binding to C-terminal, N-terminal, or mid-part of Aβ); conformation-specific antibodies; antibodies able to target intraneuronal Aβ.</td>
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<td>Increasing brain resistance to Aβ</td>
<td>Genetic ablation or reduction of GIVA-PLA2 prevents Aβ-dependent cognitive impairment in AD animal models.</td>
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<td>Compounds that decrease tau levels: tau protein might mediate Aβ-induced neuronal dysfunction; no specific drugs have been proposed.</td>
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<tr>
<td>Genetic ablation or reduction of tau prevents Aβ-dependent cognitive impairment, without affecting plaque burden or Aβ oligomer levels in AD animal models.</td>
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<td>Minocycline: modulates tau phosphorylation and aggregation.</td>
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<td>Decreased tau phosphorylation and tau aggregation, associated with inhibition of caspase-3-mediated tau cleavage; decreased Aβ-induced neuronal death and cognitive decline in animal models of AD and tayopathies.</td>
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<td>Tau anti-aggregants: N-744, rhodamines, phenylthiazolylhydrazide</td>
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<tr>
<td>Decreased tau aggregation in cellular models.</td>
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<td>Anti-phospho tau antibodies: bind to the pathological hyperphosphorylated tau, limiting its neurotoxic effect.</td>
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<tr>
<td>Decreased brain aggregated tau and decreased progression of tangle-related behavioural phenotype in mouse models.</td>
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<td>Brain-derived neurotrophic factor: involved in synaptic plasticity and neuronal survival, and reduced concentrations found in the brains of patients with AD.</td>
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<td>Gene delivery reversed synaptic loss, restored normal cell signalling and gene expression, and improved learning and memory in animals.</td>
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<th>Modulation of synaptic plasticity and nerve growth</th>
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<tr>
<td>Modulators of Nogo signalling system: Nogo signalling is involved in synaptic plasticity, and Nogo receptor has a role in long-term memory formation and binds to APP and Aβ; no specific drugs have been proposed.</td>
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<tr>
<td>Disruption of Nogo receptor expression promotes increased Aβ brain levels, plaque deposition, and dystrophic neurites in a mouse model of AD; all these expressions are reduced by infusion of a soluble Nogo receptor fragment; brain Nogo receptor overexpression has increased long-term memory in a mouse model.</td>
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APP-amyloid precursor protein; Aβ-amyloid beta; AD-Alzheimer’s disease; PnPnormal form of the prion protein; GSM-gamma-secretase inhibitor; GIVA-PLA2-group IV isof orm of phospholipase A2. NSAID=non-steroidal anti-inflammatory drug; AChEI=acetylcholinesterase inhibitor.

Table 2: Therapeutic strategies being tested in preclinical studies for AD.
increase over baseline, when drug concentration declines.\(^7\) The source of this transient Aβ increase in the plasma seems peripheral rather than central, because semagacestat can reduce CNS Aβ production and concentrations.\(^8\) In 2008, two large phase 3 RCTs were started to study semagacestat in patients with mild-to-moderate Alzheimer’s disease (NCT00594568, NCT00762411); patients who initially receive placebo will later switch to semagacestat to detect any disease-modifying effects. Study NCT00594568 is due to be completed in 2011 and NCT00762411 is due to be completed in 2012.

Notch-sparing γ-secretase inhibitors (second-generation inhibitors) are under development: begacestat was tested in a phase 1 RCT (NCT00959881) and BMS-708163 in two phase 2 RCTs in patients with prodromal or mild-to-moderate Alzheimer’s disease (NCT00810147, NCT00890890). Begacestat reduced Aβ concentrations in the plasma (with delayed rebound)\(^10\) but did not substantially affect CSF Aβ\(_{1-40}\), whereas BMS-708163 promoted a dose-dependent decrease of Aβ\(_{1-40}\) in the CSF.\(^6,65\)

PF-3084014 is a γ-secretase inhibitor with high selectivity for APP compared with Notch, and results from animal studies showed decreases in Aβ in the plasma, CSF, and brain, without a rebound effect on plasma Aβ.\(^11\) In a small phase 1 study on healthy volunteers, PF-3084014 promoted a dose-dependent reduction in plasma Aβ concentrations, although effects on CSF concentrations were small.\(^67\)

