



Alzheimer's disease: clinical trials and drug development

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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.¹ 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).^{2–15} 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 [$A\beta$] 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.⁴⁰ 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 $A\beta$ precursor protein (APP) and $A\beta$ concentrations by decreasing the translation of APP mRNA.⁴¹ 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+),⁴¹ and further RCTs for this drug in Alzheimer's disease have not been initiated.

Development of muscarinic receptor agonists 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 $A\beta$ production.⁴² Talsaclidine and AF-102B decreased CSF $A\beta$ concentrations in patients with Alzheimer's disease,^{43,44} 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.⁴⁵

Enhancement of cholinergic transmission with nicotinic receptor agonists has also been investigated. Ispronicline (AZD-3480) is a selective agonist of the nicotinic receptor $\alpha 4\beta 2$ that has had positive effects on cognition in healthy individuals and in people with age-associated memory impairment.⁴⁶ This drug has been studied in patients with mild-to-moderate Alzheimer's

Disease stage	Symptomatic activity*	Potential neuroprotective activity
AChEIs: improve cognition, behaviour, and functional and global clinical state		
Donepezil†	All stages ^{3,4,15}	AChEI
		Possibly decreases Aβ production and Aβ-induced toxicity; modulates expression of AChE isoforms; increases expression of nicotinic receptors ⁷
Rivastigmine	Mild to moderate ³	AChEI and BChEI
		Possibly decreases Aβ production and Aβ-induced toxicity; modulates expression of AChE isoforms; increases expression of nicotinic receptors ⁷
Galantamine	Mild to moderate ³	AChEI (nicotinic receptor modulation)
		Possibly decreases Aβ production and Aβ-induced toxicity; modulates expression of AChE isoforms; increases expression of nicotinic receptors ⁷
Huperzine-A‡	Approved in China for mild-to-moderate stages; dietary supplement in some countries ⁵	AChEI
		Modulates APP processing by enhancing soluble APPα secretion; antioxidant, anti-apoptotic effects; mitochondrial protection ^{2,6}
NMDA receptor antagonists: improve cognition, behaviour, and functional state		
Memantine	Moderate to severe (monotherapy and in combination with AChEI) ¹⁰⁻¹⁴	Uncompetitive, voltage-dependent NMDA receptor antagonist
		Decreases Aβ toxicity; prevents hyperphosphorylation of tau; decreases microglia-associated inflammation; increases release of neurotrophic factors from astroglia ⁷⁻⁹

The proposed mechanisms of activity for the different drugs and the indicated disease stage for treatment are shown.²⁻¹⁵ Aβ=amyloid β. APP=amyloid precursor protein. AChEI=acetylcholinesterase inhibitor. BChEI=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.

Table 1: Approved drugs for treatment in Alzheimer's disease

disease (versus placebo and donepezil), with equivocal findings: in the phase 2 RCT neither ispronicline nor donepezil showed a significant effect on the primary outcome (ADAS-cog) after 12 weeks of treatment; however, patients who received ispronicline had some improvement on the mini-mental state examination, and a post-hoc analysis suggested a positive effect on ADAS-cog at the 20 mg dose.⁴⁷

ABT-089 is a partial agonist of nicotinic α4β2 and α6β2 receptors that is able to reverse cognitive deficits induced by hyoscine (scopolamine) in healthy volunteers;⁴⁸ however, no efficacy was reported in a phase 2 RCT in patients with mild-to-moderate Alzheimer's disease on stable acetylcholinesterase inhibitor therapy.⁴⁹ EVP-6124 is an agonist of the nicotinic α7 receptor that is safe and well tolerated in patients with Alzheimer's disease, with evidence of cognitive improvement.⁵⁰ This drug is being tested in patients with mild-to-moderate disease in a phase 2 RCT (NCT01073228). Other drugs with cholinergic activity, including multifunctional compounds, are still in preclinical stages (table 2).

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. Aβ₁₋₄₀ 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.⁵¹ 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.^{52,54} 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.

