Alzheimer's disease: strategies for disease modification

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Abstract | Alzheimer's disease is the largest unmet medical need in neurology. Current drugs improve symptoms, but do not have profound disease-modifying effects. However, in recent years, several approaches aimed at inhibiting disease progression have advanced to clinical trials. Among these, strategies targeting the production and clearance of the amyloid-β peptide — a cardinal feature of Alzheimer's disease that is thought to be important in disease pathogenesis — are the most advanced. Approaches aimed at modulating the abnormal aggregation of tau filaments (another key feature of the disease), and those targeting metabolic dysfunction, are also being evaluated in the clinic. This article discusses recent progress with each of these strategies, with a focus on anti-amyloid strategies, highlighting the lessons learned and the challenges that remain.

Alzheimer's disease (AD) — the most common form of irreversible dementia — is placing a considerable and increasing burden on patients, caregivers and society, as more people live long enough to become affected (BOX 1). AD is clinically characterized by a progression from episodic memory problems to a slow global decline of cognitive function that leaves patients with end-stage AD bedridden and dependent on custodial care, with death occurring on average 9 years after diagnosis[1].

The current standard of care for mild to moderate AD includes treatment with acetylcholinesterase inhibitors to improve cognitive function. The NMDA (*N*-methyl-D-aspartate) antagonist memantine has also been shown to improve cognitive function in patients with moderate to severe AD. In addition, the common non-cognitive neuropsychiatric symptoms of AD (such as mood disorder, agitation and psychosis) often require the introduction of medication, even though no existing drug is specifically indicated for their management. However, at this point, there is no approved treatment with a proven disease-modifying effect.

This Review provides an overview of the rationale and the issues that underlie the development of disease-modifying approaches to treat AD, and discusses the current status of the field. Space constraints make it impossible to cover all preclinical approaches or ongoing clinical trials, and so this article should not be viewed as a comprehensive list. Instead, the focus here is on the strategies aimed at slowing or halting disease progression, which has implications for the design of clinical studies (BOX 2). Only some of the more advanced clinical programmes are mentioned in the context of each mechanistic approach, and inclusion or omission from this Review should not be construed as endorsement or rejection of a specific programme.

Rationale for disease-modifying strategies

Disease-modifying strategies currently being pursued for AD are based on at least one line of evidence that supports the notion that the targeted process is important in AD, which can be grouped into the following categories: pathology, genetics and epidemiology.

Pathology. Post-mortem analysis of human AD brains provided the first clues to the mechanisms of disease and potential interventions. It led to the description of the disease by Alzheimer a century ago[2], and the identification of the hallmark lesions of AD — senile plaques composed of extracellular deposits of amyloid-β (Aβ) and neurofibrillary tangles formed by accumulation of abnormal filaments of tau — in brain regions that serve memory and cognition. Besides these hallmarks, prominent activation of inflammatory processes and the innate immune response are observed (for a review see REF. 3). However, determining whether a given pathological structure drives the disease, is a neutral bystander, or just represents an unsuccessful repair attempt remains challenging. Moreover, in an end-stage AD brain there are so many biochemical changes relative to a normal brain that numerous strategies can be rationalized by differences in gene expression or protein concentration between them.

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Box 1 | The emerging Alzheimer's disease epidemic

The socioeconomic impact of dementia disorders worldwide is enormous, but difficult to quantify exactly. More than 25 million people are suffering from dementia and the annual total worldwide costs have been estimated to exceed US$200 billion[104]. According to the Alzheimer's Association, in 2009 an estimated 5.3 million people in the United States of America have Alzheimer's disease (AD), which is now the sixth leading cause of death in the United States. As increasing age is the biggest risk factor for the disease, the incidence will increase to an estimated 7.7 million cases in 2030 and 11–16 million cases in the United States in 2050. These numbers do not include the large number of people with mild cognitive impairment, a significant proportion of whom will progress to AD. Patients with AD are high users of health care and long-term care services. In the United States there are currently 9.9 million unpaid family caregivers under great emotional burden. AD and other dementias cost Medicare $91 billion per year and Medicaid $21 billion. The total annual costs of AD in the United States are estimated at $148 billion[105].

Genetics. Mutations in three genes — amyloid precursor protein (*APP*), presenilin 1 (*PS1*; also known as *PSEN1*) and *PS2* (also known as *PSEN2*) (reviewed in REF. 4) — and duplication of the *APP* gene[5] all lead to early-onset autosomal dominant AD. From a therapeutic perspective, targeting the mechanisms of familial early-onset AD makes the implicit assumption that this disease is fundamentally similar to the common sporadic late-onset form. The genetics of the more common late-onset AD is an active area of investigation. The ε4 allele of the apolipoprotein E (*APOE*) gene has been identified as the major risk factor for late-onset AD[6]. Exactly how the mutated genes or different isoforms increase the risk of disease risk is not clear, and, at least in the case of *APOE4*, a consensus mechanism of pathogenesis has not emerged in more than a decade after the discovery of its role in AD.

Epidemiology. No specific environmental toxin has been found to be consistently associated with AD, and there have been no randomized clinical trials as yet to support any specific dietary intervention. Epidemiological studies point to depression, traumatic head injury and cardiovascular and cerebrovascular factors (for example, cigarette smoking, midlife high blood pressure, obesity and diabetes) as increasing disease risk, while anti-inflammatory medications seem to reduce risk (see below). Some studies even suggest a beneficial role of psychosocial factors (for example, higher education, physical exercise and mental activity) (for a review see REF. 7). Such studies may point to a role of previously unconsidered pathways in the aetiology of the disease, but the mechanistic interpretation of retrospective epidemiological studies is challenging.

Box 2 | Disease modification versus symptomatic improvement

The treatment approaches discussed in this article all aim to interfere in the mechanisms that drive the progression of Alzheimer's disease (AD). In contrast to currently approved drugs, such treatments are not expected to lead to rapid symptomatic improvement (clinical trials of 3 to 6 months), but to block or reduce the progressive, but slow cognitive decline of patients with AD. It follows that formal demonstration of efficacy requires trials of extended duration (18 months or more) with a large number of participants to demonstrate a statistical difference in the slope of cognitive decline[106]. Biomarkers that reflect pathogenesis and the effects of drug should be measured concurrently[107]. It also follows that for disease modification, Phase II trials with small numbers of participants can inform about safety and biomarker changes, but they cannot predict efficacy, even though they are occasionally overinterpreted that way.