NIC5-15, a naturally occurring monosaccharide found in many foods, can act as a Notch-sparing γ-secretase inhibitor and insulin sensitiser (ie, it increases the sensitivity of the tissue to insulin).\(^68,69\) It is being tested in patients with Alzheimer’s disease in a phase 2 study (NCT00470418) and preliminary results have shown that NIC5-15 is safe and well tolerated.\(^70\)

γ-secretase modulators can selectively block APP proteolysis without any Notch-based adverse effects. A subset of non-steroidal anti-inflammatory drugs (NSAIDs), including ibuprofen, indomethacin, and sulindac sulfide, bind to APP and act as γ-secretase modulators, decreasing Aβ\(_{1-40}\) and Aβ\(_{1-42}\) production, with increased generation of Aβ\(_{1-38}\) fragments.\(^8\) Among these compounds, known as selective β-amyloid-lowering agents (SALAs), tarenflurbil (R-enantiomer of flurbiprofen) was tested in phase 3 RCTs in patients with mild Alzheimer’s disease, but did not show clinical effects.\(^71\) Negative results could be due to low γ-secretase modulator potency, poor CNS penetration, and inhibition of microglia-mediated Aβ clearance by residual NSAID activity.\(^72\) Another γ-secretase modulator, CHF-5074, reduced Aβ brain load and improved behavioural deficits in animals,\(^73\) and a phase 1 study to evaluate drug safety and tolerability in healthy volunteers is ongoing (NCT00954252).

**α-secretase activators**

Upregulation of α-secretase activity, and non-amyloidogenic cleavage of APP, can decrease Aβ formation and increase production of a soluble domain (sAPPα), which is potentially neuroprotective.\(^74\) Several drugs can stimulate α-secretase activity (agonists of muscarinic, glutamate, and serotonin receptors; statins; oestrogens; testosterone; and protein kinase C activators) and have been tested in clinical trials, but no evidence supports their use in Alzheimer’s disease yet.\(^75\)

Etazolate (EHT-0202), a selective GABA\(_A\) receptor modulator, stimulates neuronal α-secretase and increases sAPPα production.\(^76\) This drug, which is orally bioavailable, has been recently tested in a phase 2 RCT in patients with mild-to-moderate Alzheimer’s disease (NCT00880412, results not available).\(^77\)

Bryostatin-1 is a macrocyclic lactone that has already been investigated as an antineoplastic drug; bryostatin-1 and its synthetic analogue picolog can stimulate α-secretase by activating protein kinase C and promoting sAPPα secretion, restoring the healthy phenotype of fibroblasts isolated from patients with Alzheimer’s disease.\(^78\) Bryostatin-1 can reduce brain Aβ\(_{1-40}\) and Aβ\(_{1-42}\) and improve behavioural outcomes in mouse models of Alzheimer’s disease;\(^79\) a phase 2 study to evaluate bryostatin-1 safety in patients with mild-to-moderate Alzheimer’s disease is planned (NCT00606164).

Exebryl-1 modulates β-secretase and α-secretase activity, causing substantial reduction of Aβ formation and accumulation in the mouse brain, with memory improvements. A phase 1 RCT was approved in 2008.\(^80\)

**Drugs to prevent Aβ aggregation**

Evidence for the neurotoxic and synaptotoxic activity of Aβ oligomers\(^81\) constitutes the scientific basis for the development of compounds that inhibit Aβ aggregation or destabilise Aβ oligomeric species. Non-peptidic anti-aggregants tested so far are chemically heterogeneous, and their pharmacodynamics are not clear. They can act by binding to Aβ monomers, thus preventing oligomerisation and allowing elimination; alternatively, anti-aggregants can react with Aβ oligomers, neutralising their toxicity and promoting clearance.\(^7\) The challenge is to obtain compounds with high CNS bioavailability and low immunogenicity and toxicity.

