Preclinical and clinical challenges in the development of disease-modifying therapies for Alzheimer’s disease

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The prevalence of Alzheimer’s disease (AD) approximately doubles with every five years over the age of 65. Overall, ~5–10% of those over 65 years of age are affected. Alzheimer’s Disease International estimates that there are 18 million cases of dementia worldwide, of which 12 million are AD (http://www.alz.co.uk/alz/index.html). Given the ageing population, it has been estimated that there will be 36 million cases of AD in the developed world and 68 million cases in developing countries by 2050 (Ref. 1). The total societal costs for AD in the USA alone have been estimated to be ~US$67 billion for 1991 (Ref. 2). Current treatments for AD provide only modest symptomatic relief. There is a real need for ‘disease-modifying’ agents that slow the course of the disease and prevent or delay the disease in susceptible individuals. The development of such agents requires progress in four key areas:

• improvement in the understanding of the molecular basis of the disease;
• development of animal models;
• identification of tractable targets for therapeutic intervention; and
• establishment of new clinical trial paradigms involving predictive and correlative biomarkers.

Molecular and genetic basis of AD

Our understanding of the molecular basis of AD has arisen from both biochemical work and genetic studies in rare early-onset familial AD (reviewed in Refs 3–5). Much information came from the early biochemical characterization of the two major pathological lesions characteristic of AD, known as ‘plaques’ and ‘tangles’.

Neuritic (or senile) plaques are extracellular deposits comprising a core of aggregated fibrillar β-amyloid (Aβ) protein surrounded by degenerating neurites, activated microglia and reactive astrocytes. Aβ is generated from the larger Aβ precursor protein (AβPP) through sequential proteolytic cleavage by β- and γ-secretases (Fig. 1). A 40-amino acid form of Aβ (Aβ40) predominates, but a small proportion is the longer 42-amino acid species (Aβ42), which is thought to be the major pathogenic form in AD. Neurofibrillary tangles are intraneuronal aggregates of paired helical and straight filaments of hyperphosphorylated microtubule-associated protein, tau. The increasing cognitive and memory impairment in AD is a result of the death of neurons in brain regions containing high levels of plaques and tangles – primarily the neocortical and limbic regions.

Mutations in three genes – βAPP and presenilins-1 and -2 (PS-1 and PS-2) – have been shown to cause early-onset familial AD (FAD) (reviewed in Ref. 5). All FAD mutations identified to date have been shown either to
cascade of events that eventually leads to neuronal dysfunction and dementia. (See http://www.alzforum.org/members/forums/selkoe/Seminar/index.html.) The Amyloid Cascade hypothesis predicts that interventions that reduce amyloid production or increase its clearance would be disease-modifying.

Multiple cellular pathways for potential disease-modifying therapeutic intervention

For a treatment to slow or halt the course of a disease (i.e., be disease-modifying) it must interfere with a central pathway in the pathophysiological process. Although biochemical and genetic studies have facilitated a partial understanding of this process in AD, it remains incompletely understood. Amyloid has gained the most attention, however, studies of post-mortem tissue clearly reveal additional aspects of pathology that could also form the basis for therapeutic intervention.

Identification of tractable targets for disease-modification in AD

Reduction of β-amyloid production or deposition

Despite substantial progress in the understanding of AD pathology, the identification of potential drug targets for disease-modifying agents has been difficult. The Amyloid Cascade hypothesis predicts that agents that inhibit amyloid production could slow the course of the disease. Therefore, two potential targets are the β- and γ-secretases, which cleave βAPP to yield Aβ (Fig. 1).

β-Secretase was identified, following a long search, late in 1999 (Refs 13–16). This aspartyl protease (known as BACE1, Asp2 or memapsin 2) represents a tractable anti-amyloid target for AD. Little is known about this novel membrane-bound protease, but rapid progress has been made in its characterization. Recent advances cause optimism...
for drug discovery. First, it has recently been confirmed that BACE1 is the major β-secretase in neurons. Mice deficient in BACE1 have been shown to lack brain Aβ (Refs 17,18), even when crossed with transgenic mice overexpressing the substrate, βAPP, in the brain17. Moreover, the BACE1 knockout mice have normal phenotype, indicating that there are no apparent adverse effects associated with BACE1 deficiency in these animals17,18. Secretion of Aβ was also shown to be deficient in embryonic cortical neurons lacking BACE1 (Ref. 19), again confirming that BACE1 is the principal neuronal protease required for cleavage of βAPP at the β-secretase site. These findings increase the validity of BACE1 as a drug target for AD, by demonstrating that it is crucially required for Aβ production in neurons and by reducing the early fears that inhibition might cause mechanism-based side effects, particularly in the pancreas, where high levels of BACE1 mRNA are found. Second, the publication of a high-resolution crystal structure of the BACE1 protease domain complexed to an eight-residue inhibitor20 will assist with small-molecule-inhibitor design. Third, the structures of some potent inhibitors, albeit peptide-based compounds, have now been published15,21, providing tool compounds for further analysis of β-secretase. It is still unclear, however, whether selectivity over the related aspartyl protease, BACE2 (Ref. 22), is achievable or necessary. The development of non-peptidic small-molecule inhibitors of β-secretase, which can be tested in cellular and animal models, is eagerly awaited.