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.⁵⁵

BMS-708163, PF-3084014, begacestat (GSI-953), and NIC5-15. Semagacestat reduces A β concentrations in the plasma and A β production in the CNS.^{61,62} Plasma A β concentrations have a biphasic pharmacokinetic response: first, they decrease, concomitant to increasing semagacestat concentrations, then there is a rebound

In vivo and in vitro results	
Targeting of neurotransmission with multi-target-directed ligands	
Ladostigil (TV-3326) 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	Increased cholinergic transmission; increased dopamine, serotonin, and adrenaline levels in the brain; decreased A β levels and neuroprotection in cellular and animal models of AD ¹⁶
M-30 Derivative of rasagiline, acts as a monoamine oxidase inhibitor; also has antioxidant and iron-chelating properties and can modulate APP processing	Increased dopamine, serotonin, and adrenaline levels; decreased A β levels in the brain and neuroprotection in cellular and animal models of AD ¹⁶
Memoquin Derivative of the polyamineamide caproctamine and the synthetic derivative of coenzyme Q, idebenone; acts as an AChEI, β -secretase inhibitor, and A β anti-aggregant; has antioxidant properties and decreases tau hyperphosphorylation	Increased cholinergic transmission; decreased A β levels and neuroprotection in cellular and animal models of AD ¹⁷
Targeting of Aβ production	
β-secretase inhibition	
Anti- β -site antibodies (BBS1): mask the β -secretase cleavage site on APP, thus limiting APP processing by β -secretase	Decreased brain A β_{1-40} and A β_{1-42} , including vascular fibrillar A β deposits; increased cognition and good safety profile (no inflammatory or autoimmune response) in animal models of AD ^{18,19}
Anti- β -secretase antibodies: inhibit processing of APP by β -secretase	Decreased brain and plasma A β ; increased cognition without significant increase of glial and T-cell response in AD mouse models ²⁰
Modulators of PrP ^C (normal form of the prion protein): PrP ^C can inhibit β -secretase activity and can bind to A β oligomers, thus mediating their synaptotoxicity; no specific drugs have been proposed	Increased A β production in PrP ^C knockout mice; ²¹ anti-PrP ^C antibodies rescue synaptic plasticity in mouse hippocampal neurons ²²
γ-secretase modulation	
NGX series compounds: act as GSM	Shifted γ -secretase cleavage from A β_{1-42} to shorter and less toxic variants in vitro ^{23,24}
NSAIDs: target the γ -secretase cleavage site of APP	Decreased A β production and aggregation in cellular models ²⁵
Targeting of Aβ aggregation	
Peptidic anti-aggregants: short, modified (alanine substitution) peptidic compounds corresponding to a self-recognition element within the target sequence that drives A β fibril growth; they can bind to A β and block aggregation.	Decreased A β_{1-40} aggregation in cellular models ²⁶
Non-peptidic anti-aggregants: RS-0406, SEN1269, SP-233	Decreased A β aggregation in cellular models ²⁷
Targeting of Aβ clearance	
Anti-A β antibodies: promote A β removal from the brain; several are in preclinical testing	Target specific antibodies, including: site-directed humanised monoclonal antibodies (binding to C-terminal, N-terminal, or mid-part of A β); conformation-specific antibodies; antibodies able to target intraneuronal A β ²⁸
Increasing brain resistance to Aβ	
Inhibitors of group IV phospholipase A2 (GIVA-PLA2): GIVA-PLA2 seems to be involved in A β -induced neurotoxicity; no specific drugs have been proposed	Genetic ablation or reduction of GIVA-PLA2 prevents A β -dependent cognitive impairment in AD animal models ²⁹
Compounds that decrease tau levels: tau protein might mediate A β -induced neuronal dysfunction; no specific drugs have been proposed	Genetic ablation or reduction of tau prevents A β -dependent cognitive impairment, without affecting plaque burden or A β oligomer levels in AD animal models ³⁰
Targeting of tau-protein	
Minocycline: modulates tau phosphorylation and aggregation	Decreased tau phosphorylation and tau aggregation, associated with inhibition of caspase-3-mediated tau cleavage; ³¹ decreased A β -induced neuronal death and cognitive decline ³² in animal models of AD and tauopathy
Tau anti-aggregants: N-744, rhodanines, phenylthiazolylhydrazide	Decreased tau aggregation in cellular models ³³
Anti-phospho tau antibodies: bind to the pathological hyperphosphorylated tau, limiting its neurotoxic effect	Decreased brain aggregated tau and decreased progression of tangle-related behavioural phenotype in mouse models ³⁴
Neurotrophins	
Brain-derived neurotrophic factor: involved in synaptic plasticity and neuronal survival, and reduced concentrations found in the brains of patients with AD ³⁵	Gene-delivery reversed synaptic loss, restored normal cell signalling and gene expression, and improved learning and memory in animals ³⁵
Modulation of synaptic plasticity and nerve growth	
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 A β ^{37,38} no specific drugs have been proposed	Disruption of Nogo receptor expression promotes increased A β brain levels, plaque deposition, and dystrophic neurites in a mouse model of AD; all these events are reduced by infusion of a soluble Nogo receptor fragment; ^{38,39} brain Nogo receptor overexpression is associated with decreased long-term memory in a mouse model ³⁷