The first generation of non-peptidic anti-aggregants did not meet this challenge. Despite promising preclinical and human phase 2 studies,\(^82\) tramiprosate (homotaurine, Alzhemed; a small orally administered compound that binds preferentially to soluble Aβ and maintaining it in non-fibrillar form) did not show clinical efficacy in a North American phase 3 RCT (Alphase study) in patients with mild-to-moderate Alzheimer’s disease.\(^83\) Negative results of the Alphase study could be due to methodological problems such as large variability among clinical sites, changes in the treatment and control groups because of the concomitant treatment with cognitive-enhancing drugs, which affected the primary cognitive endpoints, and weak potency and low CNS bioavailability of the drug.\(^7\) As a consequence of the outcome of this study, a
Active immunotherapy

In a phase 2 RCT of AN-i792 (QS-21), an anti-Aβ vaccine, in patients with mild-to-moderate Alzheimer’s disease, patients responded to immunisation with Aβ1–42, developing significant Aβ-antibody titres, although not all individuals produced high IgG concentrations. However, this study was stopped because of aseptic meningoencephalitis in some patients, which was attributed to cytotoxic T cells and/or autoimmune reactions to AN-i792. To avoid neuroinflammation and toxicity, new vaccines that selectively target B-cell epitopes without stimulating T cells have been developed. Different adjuvants and mechanisms of vaccine delivery are used, such as adenoviruses, DNA vaccines, and single-chain antibody fragments.

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indicated that AD-01 and AD-02 are safe and well tolerated (NCT00495417, NCT00633841). Long-term follow-up of participants is planned (NCT01093664, NCT00711321), and further testing will focus on identifying the optimum dosage. Affitope AD-02 recently progressed to phase 2 clinical testing (NCT01117818).

**Passive immunotherapy**

Passive immunotherapy is based on monoclonal antibodies or polyclonal immunoglobulins targeting Aβ to promote its clearance. Results from animal studies have shown that anti-Aβ antibodies can prevent oligomer formation and reduce brain amyloid load with improvement in cognitive functions. Several monoclonal antibodies, generally given intravenously, are being tested in patients with Alzheimer’s disease: bapineuzumab (AAB-001), solanezumab (LY-2062430), PF-04360365, GSK-933776, R-1450 (RO-4909832), and MABT-5102A.

Bapineuzumab is a humanised anti-Aβ monoclonal antibody; a phase 2 RCT in patients with mild-to-moderate Alzheimer’s disease that had a follow-up period of longer than 18 months reported no significant effects on the primary measures of cognition and activities of daily living, as measured in prespecified within-dose cohort analyses. However, post-hoc analyses of clinical and neuroimaging data from all dose cohorts showed non-significant improvements in cognitive endpoints and signs of efficacy in APOE ε4 non-carriers. A dose-related transient vasogenic oedema was a more common side-effect in APOE ε4 carriers. Phase 3 studies are ongoing, including separate RCTs for APOE ε4 carriers and non-carriers (NCT0099675, NCT00574132, NCT00996918, NCT00998764).

Solanezumab, a monoclonal antibody that binds specifically to soluble Aβ, promotes Aβ clearance from the brain through the blood. In a phase 2 trial, the correlation between total plasma Aβ40,42 after treatment (dose-dependent increase) and baseline amyloid plaque burden shown by single photon emission CT scanning, together with the dose-dependent increase in unbound CSF Aβ40,42, suggest that solanezumab might mobilise Aβ40,42 from plaques, and might normalise soluble CSF Aβ40,42 in patients with Alzheimer’s disease. Two phase 3 RCTs have been initiated to study the efficacy of this drug in patients with mild-to-moderate Alzheimer’s disease (NCT00905372, NCT00904683). PF-04360365 is a humanised, modified IgG2 antibody that binds to the C terminus of Aβ40,42. Preliminary results on a single-dose regimen indicate that this antibody is well tolerated in patients with Alzheimer’s disease, and two long-term RCTs of multiple doses are ongoing (NCT00722046, NCT00945672).

GSK-933776, R-1450 (RO-4909832), and MABT-5102A are monoclonal antibodies that target Aβ and have been tested in patients with Alzheimer’s disease in phase 1 studies (NCT00495550, NCT00531804, NCT00736775).

Passive immunisation can also be achieved by intravenous infusion of immunoglobulins (IVIg), from healthy donors, which include naturally occurring polyclonal anti-Aβ antibodies. IVIg is already approved as therapy for immune deficiency, with good safety and tolerability evidence. In two small studies, short-term immunoglobulin administration in patients with Alzheimer’s disease was well tolerated, promoted a decrease of total Aβ CSF concentrations, and increased plasma total Aβ concentrations, with evidence of improvement or stabilisation of cognitive functions. Preliminary data from a phase 2 RCT confirmed the positive effects on cognition; a phase 3 study is ongoing (NCT00818662).