Recently, in a tale of exciting twists and turns, γ-secretase activity has been associated with presenilins. Presenilins are integral membrane proteins thought to comprise a six- or eight-transmembrane topology. Mutations in the genes encoding PS-1 and PS-2 are the most common cause of early-onset FAD. Presenilins undergo endoproteolysis to yield N- and C-terminal fragments that associate into a heterodimer (reviewed in Ref. 23). It is thought that endoproteolysis of wild-type presenilin is required for activity; however, some mutant forms do not undergo endoproteolysis yet do maintain some function, perhaps because they adopt a conformation that mimics an active, endoproteolysed, wild-type form. Despite the finding that FAD mutations in presenilin genes led to altered γ-secretase cleavage of βAPP (and thus increased production of the pathogenic Aβ42), for many years it was unclear how presenilins influenced this process. Mice deficient in PS-1 (PS-1−/− mice) were found to die late in embryogenesis, displaying a Notch-like phenotype24. Significantly, neuronal cultures derived from these animals showed almost no γ-secretase cleavage, further emphasizing the key role of presenilins in this process24. However, it was the discovery that two highly conserved transmembrane aspartate residues in PS-1 were required for γ-secretase activity25 that most closely linked presenilins with this activity. A debate raged within the AD research community over whether presenilin, which looked nothing like known proteases, could possibly be the long-sought-after γ-secretase, or whether it was instead a co-factor or trafficking molecule for the enzyme. Interestingly, it was subsequently noted that presenilins have homology with a bacterial protease type four prepylin peptidase26,27 in the region of one of the

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**Figure 2.** Amyloid cascade hypothesis of Alzheimer’s disease pathophysiology. Increased production of either total β-amyloid (Aβ) or Aβ42 leads to progressive aggregation and deposition of Aβ. This leads to an inflammatory response, accumulation of abnormal tau, and neuronal damage and death, resulting in dementia.

**Abbreviations:** APP, amyloid precursor protein; PS, presenilin.
conserved transmembrane aspartyl residues of presenilin. Key functional evidence came from two groups who, in elegant work, used potent \( \gamma \)-secretase inhibitors designed to function as transition-state analogues directed to the active site\(^{28,29} \). Labelling of PS-1 and PS-2 by the inhibitors was observed, supporting the conclusion that presenilin contains the active site of \( \gamma \)-secretase.

Thus, a second anti-amyloid target has been proposed: presenilin-\( \gamma \)-secretase. However, several potential issues could arise from inhibition of this target. First, given that \( \gamma \)-secretase activity is not sequence-specific, it is likely that other substrates exist. Indeed, it has been shown that presenilin-\( \gamma \)-secretase is required for the production of Notch-1 intracellular domain (NICD) (Ref. 30), leading to fears of unwanted side effects from \( \gamma \)-secretase inhibition; receptors of the Notch family mediate cell-cell interactions that specify cell fate during development. Encouragement comes from the observation that the presence of only a single PS-1 allele significantly reduced \( \gamma \)-secretase activity but maintained significant NICD levels and did not produce Notch-related defects in vivo\(^{30} \). Nevertheless, \( \gamma \)-secretase inhibitors have been shown to interfere with T-cell development in a foetal thymus organ culture\(^{31} \). In addition, further complexity about the nature of the target developed with the discovery that a novel transmembrane glycoprotein, nicastrin, is associated with presenilin in the \( \gamma \)-secretase complex\(^{32} \). Nicastrin modulates presenilin-mediated Notch signalling and \( \beta \)APP processing by a mechanism that is not yet clear. A second concern regarding potential side effects from \( \gamma \)-secretase inhibition centres around substrate accumulation; inhibition of \( \gamma \)-secretase leads to an accumulation of \( \beta \)APP C-terminal fragments, which have been suggested to be toxic\(^{33,34} \). Optimism arises from work showing that inhibitors can distinguish between \( \beta \)\( \beta_{40} \) and \( \beta \)\( \beta_{42} \) production\(^{35} \), raising the possibility of therapeutic approaches that target the more toxic \( \beta \)\( \beta_{42} \) production, but spare other \( \gamma \)-secretase substrates, thus potentially reducing side-effect liability.