Models of disease and their limitations. Ideally, one would use animal models to decide which disease-modifying strategy to pursue. Unfortunately, so far, there is no animal model that replicates all or most of the major aspects of AD pathology and symptomatology. Instead, models based on postulated disease pathways are widely used to explore target biology and to test pharmacodynamic effects of potential treatments. For example, transgenic mice based on the amyloid hypothesis of AD, such as Tg2576 (REF. 8) — which overexpresses a mutant form of *APP* and deposits Aβ in a temporal and spatial pattern similar to human AD, but does not develop neuronal tangles or major neuronal loss — are widely used to study the effect of anti-amyloid therapies. When interpreting data from such models it is important to be aware of potential confounding factors due to *APP* overexpression, including effects of APP itself, increased levels of APP metabolites other than Aβ, and changes in relative Aβ levels in different compartments. Similarly, tau transgenic models, such as Tg4510 (REF. 9) — which overexpresses a mutant form of tau and develops neurofibrillary tangles, brain atrophy and functional deficits — are being investigated to study effects of treatments on tau pathology. However, the success of treatments in an animal designed to model a pathway and drive pathology and/or behavioural changes predicts only that the treatment may successfully interfere with the pathway in patients, not that interference with the pathway will have efficacy in AD, which can only be established in clinical trials. The remainder of this article will focus on those approaches that have progressed to this stage.

Aβ-related treatment approaches

Genetic and pathological evidence strongly supports the amyloid cascade hypothesis of AD, which states that Aβ, a proteolytic derivative of the large transmembrane protein APP, and in particular the least soluble 42 amino-acid long $A\beta_{42}$ isoform, have an early and vital role in all forms of AD (FIG. 1). Five key arguments support a crucial role of Aβ in the pathogenesis of AD (for reviews see, for example, REFS 10–12). One, amyloid deposits provide early pathological evidence of AD and neuritic plaques are a key diagnostic criterion. Two, in peripheral amyloidoses (unrelated to Aβ and AD), amyloid burden drives tissue dysfunction, thereby suggesting that brain amyloid is pathogenic as well. Three, Aβ oligomers show acute synaptic toxicity effects, whereas plaque-derived Aβ fibrils have pro-inflammatory effects and cause neuronal toxicity. Four, the most important genetic risk factor, *APOE4*, is associated with increased amyloid burden. Five, most importantly, all mutations that cause familial early-onset AD increase $A\beta_{42}$ production or the ratio of $A\beta_{42}$ compared to the less aggregation-prone $A\beta_{40}$ isoform. All these mutations directly enhance amyloidogenic APP processing: *APP*

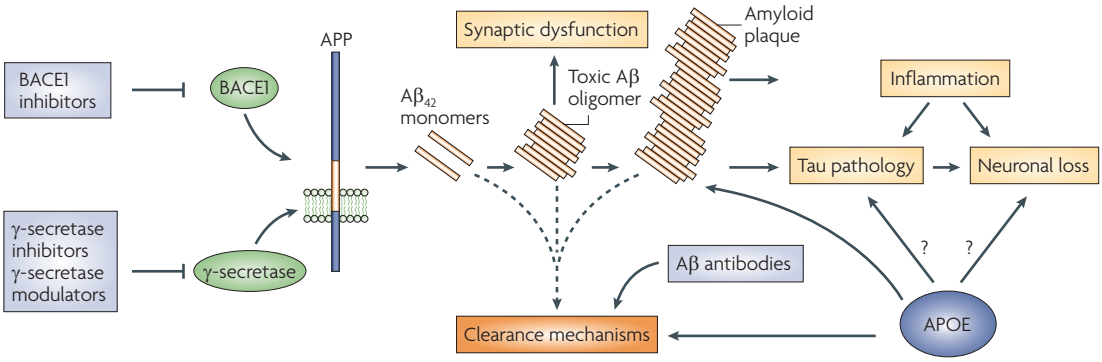

Figure 1 | **The amyloid cascade and major therapeutic approaches.** The transmembrane protein amyloid precursor protein (APP) is sequentially cleaved by two proteases, β-secretase (also known as β-site APP cleaving enzyme 1; BACE1) and γ-secretase, to release various isoforms of the amyloid-β (Aβ) peptide. The most aggregation-prone $Aβ_{42}$ isoform aggregates to form toxic oligomers and deposits in amyloid plaques. Oligomers have acute synaptotoxic effects, whereas amyloid plaques lead to an inflammatory response. The amyloid cascade is thought to trigger downstream tau pathology (FIG. 3). Apolipoprotein E (APOE) directly affects the amyloid cascade via effects on Aβ deposition and/or clearance. The fact that the ε4 allele of APOE is a risk factor in a number of neurological disorders suggests a direct effect on neurodegeneration[86]. A major therapeutic effort is aimed at reducing $Aβ_{42}$ production with BACE1 inhibitors, and with γ-secretase inhibitors and modulators. A different class of therapeutics aims to enhance the clearance of Aβ (FIG. 2). Most of these are therapeutic antibodies or vaccines directed at soluble monomeric Aβ and/or oligomers and/or plaques. Some efforts are directed at reducing Aβ aggregation (not shown).

mutations by changing the substrate properties of APP and *PSEN* mutations by changing the properties of the γ-secretase complex.

Based on this evidence, several Aβ-targeted therapeutic strategies are being pursued, including modulation of Aβ production, inhibition of Aβ aggregation, enhancement of Aβ degradation, and immunotherapy targeted at Aβ.

Modulation of Aβ production. The most direct approach in anti-amyloid therapy is reduction of $Aβ_{42}$ production. Aβ is generated proteolytically from a large precursor molecule, APP, by the sequential action of two proteases: β-secretase (also known as β-site APP cleaving enzyme 1; BACE1) and γ-secretase (FIG. 1). A third protease, α-secretase, which competes with β-secretase for the APP substrate, can preclude the production of Aβ by cleaving the peptide in two. This scenario immediately suggests three strategies to reduce Aβ: inhibition of γ-secretase, inhibition of β-secretase, or stimulation of α-secretase. All these strategies have been actively pursued for more than a decade.

γ-secretase was the first target in the amyloid pathway to be intensely pursued for drug development. Efforts began in the early 1990s, when it was demonstrated that tissue cultured cells express β-secretase and γ-secretase to constitutively generate Aβ peptide[13]. This finding triggered screening campaigns to identify non-toxic inhibitors of cellular Aβ production. Several different classes of molecules were identified, and secondary assays demonstrated that these compounds inhibited the production of all Aβ isoforms via the γ-secretase but not the β-secretase pathway. Medicinal chemistry programmes ultimately led to drug-like molecules that could reduce plasma and soluble brain Aβ in mice after only a few hours and with only single administration[14]. Data from several groups have now demonstrated that γ-secretase is an unusual transmembrane protease complex, consisting of at least four proteins: presenilin (PSEN), nicastrin (NCSTN), alphaprotein 1A (APH-1A) and presenilin enhancer 2 (PEN2) (for a review see REF. 15). Owing to this complex structure, it will be difficult to obtain high resolution structural information on the active site and to understand the enzyme in depth.