**Advantages, disadvantages, and future expectations**

Immunisation strategies have advantages and disadvantages. Active immunotherapy guarantees constant high antibody concentrations, requiring few follow-up visits, with reduced costs. However, rapid reduction of antibody concentrations, to limit adverse effects, is difficult. With passive immunotherapy, specific Aβ epitopes can be targeted more easily, and more rapid control of antibody titres is possible. Passive immunisation could be more effective in elderly people than active immunotherapy, because these individuals have reduced responsiveness to vaccines. Nevertheless, administration of antibodies is time consuming and costly, and, in our experience, the risk of vasogenic oedema and cerebral amyloid angiopathy with microhaemorrhages might be higher for passive immunotherapy than for active immunotherapy.

The role of Aβ in Alzheimer’s disease could be confirmed by ongoing RCTs. Results from the more detailed analyses of the AN-1792 trial have been mixed. There was some evidence of memory improvement and reduced CSF tau concentrations in patients with increased IgG titres. However, patients immunised with AN-1792 had a greater brain atrophy rate on MRI than did patients given placebo; this might be because of amyloid removal and cerebral fluid shifts. In a follow-up study on a subsample of participants from the phase 2 RCT, brain volume loss in antibody responders (anti-Aβ titres ≥1:2200) was not significantly different from that in patients receiving placebo at about 3-6 years from the end of the original study, and no further cases of meningoencephalitis were found. Responders maintained low, but detectable, anti-AN-1792 antibody titres at about 4-6 years after immunisation and had significantly reduced functional decline compared with placebo-treated patients. A follow-up report on a phase 1 study showed that immunisation with AN-1792 could completely remove amyloid plaques as determined by post-mortem assessment, but patients still had end-stage dementia symptoms before death. This finding suggests that clearance of amyloid plaques alone cannot repair already damaged neurons and prevent disease progression. Furthermore, intraneuronal concentrations of Aβ are high in patients with Alzheimer’s disease and how immunotherapy affects this pool of Aβ is unclear. Whether immune-mediated clearance of soluble
Aβ can restore neuronal function and hamper disease progression needs to be determined.

**Drugs to target tau protein**

Tau is a cytoplasmatic protein that binds to tubulin during its polymerisation, stabilising microtubules. In Alzheimer’s disease, tau is abnormally phosphorylated, resulting in the generation of aggregates (neurofibrillary tangles) toxic to neurons. The hypothesis that tau pathology causes Alzheimer’s disease has been the main competitor of the amyloid hypothesis. However, only one tau-directed compound (valproate; valproic acid) has so far reached phase 3 RCT (figure), with disappointing results because there were no effects on cognition and functional status.

There are two main therapeutic approaches to target the tau protein: modulation of tau phosphorylation with inhibitors of tau-phosphorylating kinases and compounds that inhibit tau aggregation and/or promote aggregate disassembly. The first approach is based on the observation that tau hyperphosphorylation and neurofibrillary tangle formation can be promoted by imbalanced activity of protein kinases (glycogen-synthase-kinase-3 [GSK3] and p70-S6-kinase and the phosphatase PP2A. GSK3 deregulation might have a role in Alzheimer’s disease pathogenesis, because GSK3 is involved in tau and amyloid processing, cellular signalling, and gene transcription.

Both lithium and valproate, well known for the treatment of psychiatric disorders, inhibit GSK3, to reduce tau phosphorylation and prevent or reverse aspects of tauopathy in animal models. Both drugs can also be neuroprotective by upregulating the anti-apoptotic factor BCL2, reducing neurotrophic factors, and hindering Aβ toxicity. However, a small RCT with lithium (10 weeks, including a 6-week titration phase) in patients with mild Alzheimer’s disease did not show any cognitive benefit, or any change in CSF biomarkers, including phosphorylated tau, total tau, and Aβ. The Alzheimer’s Disease Cooperative Study (ADCS) of valproate was designed to determine whether chronic valproate treatment could delay the onset of behavioural symptoms in outpatients with mild-to-moderate Alzheimer’s disease; a secondary aim was to test whether valproate can delay cognitive and functional decline. No effects on cognition and functional status were reported, but incidence of agitation and psychosis seemed to be reduced.