Several pharmaceutical companies have been active in presenting and publishing on \( \beta \)-secretase [including Amgen (Thousand Oaks, CA, USA), Pharmacia (North Peapack, NJ, USA), Elan (Dublin, Ireland) and GlaxoSmithKline (Stockley Park West, Uxbridge, UK)] and \( \gamma \)-secretase [including Merck (Whitehouse Station, NJ, USA) Bristol-Myers Squibb (BMS; Wallingford, CT, USA), Boehringer Ingelheim (Ingelheim am Rhein, Germany), Cephalon (West Chester, PA, USA), Roche (Basel, Switzerland) and Rhône-Poulenc Rorer (Aventis, Schiltigheim, France)], indicating the high level of industry interest in anti-amyloid approaches. Felsenstein from BMS has presented on the company’s work on \( \gamma \)-secretase inhibitors\(^{36} \), and in February 2000 stated that clinical candidates were being progressed towards First Time In Man studies.

Inhibition of amyloid aggregation or deposition is regarded as a less tractable approach than the reduction of \( \beta \)\( \beta \). However, immunization with \( \beta \)\( \beta \) has recently been shown to be remarkably effective at reducing amyloid deposition in preclinical studies (see ‘Interventions in transgenic models of AD’).

Prevention of neurofibrillary tangle formation

Intervention in the pathway leading to tau tangle formation appears less straightforward. Under normal conditions, tau co-localizes with microtubules and modulates microtubule assembly. In pathological conditions associated with neurofibrillary tangles, tau protein becomes hyperphosphorylated, reducing its affinity for microtubules and possibly leading to its aggregation (reviewed in Ref. 37). It is still unclear whether hyperphosphorylation is an absolute prerequisite for tangle formation. Moreover, the mechanism by which abnormal tau causes neurotoxicity is also unclear.

It was originally thought that tau tangles killed neurons (indeed, ‘ghost’ tangles can be detected in AD brain, which remain after the death of the neuron in which they developed), and hence prevention of neurofibrillary tangle formation was suggested to be neuroprotective in AD. However, by contrast, recent work in Drosophila expressing wild-type and mutant human tau has suggested that tau-induced neurodegeneration does not require the formation of large filamentous tau aggregates\(^{38} \). Age-dependent accumulation of abnormally phosphorylated tau, similar to that seen in AD brain, was observed in the transgenic fly brains. Neurodegeneration was also seen, but no neurofibrillary tangles or filamentous tau aggregates were detected. The correlation of neurodegeneration with the same abnormal tau phosphorylations that commonly precede tangle formation in AD, suggests that early modifications of tau could be the toxic substrates, causing neurotoxicity independently of aggregation. Accordingly, inhibition of tau phosphorylation is a potential target for disease modification in AD.

The identification of several kinases implicated in tau phosphorylation, particularly glycogen synthase kinase-3\( \beta \) (GSK-3\( \beta \)) and cyclin-dependent kinase 5 (CDK5), has suggested tractable potential targets for drug discovery. GSK-3 was originally identified as a kinase that phosphorylates and inactivates glycogen synthase, which catalyzes a regulated step in insulin-mediated glycogen synthesis. Subsequently GSK-3 has been shown to have many other cellular activities, including phosphorylation of tau at sites that are phosphorylated in neurofibrillary tangles. Active GSK-3\( \beta \) has been shown to accumulate in the tangle-bearing
neurons in AD brain39, and is associated with paired helical filaments, suggesting a direct link between GSK-3β and hyperphosphorylated tau40,41. Overexpression of GSK-3 has been shown to induce an AD-like phosphorylation of tau in neurons42, whereas inhibition of GSK-3 by lithium has been shown to reduce tau phosphorylation in cultures of human NT2N cells43. Thus it has been proposed that inhibitors of GSK-3 could have a use in AD.

Another kinase that has been shown to phosphorylate tau and accumulate in tangle-bearing neurons is CDK5. Association of CDK5 with its regulatory subunit, p35, is crucial for kinase activation. It has recently been shown that a proteolytic fragment of p35, termed p25, accumulates in neurons in the brains of AD patients and correlates with an increase in CDK5 activity44. p25 is more stable than p35, and is able to activate CDK5 constitutively. Moreover, binding of p25 to CDK5 changes its cellular location and alters its substrate specificity. The deregulated CDK5-p25 complex is able to hyperphosphorylate tau and can lead to irreversible damage to the cytoskeleton and neuronal death44. Thus, drugs inhibiting CDK5 might be predicted to reduce tau phosphorylation and possibly also reduce neurofibrillary tangle formation. However, the role of CDK5 in other cellular processes, such as central nervous system (CNS) development, dopamine signalling and synaptic vesicle exocytosis, necessitates a cautious approach for fear of unwanted side effects.