A more pressing concern from the drug development perspective is the effects of γ-secretase inhibition on substrates other than APP. For most of these substrates[16], there are no data showing that reduced cleavage due to γ-secretase inhibition would have adverse consequences in an adult animal (which does not rule out the possibility that long-term studies may still show problems). However, the crucial importance of γ-secretase cleavage of one substrate, the Notch receptor, has slowed the development of γ-secretase inhibitor drugs. Concerns about mechanism-based liabilities were triggered by the finding that deletion of the γ-secretase component PSEN1 caused a lethal phenotype similar to a *Notch1* knock out[17], indicating that γ-secretase cleavage of Notch1 is essential during embryonic development. Studies with several structurally different γ-secretase inhibitors at high doses have shown that inhibition of Notch1 cleavage blocks thymocyte differentiation and splenic B-cell maturation, and causes intestinal goblet-cell metaplasia in adult animals[18,19].

How can one accomplish significant Aβ reduction without clinical safety problems due to Notch inhibition? Several molecules, currently progressing in clinical trials, seem to overcome this issue. Eli Lilly and Company (Lilly) recently announced advancement of its γ-secretase inhibitor semagacestat into pivotal Phase III studies based on safety, tolerability and biomarker data from Phase II studies, which demonstrated safe lowering

Table 1 | **Proposed mechanisms of action of compounds in trials for Alzheimer's disease modification***

Name (initial sponsor)	Description	Proposed mechanism of action	Selected refs
Semagacestat (Eli Lilly and Company)	γ-secretase inhibitor	Reduces Aβ synthesis	20,108
Bapineuzumab (Elan and Wyeth)	Humanized monoclonal antibody to Aβ	Binds to Aβ deposits and reduces amyloid load primarily through microglial clearance	59,67
Solanezumab (Eli Lilly and Company)	Humanized monoclonal antibody to Aβ	Binds to soluble Aβ and reduces amyloid load via peripheral sink mechanism	61,65
Intravenous immunoglobulin G (Baxter)	Human immunoglobulin preparation containing endogenous polyclonal antibodies to Aβ	Primarily binds to soluble Aβ and reduces amyloid load via peripheral sink mechanism	54,66

Aβ, amyloid-β. *This table lists the four molecules that are currently in Phase III trials for Alzheimer's disease modification. In addition, dimebon (an antihistamine with neuroprotective properties[100] developed by Medivation and Pfizer, is currently in several Phase III trials to confirm the symptomatic benefits that were observed in Phase II trials[99].

of both plasma[20] and cerebrospinal fluid Aβ levels[21] (TABLE 1). In addition, both Wyeth and Bristol–Myers Squibb have disclosed information on γ-secretase inhibitors that are advancing into Phase II clinical studies[22,23].

In an ideal scenario, $A\beta_{42}$ production would be blocked without suppressing Notch cleavage at all. This is possible in principle, as at high concentrations, certain non-steroidal anti-inflammatory drugs (NSAIDs) have been shown to modulate γ-secretase cleavage such that $A\beta_{42}$ is reduced, while at the same time the production of smaller Aβ isoforms that are expected to be less prone to aggregation than $A\beta_{42}$ is increased[24]. However, Notch cleavage was not found to be blocked[25]. Further studies with $A\beta_{42}$-selective modulators revealed that the $A\beta_{42}$ reduction is not mediated by cyclooxygenase (COX) inhibition or other known non-COX targets of NSAIDs, but by direct interaction of the compounds with γ-secretase[26] or its substrate[27]. These studies may provide a mechanistic explanation for the finding from epidemiological studies that chronic intake of some NSAIDs can decrease the risk of developing AD by more than 50%[28].

Two approaches for the development of Notch-sparing γ-secretase modulators are being pursued. Myriad advanced *R*-flurbiprofen (the enantiomer of the NSAID flurbiprofen that has almost no COX activity) into clinical development despite its low *in vitro* potency. It culminated in the largest 18-month Phase III clinical trial in AD completed so far. However, patients treated with *R*-flurbiprofen did not show differences from patients receiving placebo in their cognitive decline over time, and primary end points were not met[29]. The study did not address pharmacodynamic effects of *R*-flurbiprofen on plasma or cerebrospinal fluid $A\beta_{42}$ levels[29]. However, a previous Phase I study of *R*-flurbiprofen at the same dose had not detected plasma or cerebrospinal fluid $A\beta_{42}$ effects, suggesting that in the Phase III study $A\beta_{42}$ in the central nervous system (CNS) may not have been lowered either[30]. Alternatively, companies are trying to identify γ-secretase modulators that have qualitatively similar effects on Aβ and Notch as NSAIDs, but that are orders of magnitude more potent. Eisai has disclosed the advancement of such a compound, its γ-secretase modulator E2012, into clinical development[23].

The second main strategy for targeting Aβ production involves targeting β-secretase, which was identified in 1999 as the transmembrane aspartic protease BACE1. Although the transmembrane domain is a new feature of a mammalian aspartic protease, β-secretase seems to be a regular aspartic protease. It is a type I transmembrane protein with the active site on the luminal side of the membrane where it cleaves APP. Tissue culture and animal studies indicate that β-secretase is expressed in all tissues, but the levels are higher in the brain (for a review see REF. 31). No mutations in *BACE1* have been reported to cause AD, but enhanced β-secretase activity has been detected in the brains of patients with sporadic AD. At this point it is unknown whether the increased β-secretase activity leads to the observed pathology or whether it is just a sequela of late-stage AD pathology. However, in preclinical models, increased brain *BACE1* expression can also be triggered by energy deprivation[32], leading to the intriguing possibility that brain glucose hypometabolism, as observed in *APOE4* carriers, could directly trigger the amyloid cascade.

The normal biological role of β-secretase is still unclear. As expected, *BACE1*-knockout mice are deficient in Aβ production, indicating that there are no compensatory mechanisms for β-secretase cleavage in mice. More surprisingly, the knockout mice did not seem to show serious problems due to β-secretase deletion: they were healthy and fertile, and clinical chemistry parameters were normal in both young and aged animals. Over the past couple of years, some β-secretase substrate candidates have been identified, but they have not been correlated with distinct pathology in the *BACE1*-knockout mice (for a review see REF. 31). One notable exception is type III neuregulin 1 (NRG1), a molecule that requires β-secretase-mediated cleavage for peripheral nerve myelination. *BACE1*-knockout mice show hypomyelination of peripheral nerves, because of reduced type III NRG1 cleavage early on postnatally, when *BACE1* expression is highest and when nerves are myelinated[33,34]. Interestingly, the only study so far that has addressed the issue with pharmacological inhibition of β-secretase in adult mice reported no significant effect on brain NRG1 processing despite significant lowering of Aβ levels[35]. Finally, a

recent sciatic nerve crush study suggested delayed remyelination in *BACE1*-knockout mice versus wild-type mice[36]. It is currently unknown whether administration of β-secretase inhibitors to an adult animal would affect remyelination after injury.