Several GSK3 inhibitors are under development. NP-031112 (NP-12) is a thiazolidinone-derived compound, a non-ATP competitive inhibitor of GSK3, which can reduce brain concentrations of phosphorylated tau and amyloid deposition, and prevent neuronal death and cognitive deficits in animals. This drug has been tested in patients with Alzheimer’s disease in a phase 2 RCT (NCT00948259); no results have yet been published.

Methylthioninium chloride (methylene blue, Rember), a widely used histology dye, acts as a tau anti-aggregant. This compound also has antioxidant properties, enhances mitochondrial function, and was effective, alone and in combination with rivastigmine, in reversing learning deficits and hyoscine-induced memory impairments in animals. Different doses of methylthioninium chloride (up to 100 mg) were tested in a phase 2 study in patients with moderate Alzheimer’s disease. The group given the 60 mg dose had improved cognitive function and, after 1 year, evidence of slower disease progression compared with placebo. The ineffectiveness in the group on the 100 mg dose was attributed to drug formulation defects, limiting release. A new formulation (leuco-methylthioninium), with a higher bioavailability, was recently announced, and phase 3 RCTs are needed to confirm its safety and clinical efficacy.

Davunetide (AL-108, NAP), an intranasally administrated, eight-aminoacid peptide fragment derived from the activity-dependent neuroprotective protein, and AL-208, an intravenous formulation of davunetide, are being developed. Davunetide has been tested in animal models of Alzheimer’s disease and tauopathy, and its neuroprotective activity includes regulation of microtubule dynamics, as well as inhibition of tau hyperphosphorylation and protection against Aβ toxicity. Davunetide was studied in patients with amnestic mild cognitive impairment in a 12-week, phase 2 RCT and was safe, well tolerated, and had positive effects on cognition, although confirmatory studies are needed.

Nicotinamide is the biologically active form of niacin (vitamin B3), and the precursor of coenzyme NAD+. Orally administered nicotinamide can prevent cognitive deficits in a mouse model of Alzheimer’s disease and can reduce brain concentrations of a species of phosphorylated tau (Thr231) that inhibits microtubule polymerisation. Furthermore, nicotinamide inhibits brain sirtuin deacetylase and upregulates acetyl-α-tubulin, protein p25, and MAP2c; all these interactions are associated with increased microtubule stabilisation. Nicotinamide has been used in several clinical studies, including RCTs in patients with neurodegenerative disorders, and is generally safe and well tolerated; a phase 2 RCT is ongoing in patients with mild-to-moderate Alzheimer’s disease (NCT00580931).

**Drugs to target mitochondrial dysfunction**

Targeting of organelles (eg, mitochondria) is a new approach to Alzheimer’s disease therapy, different from the protein-focused strategies that currently dominate research. Mitochondrial dysfunction occurs early in Alzheimer’s disease, can promote synaptic damage and apoptosis, and is thought to have a causal role in neurodegeneration. APP and Aβ can be imported into mitochondria, where they can interact with mitochondrial components, impair ATP production, and increase oxidative damage.

Latrepirdine was introduced in Russia as dimebon (or dimebolin), a non-selective antihistamine. In a phase 2 RCT, latrepirdine was safe, was well tolerated, and...
Neurotrophins

Neurogenesis can occur in the adult brain and in response to damage. Basal forebrain cholinergic neurons depend on NGF for survival and fibre outgrowth, and recent findings have suggested a causal link between NGF imbalance, activation of the amyloidogenic pathway, and neurodegeneration in Alzheimer’s disease. Targeted delivery of NGF to basal forebrain cholinergic neurons prevented cell death, stimulated synaptic cholinergic function, and promoted cognitive improvement in animals. Studies in patients with Alzheimer’s disease were initially based on intracerebroventricular infusion of NGF. The positive results on cognition and physiological measurements of brain functioning were counterbalanced by adverse effects (eg, pain, weight loss), leading to interruption of intracerebroventricular administration. An alternative method, gene therapy, has been developed: NGF delivery, based on intracerebral injection of autologous fibroblasts genetically modified to produce human NGF, was tested in eight patients with early-stage Alzheimer’s disease in an 18-month phase 1 study. Although two individuals had subcortical haemorrhage during implantation, an overall lower rate of cognitive decline and increased cortical glucose uptake were reported. Another ongoing phase 1 study and a planned phase 2 RCT will use in-vivo NGF brain delivery, via an adeno-associated virus-vector system (CERE-110; NCT00876863, NCT00087789).