Despite these potential opportunities for intervention, tau has received less attention from the pharmaceutical industry than amyloid. This is partly because the role of tau phosphorylation in paired helical filament formation and neurodegeneration is still not fully understood. Disaggregation of tau is another potential avenue for therapy, but is less tractable and might be ineffective, given the studies in Drosophila38. Moreover, the genetic findings suggesting that amyloid could be upstream of tau in the cascade of damage associated with AD has led many researchers to focus on upstream points of intervention.

Reduction of CNS inflammation
Examination of AD brain reveals the presence of a widespread inflammatory process centred upon the chronic activation of microglial cells, which are mainly associated with amyloid plaques. There has been extensive debate on the role of inflammation in AD pathology (see review45 and commentaries46,47). A key question is whether inflammatory mechanisms are neuropathic or neuroprotective; that is, whether they actually cause damage in AD, or whether they merely clear up the damage from other, more primary pathological processes. Overall, there is more evidence to suggest that inflammatory processes contribute to neurodegeneration45, suggesting that anti-inflammatory therapies might have disease-modifying activity.

It has been demonstrated in culture systems that treatment with fibrillar Aβ can lead to microglial activation, and that this activation results in the release of a range of damaging chemical species, including free radicals (e.g. superoxide and nitric oxide) and various cytokines, including interleukin-1 and tumour necrosis factor (reviewed in Ref. 45). Amyloid, in combination with products of activated microglial cells, also interacts with astrocytes, leading to their conversion to a ‘reactive’ phenotype that further contributes to the inflammatory process48. Evidence suggesting that inflammation might contribute significantly to AD progression comes from numerous retrospective epidemiological studies. These suggest that long-term use of non-steroidal anti-inflammatory drugs (NSAIDs) to treat conditions such as rheumatoid arthritis result in a subsequent delay in the age of onset of AD (e.g. Refs 49–51). However, further statistical analysis taking into account the duration of NSAID usage did not reveal a significant effect in one large study52.

Hence, anti-inflammatory approaches to AD have received growing attention, but the approach remains to be proven and the preferred molecular targets for such therapies remain unclear. A recent clinical trial with the glucocorticoid prednisone suggests that such agents are not useful in AD (Ref. 53). Also disappointing is the negative result from a 52-week trial of celecoxib, the selective cyclo-oxygenase-2 (COX-2) inhibitor from Pfizer (New York, NY, USA), in which no effect on disease progression was noted54. However, conventional non-selective NSAIDs interact with several molecular targets, including COX-1, COX-2 and the nuclear receptor PPARγ. It is unclear which anti-inflammatory target is most relevant for AD. Further clarification should come from the Alzheimer’s Disease Anti-inflammatory Prevention Trial (ADAPT), which will study a high-risk population of people over the age of 70 with a family history of dementia. Participants will receive either a conventional NSAID (naproxen), a COX-2 inhibitor (celecoxib) or placebo. Enrolment has commenced and interim analysis will be performed after all participants have completed an initial 30 months. In addition, attention has also focused on other inflammatory pathways, such as the complement system55 and the inflammatory mediator CD40 (Ref. 56).

Reduction of neurodegenerative processes: apoptotic cell death and neuroprotection
A late event in the disease cascade in AD involves the death of neurons in cortical and limbic brain regions. The progressive nature of neuronal dysfunction and death in AD is
consistent with an apoptotic (programmed cell death), rather than necrotic, mechanism. Despite the problems associated with the detection of apoptotic cells in post-mortem tissue, there is now increasing evidence to support an apoptotic component to AD neurodegeneration. Changes in expression of genes involved in the apoptotic process have been measured in AD brains and neurons displaying DNA fragmentation, a characteristic feature of apoptosis, have been observed\(^{57,58}\). Aggregated A\(_\beta\) is neurotoxic when applied to neurons in culture. When delivered directly to the brains of animals, differential sensitivity seems apparent, with both age- and species-dependent responses. A\(_\beta\) is neurotoxic in aged but not young pri-mates\(^{59}\); rodents appear substantially more resistant. Presenilin mutations could contribute to apoptosis by increasing A\(_\beta\) production and by increasing Ca\(^{2+}\). Presenilin mutations have been shown to increase neuronal vulnerability to apoptosis\(^{60}\) and presenilin is itself cleaved during apoptosis by caspases\(^{61}\). Tau is hyperphosphorylated in apoptotic cells, a process thought to be required for pathological tau aggregation. Furthermore, like presenilin, tau is itself a substrate for caspase-mediated proteolysis.