Behavioural consequences of *BACE1* knock out have been addressed in mouse studies in the absence or presence of *APP* transgenes. A clear consensus picture has not yet emerged: one study[37] reported more timid behaviour in *BACE1*-knockout mice compared with wild-type mice, whereas another[38] observed a lower level of anxiety. It is always difficult to extrapolate from a mouse knockout phenotype the effect of pharmacological intervention in humans due to developmental effects, compensatory changes and species differences. Therefore, whether any of the behavioural alterations seen in knockout mice will be predictive of the effects of β-secretase inhibition in humans remains unclear at this point, in particular given the major effects of strain background on behavioural phenotypes[39].

The absence of Aβ production and the distinct pathology in the *BACE1*-knockout mice is encouraging for β-secretase drug development. However, inhibitor development has proved to be highly challenging; so far, only one company has reported clinical data with a β-secretase inhibitor[40]. The most potent aspartic protease inhibitors are large hydrophilic peptides[41] and the need for blood–brain barrier penetration adds an additional hurdle on the path towards development of a β-secretase inhibitor (for a review see REF. 42).

Turning to the third strategy, α-secretase pathway stimulation leads to a reduction of the APP substrate that is available for the amyloidogenic pathway, and it was demonstrated early on that this pathway can be stimulated through cell-surface receptors (see, for example, REF. 43). However, much more APP enters the α-secretase pathway than the β-secretase pathway, so the desired reduction in Aβ requires a marked change in the metabolism of both APP and various other membrane proteins that are α-secretase substrates. The potential side effects of this approach are unknown. Stimulation of α-secretase has been explored in depth in the context of M_1 muscarinic receptor agonists, which could function as cognition enhancers and which have been reported to reduce Aβ production in a small clinical trial[44]. However, development of M_1 muscarinic receptor agonists has been hampered by the difficulty of generating M_1-specific molecules that do not cause side effects by activating other muscarinic receptors. No such molecules have been reported to be currently in clinical trials for AD.

Inhibiting Aβ aggregation. Normal cells constitutively generate small amounts of the various Aβ isoforms. Monomeric Aβ molecules, in particular $Aβ_{42}$, can form oligomeric aggregates that are thought to initiate the pathogenic cascade. It was originally assumed that only Aβ that had aggregated into the large fibrils that constitute the mature neuritic amyloid plaques would exert toxic properties. However, in recent years small soluble oligomeric assemblies of Aβ have attracted a lot of attention, as it was demonstrated that they can directly induce synaptic dysfunction. The exact nature of the pathogenic oligomeric species remains unclear (for a review see REF. 12) and a consensus pathogenic oligomer assembly mechanism has not yet emerged.

Nevertheless, in principle, developing brain penetrable small-molecule drugs that interfere with Aβ–Aβ peptide interactions seems an attractive approach. If the peptide interactions are the same in oligomers and in larger fibrils, then such molecules could inhibit both the formation of toxic oligomers and of neuritic plaques. If the peptide–peptide interactions were different in both aggregates, then one could theoretically identify molecules that interfere with just one or the other process. In this case, the assay set-up would be key to find molecules that block only formation of oligomers or molecules that block only formation of large fibrils. In the 1990s, several different assay formats for the identification of nucleation and deposition inhibitors that would block the formation of large fibrils were described. However, very few aggregation inhibitors have moved into clinical testing. One can only speculate whether it was simply not feasible to generate potent drug-like molecules that block Aβ–Aβ peptide interactions in a specific manner or whether decision-makers felt uncomfortable committing to this unvalidated mechanism of action for a drug.

Neurochem's tramiprosate — a small molecule reported to bind to Aβ monomers and maintain it in a non-fibrillar form[45] — progressed into large Phase III trials, but did not demonstrate efficacy. Drawing mechanistic conclusions from this trial is difficult, because it is not known whether the drug blocked Aβ aggregation in the brain. $Aβ_{42}$ reduction in cerebrospinal fluid had been reported in a previous Phase II trial of the drug[46], but whether this represents a desirable pharmacodynamic effect of an aggregation inhibitor is not clear. A different class of molecule, cyclohexanehexol isomers, has been suggested to stabilize Aβ into non-toxic conformers and inhibit Aβ fibril assembly *in vitro*, translating into the amelioration of several AD-related phenotypes in *APP* transgenic mice[47]. Elan is currently testing one of these isomers, ELND005, in Phase II trials for AD.

Another approach to interfere with toxic Aβ species is based on the notion that trace metals, in particular zinc and copper, contribute to amyloid pathology[48]. This has led to the investigation of orally available brain-penetrant 'metal–protein attenuating compounds'. The first of these compounds, clioquinol, has been reported to drastically reduce amyloid pathology in *APP* transgenic mice[49]. Prana is advancing a second-generation compound, PBT2, in Phase II trials[50].

Enhancing Aβ clearance. Over the past couple of years, several key enzymes involved in Aβ degradation have been identified, most notably the proteases neprilysin, insulin-degrading enzyme and plasmin[51]. From a drug development perspective, specific activation of enzymes is much more challenging than inhibition. At Wyeth, researchers have circumvented the problem of direct protease activation by blocking the inhibitor of a protease that is required to activate an Aβ-degrading

enzyme. Based on the finding that plasmin cleaves Aβ *in vitro* and that tissue plasminogen activator (required to generate plasmin from plasminogen) is inhibited *in vivo* by plasminogen activator inhibitor 1 (PAI-1), the authors generated orally available PAI-1 inhibitors that lower plasma and brain Aβ levels in transgenic mice[52].

It would not be necessary to directly activate Aβ-degrading proteases in the brain if one could move Aβ from the CNS to the periphery for degradation. Two potential targets, the receptor for advanced glycation end products (RAGE; also known as AGER), which mediates the influx of Aβ into the brain, and the low-density lipoprotein receptor-related protein 1 (LRP-1), which mediates efflux of Aβ from the brain, have been proposed to dominate Aβ transport at the blood–brain barrier. Moreover, Aβ–RAGE interactions have been proposed to activate nuclear factor-κB signalling pathways, which may promote apoptosis and inflammatory responses (for a review see REF. 53). If this model is correct, a RAGE inhibitor could lower amyloid load in the brain and also block the other detrimental effects of Aβ–RAGE signalling. Pfizer is currently testing PF-04494700, an oral small-molecule RAGE inhibitor, in Phase II trials for mild to moderate AD.

Immunotherapy. Over the past few years, Aβ immunotherapy has become one of the most exciting areas of research in AD, and more than ten immunotherapeutic agents have entered clinical trials. Three are currently in Phase III trials: Elan's bapineuzumab (humanized 3D6), Lilly's solanezumab (humanized 266) and Baxter's intravenous immunoglobulin G (IVIG), a preparation of human serum immunoglobulin that contains naturally occurring antibodies directed against Aβ[54] (TABLE 1).