APP=amyloid precursor protein. A β =amyloid beta. AD=Alzheimer's disease. PrP^C=normal form of the prion protein. GSM=gamma-secretase inhibitor. GIVA-PLA2=group IV isoform 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.⁶¹ The source of this transient A β increase in the plasma seems peripheral rather than central, because semagacestat can reduce CNS A β production and concentrations.⁶² 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 A β concentrations in the plasma (with delayed rebound)⁶³ but did not substantially affect CSF A β_{1-40} , whereas BMS-708163 promoted a dose-dependent decrease of A β_{1-40} in the CSF.^{64,65}

PF-3084014 is a γ -secretase inhibitor with high selectivity for APP compared with Notch, and results from animal studies showed decreases in A β in the plasma, CSF, and brain, without a rebound effect on plasma A β .⁶⁶ In a small phase 1 study on healthy volunteers, PF-3084014 promoted a dose-dependent reduction in plasma A β 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 A β_{1-40} and A β_{1-42} production, with increased generation of A β_{1-38} fragments.⁶⁰ 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 A β clearance by residual NSAID activity.⁷² Another γ -secretase modulator, CHF-5074, reduced A β 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 A β formation and

increase production of a soluble domain (sAPP α), 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 sAPP α 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 sAPP α secretion, restoring the healthy phenotype of fibroblasts isolated from patients with Alzheimer's disease.⁷⁸ Bryostatin-1 can reduce brain A β_{1-40} and A β_{1-42} and improve behavioural outcomes in mouse models of Alzheimer's disease;⁷⁸ 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 A β formation and accumulation in the mouse brain, with memory improvements. A phase 1 RCT was approved in 2008.⁷⁹

Drugs to prevent A β aggregation

Evidence for the neurotoxic and synaptotoxic activity of A β oligomers^{80,81} constitutes the scientific basis for the development of compounds that inhibit A β aggregation or destabilise A β oligomeric species. Non-peptidic anti-aggregants tested so far are chemically heterogeneous, and their pharmacodynamics are not clear. They can act by binding to A β monomers, thus preventing oligomerisation and allowing elimination; alternatively, anti-aggregants can react with A β oligomers, neutralising their toxicity and promoting clearance.²⁷ 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 A β 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.²⁷ As a consequence of the outcome of this study, a

European phase 3 RCT with tramiprosate was discontinued.²⁷ In 2008, Bellus Health commercialised tramiprosate as Vivimind, an over-the-counter nutritional that protects memory functions, attracting criticism from the scientific community.⁸⁴

Clioquinol (PBT1), an inhibitor of A β aggregation that works by interfering with interactions between A β , copper, and zinc, showed positive results in phase 2 RCTs, but manufacturing problems (high concentrations of toxic di-iodo impurity) stalled further development.⁸⁵ PBT2 is a second-generation inhibitor of metal-induced A β aggregation that is orally administered and has a greater blood–brain barrier permeability than does clioquinol; it binds to the A β –copper or A β –zinc complex, and studies in animals showed that PBT2 prevents A β oligomerisation, promotes A β oligomer clearance, reduces soluble and insoluble brain A β , and decreases plaque burden, with positive effects on cognition.⁸⁵ PBT2 is also thought to redistribute metals from extracellular sites, increasing availability of metal ions to neurons.⁸⁵ This effect could promote matrix metalloproteinase expression, which might increase A β degradation.⁸⁶ Furthermore, normalisation of zinc and copper concentrations in the glutamatergic synapse might improve NMDA receptor function and restore long-term potentiation.⁸⁵ In a 12-week, phase 2 RCT in patients with mild Alzheimer's disease, PBT2 was well tolerated, reduced A β_{1-42} CSF concentrations, and improved executive function.⁸⁷