The development of drugs to prevent apoptosis is com-plicated by several factors, including the low number of tractable targets in apoptotic pathways, the possible redundancy of pathways and the concern over whether a dying neuron can be rescued. Nevertheless, potential tractable targets for intervention can be identified at various levels in the apoptotic cascade, from MAP kinases through to cas-pases. Several pharmaceutical companies have developed caspase inhibitors (see Ref. 62 for review), although none has yet reached the clinic. Preclinical studies with the c-jun N-terminal caspase (JNK) pathway inhibitor, CEP-1347, suggest that neuroprotection via this mechanism could be possible, and the compound has been progressed into the clinic for AD and Parkinson’s disease by Cephalon and Lundbeck (Copenhagen-Valby, Denmark).

An alternative approach to neuroprotection is to support neuronal growth through the induction or delivery of neurotrophic growth factors. In particular, attention has focused on nerve growth factor (NGF), which can prevent metabolic or excitotoxic injury to neurons in culture. Delivery of growth factors into the brain is a major hurdle because these proteins are unable to cross the blood–brain barrier, but delivery via transplanted genetically modified cells has been suggested to be possible\(^{63}\). Another approach is to cause induction of neurotrophic factors\(^{64}\), as has been claimed for leteprinim (NeoTherapeutics, Newport Beach, CA, USA).

Other disease-modifying approaches to AD - APOE
One additional area of particular interest is APOE, given that the \(\varepsilon4\) allele significantly increases susceptibility to AD. Unfortunately, the elucidation of the mechanism through which APOE4 mediates the increased risk of AD has proved difficult, limiting progress in target identification. The APOE protein is expressed both peripherally and in the brain, and has a role in the transport of cholesterol and phospholipids. In addition, studies in animal models have shown that APOE is necessary for fibrillar amyloid deposition (see ‘Development of animal models’). Thus, it is possible that the major effect of APOE4 in mediating increased susceptibility to AD is via amyloid. The identifi-ca-tion of other molecules on the disease-associated pathway is desirable because APOE itself is not a conventional tractable drug target. Nevertheless, progress has been made, raising the possibility of future developments in this area\(^{65,66}\).

Novel disease-modifying approaches identified through clinical studies - statins
In addition to the clinical findings with NSAIDs in AD, clinical studies have also suggested potential disease-modifying activity of other agents. One finding that has caused much recent interest is the observation of decreased prevalence of AD in patients taking ‘statins’ (3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors) to lower serum cholesterol. Two major clinical studies involving different populations both indicated an \(~70\%\) reduction in the prevalence of AD in this population\(^{67,68}\). This finding has led to much speculation about the possible mechanism. Of great interest is the observation that this effect was independent of hyperlipidaemia and was not seen with exposure to non-statin lipid-lowering agents. Thus, although elevated cholesterol has previously been linked to AD, it is possible that cholesterol-lowering is not the AD-modifying mechanism in this case. \(\beta\)- and \(\gamma\)-secretases have been shown to be positively regulated by cholesterol (reviewed in Ref. 66) and simvastatin has been shown to dramatically reduce A\(_\beta\) levels both in vitro and in vivo\(^{69}\). Hence, it is possible that statins achieve their disease-modifying effect in AD through the reduction of A\(_\beta\) production. However, it has also been shown that CNS ischaemic events, which are associated with elevated cholesterol, can result in the clinical expression of AD (Ref. 70). Thus, an alternative explanation is that the reduction of cholesterol with statins decreases the risk of cerebral ischaemia and delays the conversion of preclinical AD to clinically diagnosable disease\(^{66}\). A prospective placebo-controlled study of Pfizer’s atorvastatin (Lipitor) has recently been announced.
Promise of new technologies for novel target identification
New molecular technologies to analyze gene expression profiles, such as microarrays and Serial Analysis of Gene Expression (SAGE), offer the prospect of novel target identification for AD. In these approaches, differences in gene expression between disease (or animal model) and control tissue can be explored. Recent publications of gene expression profiles in aging demonstrate the power of such techniques to identify patterns of changes in gene expression. Recently, such an approach has been applied to brain tissue from patients at high risk of AD. Candidate genes were identified whose expression was altered in cerebral cortex, including the synaptic vesicle protein synapsin II, which plays an important role in neurotransmitter release. Such approaches could highlight new pathways of relevance to early disease progression, and hence potential new drug targets, although the challenge is to identify the important changes of all those likely to be observed. Studies at the protein level – proteomics – although more resource intensive, also show promise for the identification of both biomarkers and potential new targets.

Development of animal models
Genetic findings in AD provided the opportunity for the creation of transgenic mice, and these have been of great value in the study of pathophysiological processes. A key breakthrough has been the development of transgenic mice that show age-dependent accumulation of Aβ plaques, together with neuroinflammatory and neuritic pathology. More recently, these models have been applied to the evaluation of novel disease-modifying drugs.