The field began to draw attention after the publication of the first immunization paper from Elan[55], which reported that amyloid pathology was reduced in an *APP* transgenic mouse model after vaccination with aggregated $Aβ_{42}$. The outcomes of Aβ plaque burden, neuritic dystrophy and gliosis were all shown to be significantly improved by vaccination in both young and aged animals. The mechanism that resulted in plaque reduction did not seem to produce any obvious signs of damage to the brains of $Aβ_{42}$ immunized animals. The authors proposed that $Aβ_{42}$ immunization augments a highly specific immune response to clear Aβ, which markedly reduced the pathology in the animal model[55]. The reduction in amyloid pathology was subsequently reproduced in several studies using different transgenic mouse models[56,57]. Antibody-mediated resolution of peripheral light chain-associated amyloid deposits had been demonstrated before[58], but for the Aβ vaccination experiments it was not possible to rule out direct effects of the injected Aβ aggregates, a role of the adjuvant or involvement of a T-cell response. The Elan group subsequently reported that direct peripheral administration of mouse antibodies raised against human Aβ to a mouse amyloid model mimicked the effects of vaccination on amyloid burden. It was shown that a T-cell response was not required for amyloid plaque reduction and that the animals did not have abnormal leakage at the blood–brain barrier[59].

The mechanisms of AD immunotherapy are not fully understood. Four hypotheses, which are not mutually exclusive, have been proposed. Several studies by Elan provided data supporting a mechanism based on microglial activation and phagocytosis[55,59] (FIG. 2a). In this scenario, a small proportion of peripherally administered antibody reaches the CNS, binds to amyloid deposits and triggers endogenous microglia to phagocytose the amyloid. This mechanism requires antibodies to reach parenchymal deposits, but the action of the antibodies is almost catalytic in that they just need to activate the 'waiting' microglia, which seem unable to clear the amyloid by themselves. This explains how the 0.1% of plasma antibodies that are found in cerebrospinal fluid can have profound parenchymal effects. Evidence for direct binding of peripherally administered amyloid-specific antibodies to amyloid deposits in the brain has been provided and the proposed clearance mechanism has been modelled in an *ex vivo* assay that predicted the observed *in vivo* efficacy for all antibodies studied. In this paradigm, capture of soluble Aβ was not required for reduction of amyloid pathology and neuronal dystrophy[59].

It has also been reported that antibodies can resolve *in vitro* aggregated Aβ fibrils; this direct resolution of amyloid deposits might underlie its therapeutic effects[60] (FIG. 2b). However, how small amounts of antibody would dissolve existing insoluble fibrils in the brain is not understood.

The Aβ mid-region monoclonal antibody 266, which shows picomolar affinity to soluble Aβ and does not bind to plaques, was found to reduce amyloid levels upon passive administration[61]. It was suggested that the antibody, at concentrations sufficient to produce detectable cerebrospinal fluid levels, captures soluble Aβ and produces a net flux of Aβ from the CNS to the periphery, which, over an extended time period, would lead to decreased parenchymal amyloid load (FIG. 2c).

Finally, several studies in transgenic mouse models have observed acute beneficial effects on cognitive performance[62] or cognitive effects that were much more pronounced than the reduction in amyloid load upon antibody administration. It has been proposed that the beneficial effects seen under these circumstances could be mediated by the rapid clearance of toxic Aβ oligomers (FIG. 2d). These studies raise questions about the relevance of cognitive end points in *APP* transgenic mice for the assessment of human clinical candidates. Are the observed Aβ-related impairments just a consequence of *APP* overexpression without relevance to memory loss in AD, and should one strictly focus on pathology end points in this model? Or are they meaningful? In that case a human clinical candidate may not have to demonstrate effects on Aβ load. However, if the rapid symptomatic effects in mice were predictive of the human AD situation, one would expect rapid symptomatic effects in patients, and such data have not been reported yet.

Another important issue is the role of different Aβ antibody epitopes. Although the Aβ peptide is relatively small, it is possible to raise antibodies to distinct amino-terminal, mid-region, carboxy-terminal and possibly conformational epitopes. For example, Elan's 'microglial

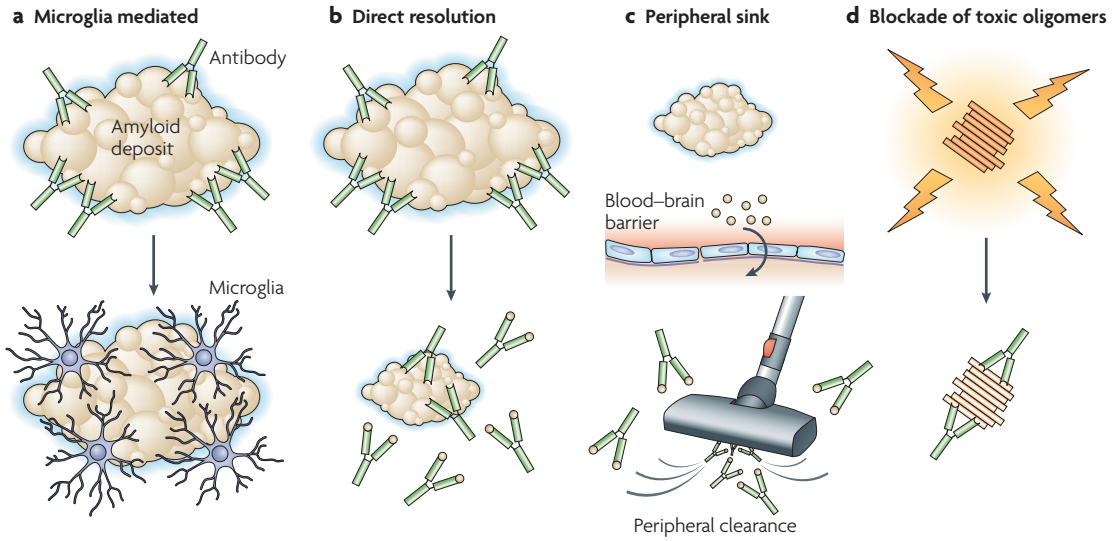

Figure 2 | **Models of antibody-mediated amyloid clearance.** Four models of antibody-mediated amyloid clearance, which are not mutually exclusive, have been proposed. **a** | Small amounts of amyloid-specific antibodies reach amyloid deposits in the brain and trigger a phagocytic response by microglia. **b** | Amyloid-specific antibodies reach amyloid deposits in the brain and resolve them directly through interaction of the antibody with the amyloid deposit. **c** | Amyloid-specific antibodies act as a peripheral sink for soluble amyloid-β (Aβ) species, leading ultimately to the resolution of brain deposits by pulling soluble Aβ into the periphery, where it is rapidly cleared. **d** | Amyloid-specific antibodies rapidly bind to oligomeric Aβ species, blocking their toxic effects without immediate impact on amyloid load.

clearance' antibody 3D6 recognizes amino-terminal epitopes, whereas Lilly's 'peripheral sink' antibody 266 recognizes a mid-region epitope. It is likely that the choice of epitope affects the predominant mechanism of action and determines which Aβ isoforms are cleared. Does the choice of epitope also determine liabilities? One concern associated with administration of certain Aβ-specific antibodies is intracerebral haemorrhage. Plaque-binding antibodies such as 3D6 have been shown to increase microhaemorrhages associated with cerebral amyloid angiopathy in *APP* transgenic mice, whereas 266 did not show this effect[63]. The mechanism of this effect is not well understood. Microhaemorrhage data have been disclosed for only a few of the plaque-binding antibodies, so it is not clear whether every plaque-binding antibody will lead to increased microhaemorrhages. A recent study in mice further illustrates the complexity of the matter: the route of administration (antibody concentration at the target) of the plaque-binding antibody could determine whether parenchymal amyloid deposit clearance is associated with microhaemorrhages[64].