Scyllo-inositol (scyllo-cyclohexanehexol, AZD-103, ELND-005), an orally administered stereoisomer of inositol, crosses the blood–brain barrier using inositol transporters. Scyllo-inositol is thought to bind to A β , modulate its misfolding, inhibit its aggregation, and promote dissociation of aggregates.⁸⁸ In studies in animals, scyllo-inositol reduced brain concentrations of soluble and insoluble A β_{1-40} and A β_{1-42} , plaque burden, synaptic loss, and glial inflammatory reaction, and improved spatial memory function.⁸⁸ This compound was well tolerated in healthy volunteers and reached the CNS at doses associated with therapeutic effects in animals.⁸⁹ The drug is being tested in a phase 2 RCT in patients with mild-to-moderate Alzheimer's disease (NCT00568776), but because of high rates of serious adverse events (including nine deaths) among patients in the two high-dose groups (1000 mg and 2000 mg twice daily), these doses have been removed from the RCT, while the study continues unchanged for patients who are assigned the lower dose (250 mg twice daily) and placebo.⁹⁰

Epigallocatechin-3-gallate (EGCg), a polyphenol from green tea, induced α -secretase and prevented A β aggregation in animals by directly binding to the unfolded peptide.⁹¹ EGCg also modulates signal transduction pathways, expression of genes regulating cell survival and apoptosis, and mitochondrial function.⁹¹ EGCg is being tested in patients with multiple sclerosis, and a phase 2–3 RCT in patients with early Alzheimer's disease is ongoing (NCT00951834).

Drugs to promote A β clearance

Active and passive immunisations were developed to inhibit generation of toxic A β aggregates, and to remove soluble and aggregated A β . At least three different immune-mediated mechanisms can promote A β removal: solubilisation by antibody binding to A β ; phagocytosis of opsonised A β by microglia; and A β extraction from the brain by plasma antibodies (the “sink” hypothesis).

Active immunotherapy

In a phase 2 RCT of AN-1972 (QS-21), an anti-A β vaccine, in patients with mild-to-moderate Alzheimer's disease, patients responded to immunisation with A β_{1-42} , developing significant A β -antibody titres, although not all individuals produced high IgG concentrations.⁹² 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-1972. 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.

CAD-106 is a vaccine that comprises the A β_{1-6} peptide coupled to the Q β virus-like particle. This vaccine can induce A β -specific antibodies and reduce amyloid accumulation in animals, without stimulating T cells and causing microhaemorrhages. In patients with mild-to-moderate Alzheimer's disease, CAD-106 induced a substantial anti-A β IgG response, was well tolerated, and did not induce meningoencephalitis;⁹³ confirmatory phase 2 RCTs are ongoing (NCT01097096, NCT01023685, NCT00795418).

ACC-001 and V-950 are vaccines that are based on the N-terminal A β fragment, which contains the B-cell epitope. ACC-001 is conjugated to the mutated diphtheria toxin protein CRM19, and is being studied in a phase 2 trial (NCT00479557). V-950 is being tested in a phase 1 trial as an aluminium-containing adjuvant with or without ISCOMATRIX (CSL Behring, PA, USA), a biological adjuvant of saponin, cholesterol, and phospholipids (NCT00464334). ACI-24 is a vaccine that contains A β_{1-15} embedded within a liposomal surface, and reduces brain amyloid load and restores memory deficits in mice.⁹⁴ This vaccine is entering a phase 1 RCT. UB-311 is a vaccine in which the immunogen A β_{1-14} is associated with the UB1Th peptide (United Biomedical, NY, USA) and a mineral salt suspension adjuvant,⁹⁵ and is being tested in patients with mild-to-moderate Alzheimer's disease in a phase 1 RCT (NCT00965588).