In 1995, Games et al. demonstrated that a transgenic mouse (termed PDAPP) expressing high levels of an FAD mutant of βAPP (V717F) developed many of the pathological hallmarks of AD, including diffuse and neuritic amyloid plaques, synaptic loss, astrocytosis and microgliosis. Several other transgenic mice expressing βAPP mutants have also been generated. Animals develop amyloid pathology from as early as six months of age. The Tg2576 line, which expresses high levels of another FAD mutation of βAPP (K670N/M671L, known as the ‘Swedish’ mutation) shows a robust and progressive cognitive decline. A line generated by scientists at Novartis (Basel, Switzerland) (APP23, also expressing the Swedish βAPP) additionally showed region-specific neuronal loss in the CA1 area of the hippocampus.

In an attempt to further explore the role of other proteins, such as presenilin and APOE in amyloid production and deposition in vivo, crosses between different transgenic and knockout animals have been made. The Tg2576 βAPP transgenic was crossed with mice expressing an FAD mutation in PS-1 (Ref. 84), resulting in accelerated pathology. Amyloid deposition was apparent from the age of six months in the dual βAPP–PS-1 transgenic, compared with 9–12 months in Tg2576. Interestingly, APOE is required for amyloid deposition; crosses of βAPP (V717F) transgenics with APOE knockout animals revealed a gene dose-dependent reduction in amyloid deposition with no Aβ immunoreactivity in the APPV717F−/−/APOE−/− animals. Subsequent studies with transgenic mice expressing different alleles of APOE showed that APPV717F expressing APOE4 (the allele shown to increase susceptibility to AD) deposited substantially more (>10-fold) fibrillar amyloid deposits than the animals expressing APOE3 (Ref. 8).

These animals provide an excellent model of amyloid deposition and allow the preclinical evaluation of potential anti-amyloid therapeutics. Readouts for disease-modification in these models include measurement of amyloid levels in plasma, cerebrospinal fluid or brain, and quantification of amyloid deposition. Alternative endpoints, although less validated, include cognitive assessment and measurement of regional brain volume using neuroimaging, where possible. A potential limitation of these models, however, is that they do not develop neurofibrillary pathology and generally show very limited neuronal loss, making them unsuitable for testing drugs targeted at these processes.

Recent progress has been made in the modeling of tau pathology in transgenic animals. Of particular note, mice expressing human tau with a mutation (P301L) that causes frontotemporal dementia with Parkinsonism in humans, develop neurofibrillary tangles and associated reactive gliosis. However, these animals are considered to be better models for the human tauopathies than for AD, because they do not additionally develop amyloid pathology.

A different approach was taken by Capsoni and colleagues in Italy, who have explored the actions of NGF (Ref. 87). Knockout of NGF leads to a lethal phenotype, and so the group generated transgenic mice that express neutralizing anti-NGF antibodies at high levels in adult, but not newborn, animals. The resultant transgenic mice developed age-dependent, progressive neurodegenerative pathology, including amyloid plaques, hyperphosphorylated tau and neurofibrillary tangles in cortical and hippocampal neurons. Aged animals displayed extensive neuronal loss throughout the cortex and showed reduced ability in spatial learning behavioural tasks. A cholinergic deficit in the basal forebrain and enlargement of lateral ventricles were also noted in aged animals. Further characterization of these animals is eagerly awaited to determine whether they will be useful for the development of disease-modifying therapeutics.
Although the βAPP and anti-NGF antibody transgenics show CNS inflammatory pathology, this is limited and is difficult to use as a primary endpoint for assessment of interventions. Chronic infusion of lipopolysaccharide (LPS) into the ventricle of young rats has been shown to reproduce aspects of AD pathology, including astrocytosis, increased activation of microglia, increased inflammatory mediator and βAPP production and a deficit in working memory98. Such a model is of value for the evaluation of anti-inflammatory approaches to AD.

Animal models of CNS apoptotic neurodegeneration in the adult are difficult to develop and often involve the use of neurotoxic agents, such as kainate or MPTP (for examples see Refs 89 and 90). For this reason, transgenic or other models of AD that also show quantifiable neuronal loss are of great interest. The use of neuroimaging techniques to assess neuronal loss in these animals91 offers a potential method for the evaluation of anti-apoptotic agents.

Interventions in transgenic models of AD: pharmaceutical, dietary and immunization

Significantly, the first reports of interventions in βAPP transgenics are starting to be published. Ibuprofen was shown to suppress plaque pathology and inflammation92, providing further optimism for NSAID-like treatments in AD. Refolo and colleagues93 explored the effect of cholesterol on the development of amyloid pathology and demonstrated that a hypercholesterolaemic diet led to accelerated amyloid pathology in a dual βAPP PSW/PS-1M146V transgenic. At the World Alzheimer Congress (9–18 July 2000, Washington, DC, USA) Pappolla and colleagues announced that melatonin (administered in drinking water for 11 months) markedly reduced amyloid accumulation in Tg2576 βAPP transgenic mice.