Although several Aβ immunotherapeutics are currently in clinical development, there are no definitive data on the efficacy of any immunotherapeutic approach, and no Phase III trial has been completed yet. At the International Conference on Alzheimer's Disease (ICAD) in 2008, data on Lilly's solanezumab was reported. This antibody was well tolerated with no evidence of treatment-related brain inflammation or bleeding. Based on biomarker results, which suggest that the antibody may mobilize Aβ in plaques, the company has initiated Phase III trials[65]. At the same meeting, interim data of Baxter's small Phase II study of IVIG was presented, suggesting improvement in cognitive measures in the treatment group after 9 months[66]. IVIG is also entering Phase III trials.

Much of the discussion about efficacy of Aβ immunotherapeutics has focused on the analysis of data from two drugs that Elan and Wyeth have moved into clinical trials. In particular, Phase II data of the amino-terminal antibody bapineuzumab (already in Phase III trials), presented at ICAD 2008, and published late last year[67]. In the Phase II study, bapineuzumab was generally safe, but the prespecified efficacy analysis did not reach significance in the total population of 240 patients with mild to moderate AD. However, the presenters pointed to a statistically significant and clinically meaningful improvement in the subgroup of *APOE4* non-carriers. The main side effect, vascular oedema, seemed to be dose-related and was more frequent in *APOE4* carriers[67]. Mechanistically, it will be important to better understand whether the increased microhaemorrhages that have been observed preclinically with some immunotherapeutics translate directly into the clinically observed vascular oedema, and how different *APOE4* isotypes may affect different outcomes.

Discussions about the efficacy of immunotherapy also focus on Elan and Wyeth's AN1792, an active immunization against synthetic $A\beta_{1-42}$ peptide, and the first Aβ directed immunotherapy to enter Phase II trials (for a review see REF. 68). Clinical development was terminated in 2002 after some patients developed meningoencephalitis, but follow-up studies have led to different interpretations. For example, a 6-year prospective follow-up of 80 patients from the Phase I trial did not show improved survival or an improvement in the time to severe

dementia in the AN1792 versus placebo group. In a subgroup of eight patients that had received AN1792 and that had post-mortem neuropathology, the two patients with extensive evidence of Aβ plaque removal had still progressed to end-stage AD[69]. These results have been interpreted as suggesting that amyloid therapeutics will not work[70]. However, the number of patients analysed by autopsy was extremely small, dosing in the study was halted more than 4 years before completion of follow up, and the data are confounded by the adverse effects of the active immunization that led to termination of the trial. By contrast, another study of AN1792 immunotherapy arrived at a positive conclusion: follow-up on 159 patients that had participated in the Phase IIa study revealed that patients defined as antibody responders demonstrated significantly reduced functional decline compared with placebo-treated patients, suggesting that Aβ immunotherapy may have long-term functional benefits[71]. However, one could argue that by selecting antibody responders one may select a 'healthier' subset of patients and that the slower progression in this group may reflect this selection rather than a treatment effect. In summary, analyses of the results of Phase III studies of Aβ immunotherapeutics will be required to understand whether such treatments have the desired effect.

Anti-inflammatory approaches

Inflammation may be the most confusing area of AD therapy, and there is currently no consensus about whether and how it should be targeted therapeutically. A recent genome-wide association study has just established a genetic link between inflammation and AD, identifying the complement receptor 1 (*CR1*) gene, which is critically important for enabling the innate immune humoral response, as a true risk factor for AD[72]. It has long been known that activated microglia are strongly associated with senile plaques and that many inflammatory mediators including prostaglandins, pentraxins, complement components, cytokines, chemokines, proteases and protease inhibitors are upregulated in affected areas of the AD brain. This has led to the hypothesis that anti-inflammatory therapy could be beneficial, and this idea is supported by lower incidence of AD in patients with arthritis, most of whom use NSAIDs (for a review see REF. 3).

Evidence from multiple case–control and population-based studies supported a roughly 50% reduction in AD risk in long-term users of NSAIDs and warranted their testing in clinical trials for AD (for a review see REF. 73). However, clinical trials in AD were disappointing: adequately powered studies of the COX2-selective compounds celecoxib and rofecoxib, and of the mixed COX1/COX2 inhibitor naproxen, all failed to show therapeutic benefit (for a review see REF. 3). This can be explained in several ways. First, the data are consistent with the idea that NSAIDs and anti-inflammatory approaches in general work only in primary prevention of AD, not in treatment[3]. Second, the trials may not have addressed the right molecular targets. For example, it has been suggested that one should focus on COX1, because — in contrast to COX2 — it is highly upregulated in microglia. It was argued that doses in the naproxen trial were too low and that future trials should use full therapeutic doses of COX1-targeted NSAIDs despite the gastrointestinal side effects[3]. Moreover, NSAIDs have molecular targets in addition to COX, which may not have been optimally engaged in the previous trials. For example, specific activation of peroxisome proliferator-activated receptor-γ (PPARγ) elicits anti-amyloidogenic, anti-inflammatory and insulin-sensitizing effects[74]. However, the recent failure of rosiglitazone in large Phase III trials[75] does not support further evaluation of this target in AD treatment. It has also been proposed that the epidemiologically promising NSAIDs — in contrast to the NSAIDs tested in large trials — show direct γ-secretase modulating activity (unrelated to their COX effects) and that this explains the failure of the NSAID trials and points to a direction for future development[25].

Although inflammation is recognized as part of the AD pathology, an increasing number of preclinical studies suggest that some aspects of the immune response may actually be beneficial[73]. In AD, microglia probably phagocytose and clear Aβ, and ongoing clinical immunotherapy studies promise to improve microglial phagocytosis of Aβ, thus reducing amyloid pathology (see above). Therefore, should one redirect rather than suppress the inflammatory machinery in AD? Preclinical data suggest that this may actually be feasible; for example, deletion of the prostaglandin E2 receptor has been shown to reduce oxidative damage and amyloid burden in an *APP* transgenic model[76]. This raises the possibility that a prostaglandin E2 receptor antagonist could upregulate microglial phagocytosis of Aβ while at the same time decreasing potential oxidative damage and secondary neurotoxicity. Clearly, distinguishing and modulating beneficial and detrimental parts of the immune response in AD will be an exciting and challenging field for many years to come.