Another study is testing encapsulated-cell biodelivery, a strategy developed to provide local NGF release while preventing adverse effects. This form of biodelivery is based on stereotactic implantation of a catheter-like device containing NGF-producing cells (NsG0202). Cells are enclosed by an immunoprotective, semi-permeable, hollow fibre membrane, enabling influx of nutrients and outflow of NGF, and preventing direct contact of cells with the host tissue and immune system. An open-label, phase 1, dose-escalation study of NGF encapsulated-cell biodelivery to cholinergic basal forebrain neurons in patients with Alzheimer’s disease is ongoing. The study will test the safety, tolerability, and effects of this approach on cognition and behaviour. Six patients with mild-to-moderate Alzheimer’s disease diagnosed in the previous 3 years and on stable acetylcholinesterase inhibitor therapy were implanted in 2008, and the implant was removed after 12 months. Preliminary results have shown good safety and tolerability, no serious adverse events (eg, no pain, no weight loss), and an increase in expression of cortical nicotinic receptors, and three patients have shown cognitive improvement.

Intranasal delivery and topical application of an NGF solution on the ocular surface are being tested in preclinical stages. Such delivery is a non-invasive, less risky, and less expensive approach for NGF therapy.

Other potential therapeutic strategies

Other approaches such as omega-3 polyunsaturated fatty acids (eg, docosahexaenoic acid) or antioxidants (eg, vitamin E) have been tested in RCTs. Some trials have reported beneficial effects of docosahexaenoic acid supplementation in elderly people with cognitive decline or Alzheimer’s disease. However, other studies have reported no effects of docosahexaenoic acid on cognitive function or behavioural disturbances in patients with mild-to-moderate Alzheimer’s disease. Treatment of patients with Alzheimer’s disease with polyunsaturated fatty acids did not modify biomarkers of inflammation and neuropathology of this disorder in the CSF or plasma.

Antioxidant supplementation has also not been effective in treatment trials. Supplement composition is still a matter of debate, because high doses of a single antioxidant (vitamin E) have been associated with increased mortality risk and no beneficial effects for patients with Alzheimer’s disease, whereas a combination of micronutrients could be more effective in neuroprotection.

RCTs that have studied statins in patients with Alzheimer’s disease have not produced any evidence of beneficial effects so far, although more detailed analyses of factors that affect response to treatment are ongoing. Statins reduce cholesterol concentrations and have several pleiotropic effects (ie, can lower Aβ production and reduce Aβ-mediated neurotoxicity, as well as having antioxidant and anti-inflammatory properties). Recently, no significant clinical benefit on cognition or global functioning was shown for atorvastatin in a 72-week, phase 3 RCT (Lipitor’s Effect in Alzheimer’s Dementia; LEADe) in patients with mild-to-moderate Alzheimer’s disease already taking...
Other RCTs, such as the Statins in Healthy, At-Risk Adults: Impact on Amyloid and Regional Perfusion (SHARP), Pitavastatin Treatment for Group of Mild to Moderate Alzheimer’s Disease (PIT-ROAD), and Simvastatin in Amnestic Mild Cognitive Impairment (SIMaMCI) studies, to test the effectiveness of statins in prevention and therapy of Alzheimer’s disease, are ongoing (NCT00548145, NCT00842920, NCT00939822).

Serotonergic drugs are another potential therapeutic approach: SB-742457 is a 5-HT₆ receptor antagonist being tested in patients with mild-to-moderate Alzheimer’s disease (NCT00710684), with some promising results, but RCTs of the 5-HT, agonist PRX-03140 were terminated when EPIX Pharmaceuticals went into liquidation (NCT00693004, NCT00672945). Drugs that target phosphodiesterase are also being studied. Phosphodiesterases are widely expressed in the brain, and phosphodiesterase 9A inhibitors are hypothesised to regulate cGMP signalling pathways involved in synaptic plasticity. PF-04447943 is a selective