Another active immunisation strategy is based on Affitopes, short peptides mimicking parts of native A β_{1-42} without its sequence identity. Affitopes AD-01 and AD-02 target the N-terminal A β fragment and both had disease-modifying properties in animal models of Alzheimer's disease.⁹⁶ Phase 1 RCTs recently finished, and the results

indicated that AD-01 and AD-02 are safe and well tolerated (NCT00495417, NCT00633841).⁹⁶ 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 A β to promote its clearance. Results from animal studies have shown that anti-A β antibodies can prevent oligomer formation and reduce brain amyloid load with improvement in cognitive functions.⁹⁷ 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-A β 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* ϵ 4 non-carriers.⁹⁸ A dose-related transient vasogenic oedema was a more common side-effect in *APOE* ϵ 4 carriers. Phase 3 studies are ongoing, including separate RCTs for *APOE* ϵ 4 carriers and non-carriers (NCT00909675, NCT00574132, NCT00996918, NCT00998764).

Solanezumab, a monoclonal antibody that binds specifically to soluble A β , promotes A β clearance from the brain through the blood. In a phase 2 trial, the correlation between total plasma A β_{1-42} 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 A β_{1-42} , suggest that solanezumab might mobilise A β_{1-42} from plaques, and might normalise soluble CSF A β_{1-42} in patients with Alzheimer's disease.⁹⁹ 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 A β_{1-40} . Preliminary results on a single-dose regimen indicate that this antibody is well tolerated in patients with Alzheimer's disease,¹⁰⁰ 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 A β and have been tested in patients with Alzheimer's disease in phase 1 studies (NCT00459550, NCT00531804, NCT00736775).

Passive immunisation can also be achieved by intravenous infusion of immunoglobulins (IVIg), from

healthy donors, which include naturally occurring polyclonal anti-A β 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 A β CSF concentrations, and increased plasma total A β concentrations,^{101,102} with evidence of improvement or stabilisation of cognitive functions. Preliminary data from a phase 2 RCT confirmed the positive effects on cognition;¹⁰³ 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 A β 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.¹⁰⁴ 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 A β in Alzheimer's disease could be confirmed by ongoing RCTs. Results from the more detailed analyses of the AN-1972 trial have been mixed. There was some evidence of memory improvement and reduced CSF tau concentrations in patients with increased IgG titres.⁹² However, patients immunised with AN-1972 had a greater brain atrophy rate on MRI than did patients given placebo; this might be because of amyloid removal and cerebral fluid shifts.¹⁰⁵ In a follow-up study on a subsample of participants from the phase 2 RCT, brain volume loss in antibody responders (anti-A β titres \geq 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.¹⁰⁶ Responders maintained low, but detectable, anti-AN-1972 antibody titres at about 4.6 years after immunisation and had significantly reduced functional decline compared with placebo-treated patients.¹⁰⁶ A follow-up report on a phase 1 study showed that immunisation with AN-1972 could completely remove amyloid plaques as determined by post-mortem assessment, but patients still had end-stage dementia symptoms before death.⁵³ This finding suggests that clearance of amyloid plaques alone cannot repair already damaged neurons and prevent disease progression. Furthermore, intraneuronal concentrations of A β are high in patients with Alzheimer's disease and how immunotherapy affects this pool of A β is unclear.¹⁰⁷ Whether immune-mediated clearance of soluble

A β 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.¹⁰⁸ 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.^{109,110}

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.⁸ 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.⁸

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.¹⁰⁹ Both drugs can also be neuroprotective by upregulating the anti-apoptotic factor BCL2, inducing neurotrophic factors, and hindering A β toxicity.¹⁰⁹ 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 A β ₁₋₄₂.¹¹¹

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.^{109,110}

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.¹¹² 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.¹¹³

This compound also has antioxidant properties, enhances mitochondrial function,¹¹⁴ and was effective, alone and in combination with rivastigmine, in reversing learning deficits and hyoscine-induced memory impairments in animals.¹¹⁵ 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.¹¹⁶ 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,¹¹⁷ and phase 3 RCTs are needed to confirm its safety and clinical efficacy.