Tau pathology approaches

Intraneuronal tangles containing hyperphosphorylated tau are a hallmark of AD pathology[2]. Tau and tangle pathology are not specific for AD, but are part of the pathology in a number of other disorders such as Pick's disease, progressive supranuclear palsy, corticobasal degeneration and motor neuron diseases. However, there is a strong correlation between cognitive dysfunction and tangle load and localization in AD[77]. Furthermore, the discovery of tau mutations that cause some forms of frontotemporal dementia provided a direct genetic link between tau and neurological disease (see, for example, REF. 78). It also allowed the generation of transgenic models that show severe tau pathology, which will be important for demonstrating pharmacodynamic effects of tau-based drugs *in vivo*. Frontotemporal dementia differs from AD both in symptoms and in pathology, but the demonstration that tau pathology alone can cause cell loss and dementia clearly indicates that tau pathology is not just a marker of dying neurons in AD.

Tau is a soluble microtubule-binding protein. Its main role is the stabilization of microtubules in axons as tracks for axonal transport and as cytoskeletal elements

for growth. The characteristic inclusions observed in AD neurons consist of hypherphosphorylated, aggregated insoluble tau (for a recent review see REF. 79). Both direct toxic effects of the aggregated tau and/or loss of axonal transport due to sequestration of soluble tau into hyperphosphorylated and aggregated forms that are no longer capable of supporting axonal transport have been proposed to contribute to disease (FIG. 3). Inhibition of tau aggregation and blockade of tau hyperphosphorylation are the main treatment strategies being explored (for recent reviews see REFS 80,81). Inhibition of aggregation is conceptually more appealing, because there seems to be general consensus that tau aggregates are detrimental[82]. However, from a drug development perspective, anti-aggregation approaches pose a lot of challenges. For instance, finding molecules with drug-like properties that specifically disrupt protein–protein interactions over large interaction surfaces is theoretically very difficult, even though tau-specific hexapeptide motifs critically contribute to the overall aggregation process[82]. In the case of AD drugs, such molecules would have to pass the additional hurdle of blood–brain barrier penetration. Nonetheless, academic investigators are pursuing anti-tau aggregation strategies: screens have been run, hits have been identified and medicinal chemistry efforts have been initiated[82].

Strategies aimed at reducing tau hyperphosphorylation, which appear to be more straightforward, are more widely pursued. However, this approach faces three major questions. First, is tau hyperphosphorylation really critical to tau pathology? Second, assuming that tau hyperphosphorylation is critical, which is the key pathogenic kinase that should be inhibited? So far, there is no broad consensus on the identity of this kinase. Several candidates have been proposed, including cyclin-dependent kinase 5 activator 1 (CDK5R1), MAP/microtubule affinity-regulating kinase 1 (MARK1) and glycogen synthase kinase 3 (GSK3), but it is not clear yet whether a single culprit kinase even exists. Third, assuming that the biological hurdles are overcome and the key pathogenic kinase is identified, it would be assumed that one would generate a small-molecule inhibitor of the enzyme. However, generation of a highly specific brain penetrant kinase inhibitor that is suitable for chronic dosing will be challenging; all marketed kinase inhibitor drugs in the United States of America and in Europe treat cancer, for which safety hurdles are lower than for mild AD. Moreover, for some of the proposed candidates, one would expect severe mechanism-based side effects upon chronic inhibition. Several kinases are being investigated in preclinical studies by various companies, but no updates on clinical trials were presented at the ICAD in 2009.

At present, the clinically most advanced tau-directed therapy is methylthioninium chloride (methylene blue), which has been reported to dissolve tau filaments isolated from AD brains *in vitro* and to prevent tau aggregation in cell-based models. Based on these findings, TauRx Therapeutics initiated a Phase II placebo-controlled clinical trial in 332 subjects with mild to moderate AD. Significant AD Assessment Scale-cognitive score differences relative to placebo were observed in the middle-dose group, but not in the low- and high-dose groups after 24 and 50 weeks of treatment, which the authors interpreted as evidence of arrested disease progression[83].

APOE-related treatment approaches

APOE is a major carrier of apolipoprotein and cholesterol in the brain. There are three major human isoforms, APOE2, APOE3 and APOE4, encoded by polymorphic alleles ε2, ε3 and ε4, respectively[84]. Carriers of the ε4 allele have a gene-dosage-dependent increase in risk of late-onset AD relative to ε3 and ε2 carriers. Although only 15% of the population carries at least one ε4 allele, 40% of all AD cases are carriers[6].

Of all the possible genetic causes of AD, ε4 is the one involved in most cases[85]. However, because of the complex biological effects of APOE and its different isoforms, progress in the development of APOE-related treatments has been slow. The fact that ε4 is also a risk factor for a number of other conditions (reviewed in REF. 86) raises the question of whether an AD-specific molecular pathway even exists. At the conceptual level, the key question is whether ε4 is a risk factor because it has gained toxic properties relative to ε3 or because it has lost beneficial ε3 function. Some investigators are convinced of the former concept and are pursuing several approaches to mitigate toxic effects of APOE4. For example, by inhibiting a neuronal protease that — according to their model — generates a toxic APOE4 fragment or by developing 'structural correctors' — small molecules that would bind to APOE4 and block the intramolecular domain interaction that is characteristic of this isoform, thus converting it into an APOE3-like structure[87].

Others favour the idea that APOE4 has partially lost the beneficial function of APOE3, at least with respect to its involvement in the amyloid pathway[88]. Analyses of Aβ deposition in which *APOE*-knockout or human ε2-, ε3- or ε4-knock-in mice were cross-bred with *APP* transgenic mice showed that APOE3 caused less Aβ deposition than APOE4, and that APOE4 caused less Aβ deposition than the knockout mice[88]. These results suggest that enhancing *APOE* expression could be a therapeutic strategy that could benefit anyone who carries at least one *APOE3* allele[89]. Progression of APOE-directed treatment approaches into clinical trials has not been reported yet.

Metabolic dysfunction approaches

Several treatment approaches are based on the idea that a metabolic defect that is not directly reflected in the hallmarks of AD brain pathology may have a major role in the disease process. Although the rationale for these approaches may be less robust than that for pathology-based efforts, approved drugs to address the metabolic defects for other indications such as diabetes already exist, and therefore the hypotheses can be immediately tested in the clinic.