The stunning observation that immunization of transgenic PDAPP mice with Aβ dramatically reduced amyloid deposition and attenuated subsequent neuropathological changes94 created great excitement and raised the possibility of a vaccine for AD. This approach has since been reproduced in other transgenic lines95,96. Reduced amyloid deposition and protection from cognitive decline was demonstrated, suggesting that the progressive cognitive decline seen in these lines was a direct result of amyloid pathology. Given the low penetration of antibodies from the periphery into the brain, much debate ensued on the mechanism of efficacy. This was clarified by the demonstration that peripherally administered antibodies against Aβ could enter the CNS and reduce pathology97. Elan has formed alliances with Wyeth-Ayerst (Madison, NJ, USA) to develop the vaccine (known as AN-1792 or Betabloc) and with Cambridge Antibody Technology (Melbourne, UK) to develop antibody-based therapeutics. Phase II clinical studies on the vaccine are now underway and results are eagerly awaited.

Animal models will increasingly allow the evaluation of disease-modifying approaches to AD, giving pharmaceutical companies the crucial increased confidence required to invest in expensive clinical trials.

Clinical challenges in the development of disease-modifying agents

One of the greatest challenges in the development of disease-modifying agents for AD is the design of clinical trials to demonstrate drug activity within a reasonable timeframe and in a cost-effective manner. Current clinical trials in AD rely on cognitive and behavioural readouts, which are improved by cognitive-enhancing drugs. The subjectivity and variability that are inherent in cognitive testing leads to a requirement for large trials of long duration. For disease-modifying drugs we face the challenge of detecting reduced deterioration rates within a reasonable timeframe. Moreover, given that amyloid accumulation is thought to commence years, or decades, before clinical manifestation of disease, it seems preferable to commence preventive intervention at a very early stage – long before the development of symptoms. Identification of those individuals most likely to benefit from such intervention, and measures of drug efficacy in such a population, are very significant challenges. Progress in this field requires the identification of sensitive measures that correlate with disease progression. It will be important to find markers of progression of disease pathology from the earliest preclinical stages through to the full disease state.

Clinical trial design

The International Working Group on Harmonization of Dementia Drug Guidelines recently examined a wide range of issues facing those wishing to develop drugs for dementia. An examination of potential protocols for the evaluation of disease-modifying drugs was included in the analysis98,99. Unanimity on trial design was not reached, indicating the complexity of this issue. Discussion continued at an AD conference the following year100. The two basic designs currently used in clinical trials are ‘survival’ analysis and staggered start–withdrawal analysis. Survival analysis measures the proportion of subjects reaching a particular endpoint or outcome over time (e.g. institutionalization, diagnosis of severe dementia, death, and so on). This has rarely been used for AD trials, however, the advantage of this approach is that there is flexibility in the definition of endpoints. Staggered start–withdrawal trials aim to show a statistically significant treatment effect at the
end of the study (for example, reduced deterioration) in the treatment arm that has the longest drug treatment period.

Populations for testing disease-modifying agents

Of key importance is the identification of target populations who will benefit from disease-modifying interventions. Clinical trials of disease-modifying agents could be conducted in patients with mild to moderate AD. However, post-mortem studies have revealed some AD-like pathology in cognitively normal elderly subjects, and evidence from neuroimaging (see ‘Alternative clinical endpoints – neuroimaging’) also indicates neuronal loss before the onset of symptoms. Given this substantial pre-symptomatic pathology, disease-modifying intervention is likely to be of greatest benefit if commenced as early as possible.

To this end, there is a growing interest in the identification of molecular diagnostics. Of particular value would be techniques for early diagnosis, or better, the diagnosis of ‘at-risk’ people or those with preclinical stages of AD. None of the current biomarkers has achieved universal acceptance, and none fully meets the criteria for an ideal biomarker101. Several candidate diagnostic markers have been proposed, including cerebrospinal fluid, Aβ42 and tau, and plasma Aβ42 (reviewed in Refs 101,102). Recent ventures to identify and map single nucleotide polymorphisms in the human genome offer the potential to better predict disease in those at risk of disease, define and diagnose sub-populations within a disease area, and target specific therapies to those patients most likely to respond well103.