Davunetide (AL-108, NAP), an intranasally administered, 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 A β toxicity.¹¹⁸⁻¹²⁰ Davunetide was studied in patients with amnesic mild cognitive impairment in a 12-week, phase 2 RCT and was safe, well tolerated, and had positive effects on cognition,¹²¹ 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.¹²² Furthermore, nicotinamide inhibits brain sirtuin deacetylase and upregulates acetyl- α -tubulin, protein p25, and MAP2c; all these interactions are associated with increased microtubule stabilisation.¹²² 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.¹²³ APP and A β can be imported into mitochondria, where they can interact with mitochondrial components, impair ATP production, and increase oxidative damage.^{123,124}

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

significantly improved the clinical course of patients with mild-to-moderate Alzheimer's disease, ameliorating all outcome measures.¹²⁵ However, these results were not confirmed in a phase 3 RCT (CONNECTION study).¹²⁶ Results from other ongoing RCTs (latrepirdine in combination with donepezil and memantine) are awaited (NCT00829374, NCT00912288). Preliminary results of a phase 1, 4-week, placebo-controlled safety study in patients with Alzheimer's disease on a stable dose of donepezil showed that the therapeutic combination was well tolerated.¹²⁷

Latrepirdine weakly inhibits acetylcholinesterase and butyrylcholinesterase. This drug also inhibits NMDA receptors and voltage-gated calcium channels,¹²⁸ but its potency for NMDA receptors is 200-times less than that of memantine, and its neuroprotective effect is mainly attributed to preserving mitochondrial structure and function. The most potent activity identified for latrepirdine so far is its enhancement of mitochondrial function both under stress and non-stress conditions. Latrepirdine was suggested to inhibit the mitochondrial permeability transition pore ($A\beta$ -induced activation of this pore can induce apoptosis)¹²⁹ and protect neuronal mitochondria from $A\beta$ -mediated toxicity and other insults.¹³⁰ Latrepirdine can also increase mitochondrial membrane potential and ATP production.¹³¹

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.¹³² Targeted delivery of NGF to basal forebrain cholinergic neurons prevented cell death, stimulated synaptic cholinergic function, and promoted cognitive improvement in animals.¹³² Studies in patients with Alzheimer's disease were initially based on intracerebroventricular infusion of NGF.^{133,134} The positive results on cognition and physiological measurements of brain functioning were counterbalanced by adverse effects (eg, pain, weight loss),^{133,134} 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.¹³⁵ Although two individuals had subcortical haemorrhage during implantation, an overall lower rate of cognitive decline and increased cortical glucose uptake were reported.¹³⁵ 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).¹³⁶ 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.¹³⁶ 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.^{136,137} 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.¹³⁷

Intranasal delivery and topical application of an NGF solution on the ocular surface are being tested in preclinical stages.¹³² 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.^{138,139} 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.¹⁴⁰⁻¹⁴² 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.¹⁴³ Antioxidant supplementation has also not been effective in treatment trials.¹⁴⁴ 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.^{145,146}

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.¹⁴⁷ Statins reduce cholesterol concentrations and have several pleiotropic effects (ie, can lower $A\beta$ production and reduce $A\beta$ -mediated neurotoxicity, as well as having antioxidant and anti-inflammatory properties).¹⁴⁷ 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

donepezil.¹⁴⁸ 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 Amnesic 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,¹⁴⁹ but RCTs of the 5-HT₄ agonist PRX-03140 were terminated when EPIX Pharmaceuticals went into liquidation (NCT00693004, NCT00672945).¹⁵⁰