Epidemiology studies suggest an association of metabolic syndrome with AD, and that association holds, even when diabetics are excluded[90]. A body of

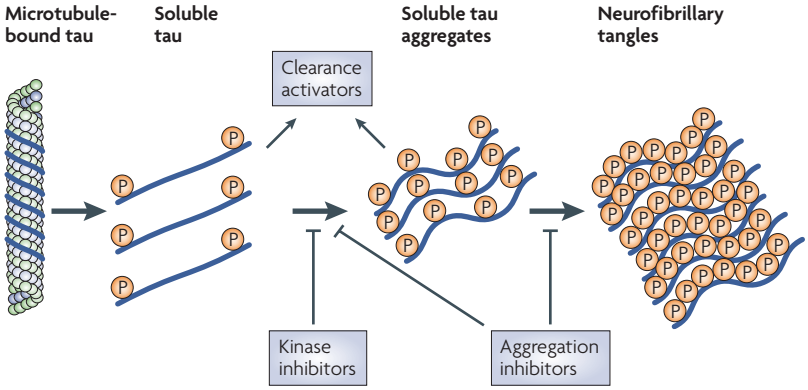

Figure 3 | **Tau pathology and major therapeutic approaches.** Microtubule-bound soluble tau supports axonal transport. Tau is hyperphosphorylated in Alzheimer's disease, which could lead to the detachment of tau from microtubules, which could then lead to the formation of soluble tau aggregates and insoluble paired helical filaments that ultimately form neurofibrillary tangles. Destabilization of microtubules (impairing axonal transport) and direct toxic effects of soluble hyperphosphorylated tau and fibrillar tau may all contribute to tau-mediated neurodegeneration. Anti-phosphorylation strategies (kinase inhibitors) aim to inhibit these processes. Aggregation inhibitors could block the formation of soluble tau aggregates and the formation of tangles. Tau toxicity could also be prevented by enhancing the clearance of tau and the degradation of tau aggregates.

literature suggests that cholesterol modulates Aβ production *in vitro* and in animal models, thus linking cholesterol-lowering approaches mechanistically to one of the hallmarks of disease pathology (reviewed in REF. 91). Specifically, studies with HMG-CoA reductase inhibitors (statins)[92] and with acyl-CoA:cholesterol acyltransferase inhibitors[93] have demonstrated lowered Aβ levels in animals. Whether a therapeutic effect requires lowering of brain cholesterol (as opposed to plasma cholesterol) is currently not known. The efficacy of statins towards AD has been examined in several small clinical trials. However, in the most recent placebo-controlled 18-month study in more than 600 mild to moderate AD cases, atorvastatin (Lipitor; Pfizer) failed to improve outcomes on symptoms and progression of AD[94].

Abnormalities of cerebral glucose metabolic rates in patients with AD are also well documented[95]. Interestingly, cognitively normal homozygous carriers of the ε4 allele have reduced glucose metabolism in the same regions of the brain as patients with probable AD[96], and in longitudinal studies cognitively normal *APOE4* heterozygotes showed significantly greater declines in cerebral glucose metabolic rate than non-carriers[97]. These abnormalities in glucose metabolism may identify early preclinical AD and could have an important role in the disease process.

Other studies suggest that insulin resistance may accelerate AD pathogenesis through various mechanisms, including direct effects of peripheral hyperinsulinaemia on memory, inflammation and regulation of Aβ and tau metabolism (for a review see REF. 98). Based on these findings, clinical studies with approved insulin-sensitizing PPARγ agonists have been initiated. These drugs could provide benefits through their various direct actions in the brain and/or through their influence on peripheral insulin levels. Depending on the proposed mechanism of action, brain penetration of the PPARγ agonists may or may not be required. Based on these findings GlaxoSmithKline had advanced rosiglitazone into Phase III studies in APOE-stratified mild to moderate AD, but as reported at the ICAD in 2009, these adequately powered studies failed to detect a significant treatment benefit in either monotherapy or in adjunctive therapy to acetylcholinesterase inhibitors[75].

Finally, pivotal trial results were just announced for dimebon, an investigational medication for AD with the promise of symptomatic and potentially disease-modifying effects. This molecule, which is approved as an antihistamine in Russia, was advancing in several Phase III studies to assess safety and efficacy across all stages of AD, as monotherapy or in combination with currently available treatments. A 12-month double-blind placebo-controlled Phase II trial of patients with mild to moderate AD in Russia demonstrated highly significant improvements in cognition, functional ability and behaviour compared with placebo[99]. How dimebon achieved its therapeutic effects is not clear. Neuroprotection via improvement in mitochondrial function is being investigated as a potential mechanism based on preclinical work that led to an initial human trial[100]. But dimebon also demonstrates a broad spectrum of activities, including weak acetylcholinesterase and butyryl-cholinesterase activity, and weak blockade of the NMDA receptor signalling pathway[100]. However, as announced in March 2010, in two Phase III trials in AD, dimebon did not meet its co-primary or secondary efficacy end points compared with placebo[101]. Co-primary end points were measures of cognition and global function.

Progress and questions

The past 5 years have seen exciting progress in disease-modifying therapies for AD. Interventions in the amyloid pathway continue to be the focus of most drug discovery efforts and several programmes have advanced into the clinic. It now seems that at least some of these treatments may be safe. But will they work? There are still many unknowns — such as which Aβ species to target with immunotherapeutics, and what degree of Aβ synthesis reduction do secretase inhibitors have to achieve — but the single biggest concern might be the timing of the intervention. Imaging studies with amyloid ligands suggest that significant plaque deposition occurs already before clinical decline[102]. On the other hand, reducing the generation or enhancing the clearance of new Aβ monomers and oligomers could be beneficial even in the presence of an existing amyloid burden, and approaches that clear existing plaques and soluble species at the same time may offer even more benefit. Nonetheless, it is possible that anti-amyloid therapy may be most efficacious in prevention paradigms, before patients meet current diagnostic criteria for AD. The development of new diagnostic criteria that include biomarkers to diagnose early forms of AD before full-blown dementia is vital for the field[103]. The situation could be reminiscent of the development of HMG-CoA reductase inhibitors (statin therapy), in which cholesterol lowering is

now widely used in primary prevention, but the initial approval required demonstration of efficacy in patients with advanced coronary heart disease.

For the non-amyloid approaches, similar considerations apply. For example, it is not clear when in the pathogenesis of AD APOE4 exerts its role. Will 'correcting' a risk factor for AD have therapeutic benefit in diagnosed patients? Therapies that address tau pathology — widely viewed as being downstream from amyloid pathology — may have an advantage in this respect, but generally accepted tractable targets have yet to emerge. Nevertheless, if only one of the many approaches discussed in this article demonstrates clinical efficacy, we may finally be able to slow the emerging AD epidemic.

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Competing interests statement
The author declares competing financial interests: see web version for details.

DATABASES
Entrez Gene:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
APOE | *APP* | *CR1* | *PS1* | *PS2*
OMIM:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM
Alzheimer's disease
UniProtKB: http://ca.expasy.org/sprot
Amyloid-β | APH-1A | BACE1 | CDK5R1 | COX1 | COX2 | GSK3 | insulin-degrading enzyme | LRP-1 | MARK1 | NCSTN | neprilysin | Notch1 | NRG1 | PAI-1 | PEN2 | PPARγ | PSEN1 | RAGE | tau

FURTHER INFORMATION
Alzheimer's Association
http://www.alz.org

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