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<th>Recommendations</th>
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<tr>
<td><strong>Patients</strong></td>
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<tr>
<td>Target group selection: patients with AD have various types of neuropathology (ie, amyloid plaques, NFTs, infants, Lewy bodies), there are mixed causes of dementia in many patients, particularly those who are older than 80 years</td>
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<td>Disease stage: in patients with mild-to-moderate AD, the disease could already be too advanced for a disease-modifying effect of a specific drug (eg, immunotherapy)</td>
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<td>Drugs</td>
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<td>Choosing the right drug: compounds with positive results in preclinical and early clinical testing failed in large phase 3 RCTs, with costly losses (eg, tramprolate)</td>
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<td>Optimisation of drug dosage and treatment duration: some RCTs could have been hindered by the inability to reach a therapeutic dosage (eg, tarenflurbil), or treatment duration could have been too short to detect a disease-modifying effect (eg, lithium, latrepirdine)</td>
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<td>Genetics: polymorphisms (eg, APOE, cytochrome P450) might affect drug response</td>
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<td>Outcome measurements</td>
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<td>Measuring effects: many RCTs are developed according to the design of AChEI RCTs, an approach that has indicated the AChEI symptomatic effect, but is not sensitive in detecting the efficacy of disease-modifying drugs, and rating scales used for monitoring clinical efficacy in RCTs might have low sensitivity towards changes (particularly if the placebo group does not worsen significantly), Furthermore, these tools have a subjective component that can be a source of error</td>
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<tr>
<td>Reliable evaluation of patients: inadequate training and monitoring of RCT rates might increase variability and introduce errors during enrolment and evaluation of patients</td>
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<td>Trial protocol</td>
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<td>RCT protocols: can be too exhausting and time-consuming for the patient and the caregiver, thus increasing withdrawal rates</td>
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<td>Informed consent: often involves a long document that is difficult to read and fully understand</td>
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<td>Optimisation of resources</td>
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<td>Consistency: multicentre RCTs done in several countries can have cultural and linguistic issues with assessment scales (eg, translation, validation), as well as infrastructure problems (technological disparities between centres)</td>
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<tr>
<td>Avoid repeating errors: unsuccessful preclinical and clinical studies are often not published and sponsors are reluctant to release information, similar planning and methodological problems occur in different RCTs</td>
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Recommendations are based on both scientific publications and our personal experience. AChEi=acetylcholinesterase inhibitor. AD=Alzheimer’s disease. NFT=neurofibrillary tangle. RCT=randomised controlled trial.
phosphodiesterase 9A inhibitor, able to increase cGMP concentrations in the CSF of healthy volunteers, and is being tested in a phase 2 RCT in patients with mild-to-moderate Alzheimer’s disease (NCT00930059).

The receptor for advanced glycation endproducts (RAGE) is a multi-ligand receptor present on the cell membrane of neurons, glia, and endothelial cells. RAGE binds to Aβ, promoting its influx into the CNS across the blood–brain barrier. This receptor can also be involved in Alzheimer’s disease pathogenesis through inflammatory and pro-coagulant activity within the endothelium, and can cause production of reactive oxygen species and induction of apoptosis. The soluble form (sRAGE) can compete for Aβ binding with the membrane-linked RAGE, thus promoting removal of circulating Aβ. A phase 2 study on the RAGE inhibitor PF-04494700 (TTP-488) is ongoing (NCT00566397).

Conclusions
Research on Alzheimer’s disease therapy has so far had some success in terms of symptomatic treatments (table 1), although it has also had several failures for disease-modifying drugs. Many clinical and experimental studies are ongoing (figure, table 2). How close we are to effective treatment of Alzheimer’s disease is difficult to estimate, but available results from RCTs are not in line with previous optimistic predictions of an imminent breakthrough. To explain the disappointing results of several RCTs, researchers have highlighted different errors, both in drug choice and development programmes. In table 3 we have summarised the many design problems and have suggested possible strategies, on the basis of both our personal experience in RCTs and the ongoing debate in the scientific community. The recent failures of phase 3 trials after positive phase 2 studies highlight the need for new guidelines in preclinical and clinical phases of drug development, such as use of validated biomarkers and error management checklists for drug developers that can identify and control sources of error in each phase of drug study.

Another problem in development of Alzheimer’s disease therapy is that design of selective compounds without undesirable and potentially toxic side-effects is difficult, and reaching the stage of clinical testing can take many years. Research on pharmacodynamics, biological aspects, or regulatory mechanisms of therapeutic targets is ongoing and will improve drug safety and efficacy. Alzheimer’s disease is a complex multifactorial disorder, and the details of its causes might not yet be understood at a level adequate for drug discovery. However, there are many examples of drugs that were developed long before their targets were known (eg, aspirin and penicillin). Faced with disappointing results from RCTs of approaches targeting several mechanisms thought to be involved in Alzheimer’s disease, the one protein, one drug, one disease hypothesis used as the basis of most Alzheimer’s disease therapy studies needs to be revised.