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

Recommendations	
Patients	
Target group selection: patients with AD have various types of neuropathology (ie, amyloid plaques, NFTs, infarcts, Lewy bodies); ¹⁵⁵ there are mixed causes of dementia in many patients, particularly those who are older than 80 years	Criteria for identifying subgroups with more homogeneous biomarker evidence of AD pathology are needed to facilitate RCTs ¹⁵⁶ but drug approval would be limited to the same criteria used to subdivide patients; the need to better identify homogeneous and responsive groups of patients does not mean that we cannot consider possible solutions for the large group of patients with dementia who have mixed causes (who are usually excluded from RCTs)
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) ⁵³	RCTs that include patients with early AD might enable detection of disease-modifying effects; however, different drugs can have different therapeutic windows: investigation into which stage of the AD process a therapeutic strategy is more effective is warranted
Drugs	
Choosing the right drug: compounds with positive results in preclinical and early clinical testing failed in large phase 3 RCTs, with costly losses (eg, tramiprosate) ⁵³	Robust proof-of-concept studies should be mandatory; investigators must take into account class efficacy evidence when available (ie, if a drug has the same mechanism of one that previously failed, justification why the new compound might be effective is needed); the use of drug-related biomarkers in preclinical and early clinical stages can help to confirm the target engagement and to assure early withdrawal of ineffective drugs
Optimisation of drug dosage and treatment duration: some RCTs could have been hindered by the inability to reach a therapeutic dosage (eg, tarenfluril), ⁷² or treatment duration could have been too short to detect a disease-modifying effect (eg, lithium, latrepirdine) ¹¹¹	Adequate dosing and titration phase is required to assure a fair test of drug efficacy; clarification of pharmacokinetics is important (ie, whether a drug reaches sufficient concentrations in human CNS) and age-related changes that can affect drug concentrations at brain targets should be taken into account; adequate duration of RCTs will enable detection of a disease-modifying effect, and also evaluation of long-term consequences of the therapy (one concern in anti-amyloid strategies is to verify that they do not stabilise or increase concentrations of toxic A β intermediates)—a duration of 18 months has been suggested for RCTs, but so far many RCTs with this duration have not reported positive results ¹⁵⁷
Genetics: polymorphisms (eg, APOE, cytochrome P450) might affect drug response ^{98,158}	Moving towards a tailored therapeutic approach: considering genetic polymorphisms that affect drug response might be useful when selecting the more effective drug class for individual patients, and can help to optimise drug dosage (eg, increased doses for individuals with a rapid metabolism)
Outcome measurements	
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, ¹⁵⁴ 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 ^{153,157}	Development and use of relevant, reliable, multidimensional measures for clinical (cognitive and functional) endpoints are key factors, as well as the use of biomarkers (neuroimaging, CSF or blood molecules) that reliably and quantitatively correlate with disease progression; collection of baseline data (clinical, biomarkers) that can be used as reference to interpret later findings is advisable; for early AD (ie, mild cognitive impairment), self-rated and observer-rated assessments of activities of daily living, instrumental activities of daily living, and quality of life are recommended
Reliable evaluation of patients: inadequate training and monitoring of RCT raters might increase variability and introduce errors during enrolment and evaluation of patients ¹⁵³	Adequate training and monitoring of RCT raters will ensure homogeneous recruitment of patients, reducing variance, and guaranteeing a more accurate rating, thus increasing confidence in observed outcomes; effective implementation of quality control on data at research sites is recommended
Trial protocol	
RCT protocols: can be too exhausting and time-consuming for the patient and the caregiver, thus increasing withdrawal rates	Frequency, duration, and complexity of visits should avoid stress to patients and caregivers and reduce withdrawals; cognitive testing should not be too long and tiring for patients (ie, limit the number of tests, set cut-off for stopping testing)
Informed consent: often involves a long document that is difficult to read and fully understand	The consent document must be informative for patients and caregivers; on the basis of our experience, it should be simplified and not exceed two pages
Optimisation of resources	
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)	Multicentre trials should use centres of excellence that are already experienced in RCTs to minimise inter-site and inter-country variability
Avoid repeating errors: unsuccessful preclinical and clinical studies are often not published and sponsors are reluctant to release information; ¹⁵³ similar planning and methodological problems occur in different RCTs ¹⁵⁴	Companies should share their experience; access to data of failed studies can positively affect drug development; more collaboration between pharmaceutical companies and clinical researchers, with information sharing, can lead to more standardised RCT protocols, reduction of errors, and decreased costs
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.	
Table 3: Challenges and problems in RCTs for AD	

phosphodiesterase 9A inhibitor, able to increase cGMP concentrations in the CSF of healthy volunteers,¹⁵¹ 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 A β , 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.¹⁵² The soluble form (sRAGE) can compete for A β binding with the membrane-linked RAGE, thus promoting removal of circulating A β .¹⁵² 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.^{153,154} 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 biomarkers¹⁵⁹ and error management checklists for drug developers¹⁶⁰ 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,¹⁶¹ 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).¹⁶² 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.¹⁶² 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,¹⁶³ 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.⁴⁰

Multi-target therapies can be designed in several ways.⁴⁰ 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:⁴⁰ 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.clinicaltrial.gov>) and the Cochrane Dementia and Cognitive Improvement Group (<http://www.medicine.ox.ac.uk/aloids>) 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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