Advances in research have shifted understanding of the disease process towards genes and proteins. In the drug development field, this has resulted in a linear and target-driven, reductionist approach: the candidate drug is connected to a single target linked to a pathogenic pathway linked to the disease. However, a single target or pathogenic pathway for Alzheimer’s disease is unlikely to be identified. The pathway from genes and proteins to Alzheimer’s disease is non-linear and hard to predict, owing to many interactions on several levels (genes, proteins, organelles, cells, organs, whole organism, environment). Additionally, many drugs bind to more than one target, and a network model of drug–protein interactions might work better than a linear drug–protein model.

Multi-target therapies can be designed in several ways. The most conventional strategy is to prescribe several individual drugs. This approach is already used in Alzheimer’s disease, where acetylcholinesterase inhibitors can be given together with NMDA receptor antagonists for better symptomatic effects. Another strategy is to develop drugs that contain two or more active ingredients delivered in the same device (ie, a pill or capsule). The main drawback for both approaches is the regulatory requirement that each drug or ingredient needs to be proven to be both safe and efficient, both individually and in combination (a requirement that reinforces the one target, one drug, one disease approach). The third strategy for multi-target therapy is to design a single compound with selective poly-pharmacology. multi-target-directed ligands are synthetic hybrids with properties that cover different

Search strategy and selection criteria
Compounds and studies in this Review were identified by systematic searches on PubMed with the terms “Alzheimer’s disease”, “therapy”, “acetyl-cholinesterase inhibitor”, “acetylcholine receptor”, “NMDA-receptor antagonist”, “memantine”, “secretase inhibitor”, “dimebon”, “mitochondria”, “amyloid”, “immunotherapy”, “vaccine”, “antibody”, “tau protein”, and “neurotrophic factors”, from 1996 until March, 2010. Additional papers were identified through a manual search of the reference lists of relevant retrieved articles. A search on the proceedings of conferences on Alzheimer’s disease (International Conference on Alzheimer’s Disease, International Geneva/Springfield Symposium on Advances in Alzheimer Therapy, and 5th Kuopio Alzheimer Symposium) held from March, 2008, to March, 2010, was also done to identify clinical trials. Only publications in English were reviewed. ClinicalTrials.gov (http://www.clinicaltrials.gov) and the Cochrane Dementia and Cognitive Improvement Group (http://www.medicine.ox.ac.uk/alois) websites and websites of pharmaceutical companies were used for information on ongoing RCTs and other compounds under investigation.
Alzheimer’s disease-related mechanisms simultaneously (eg, acetylcholinesterase inhibitors, antioxidants, APP metabolism modulation; table 2).163 Such drugs are now in preclinical stages of testing (figure).

The first signs of a shift away from linear one protein, one drug thinking have already appeared: research is moving from proteins to focus on organelles (eg, mitochondria) and also multi-target-directed ligands163 (table 2). A single cure for Alzheimer’s disease is unlikely to be found. New information on pieces of the complex Alzheimer’s disease puzzle from preclinical research161 might mean that networks of interactions instead of single potential drug targets can be identified. As reviewed in this paper, several promising RCTs are ongoing, and an increased collaboration between pharmaceutical companies, basic researchers, and clinical researchers has the potential to bring us closer to developing an optimum treatment for Alzheimer’s disease.

Contributors
All authors contributed equally to the literature search, preparation of the tables and figures, and the writing of this Review. FM coordinated preparation of this paper.

Conflicts of interest
FM and AS have no conflicts of interest. MK has received honoraria for serving on the scientific advisory boards of Elan and Pfizer, and serves as a speaker at scientific meetings organised by Janssen, Novartis, and Pfizer. PM serves on the scientific advisory board for Lundbeck and as a speaker at scientific meetings organised by Lundbeck, Medivation, Novartis, Pfizer, and Wyeth. BW has received honoraria for serving on the scientific advisory board and at workshops of Elan, Janssen, Lundbeck, Medivation, Novartis, Pfizer, and Merz.

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