It seems probable that a preclinical phase of AD exists, in which individuals show subtle cognitive deficits. Interest in the transitional state between normal ageing and AD has resulted in many studies, and the description of a range of conditions, including age-associated memory impairment, late-life forgetfulness, benign senescent forgetfulness and mild cognitive impairment104. There has been a recent effort to standardize, and a condition of Mild Cognitive Impairment (MCI) (Ref. 105) is increasingly recognized, although diagnostic criteria are not widely agreed104. Nevertheless, individuals with identified mild memory impairment have been shown to be at increased risk of developing AD, highlighting a population in whom disease-modifying agents could be beneficial. Moreover, survival trials could be performed in this population to assess any reduction in progression to AD associated with treatment.

Recent failures to demonstrate disease-modification in placebo-controlled trials of oestrogen106, celecoxib54 and prednisone53 in patients with mild to moderate AD could highlight the difficulty of slowing the course of established disease. Accordingly, studies are increasingly being conducted in people at high risk of developing AD. Several studies in MCI are ongoing or planned, to evaluate the potential of agents to delay the onset of AD. An Alzheimer's Disease Cooperative Study (http://antimoney.ucsd.edu/) is being conducted with vitamin E and donepezil (an acetylcholinesterase inhibitor). Patient enrolment has recently been completed. Rivastigmine (another acetylcholinesterase inhibitor) is also being tested in MCI with the aim of delaying progression to full-blown AD. In addition, rofecoxib, a selective COX-2 inhibitor, is reported to be in Phase II trials in MCI. An alternative ‘at risk’ population could be defined as those people aged 70 or older who have a close relative with serious age-related memory loss, dementia, senility or AD. This is the approach taken in the ADAPT trial (see ‘Reduction of CNS inflammation’) to examine the potential of anti-inflammatory drugs (naproxen or celecoxib) to delay or prevent the onset of AD. Examples of completed and ongoing clinical trials of disease-modifying agents are shown in Table 1.

Alternative clinical endpoints – neuroimaging

Early diagnosis and tracking of disease progression could also be achieved through the use of neuroimaging approaches. The field is attracting increasing attention, as reflected by the high attendance at the First International Meeting of the Alzheimer’s Imaging Consortium (8 July 2000, Washington, DC, USA). Multiple neuroimaging techniques have been applied to AD. Positron emission tomography (PET) has been used to study those at genetic risk of AD (i.e. carriers of APOE4). Reduced glucose metabolism is seen in certain brain regions of AD patients, and a similar reduction has been detected in the same brain regions in late-middle aged, cognitively normal APOE4 carriers107. Thus, changes in regional brain metabolism could constitute the earliest detectable stage of disease progression many years before disease onset – at least in APOE4 carriers. However, longitudinal studies are required to establish whether affected individuals go on to develop AD. Interestingly, Small and coworkers109 demonstrated metabolic decline over a two-year period and showed that low baseline metabolism predicted future cognitive impairment in subjects at genetic risk of AD, emphasizing the potential of this approach as a preclinical measure of disease progression.

Volumetric magnetic resonance imaging (MRI) has revealed a robust longitudinal reduction in hippocampal and global cerebral volume with disease progression. Highly accurate computerized superimposition (‘registration’) of serial cross-sectional MRI scans enables the accurate measurement of even minor atrophy110. Median loss of brain volume ranges from 5–20 ml year−1 in AD compared with
less than 2 ml year$^{-1}$ in controls$^{111}$. Fox and colleagues$^{112}$ have shown that the rate of global brain atrophy measured by registered serial MRI is strongly correlated with rate of change in cognitive function (as assessed by the Mini-Mental State Examination), implying clinical relevance of this marker to disease progression. The finding that cerebral atrophy can be detected in preclinical AD by serial volumetric MRI (Ref. 113) suggests that this method could be used to monitor the course of the disease even at the preclinical stage, and thus constitutes a promising potential endpoint in trials of disease-modifying agents for AD.

As discussed above, of importance for clinical trials is the selection of appropriate and sensitive endpoints to demonstrate disease modification. Endpoints that enable smaller, shorter trials are favoured in this high-risk area of drug development. Neuroimaging appears to offer great potential as a surrogate readout of disease progression. Indeed, reduction in total brain volume (or shrinkage of a brain region known to be involved in cognition) might be expected to be intimately linked to the causal pathway of neurodegeneration, and thus to clinical deterioration. Hence, the demonstration of drug-induced reduction of brain atrophy might be expected to predict clinical benefit – an important feature of a potential surrogate endpoint.

**Future prospects for drug development**

The past few years have seen major breakthroughs in AD research, leading to an increased understanding of the pathophysiology. New tractable targets have been identified in key disease pathways, improving the prospects for development of disease-modifying drugs for this devastating disorder. The development of improved animal models, including transgenic mice that develop multiple features of AD, is facilitating preclinical evaluation of new approaches. This allows the provision of valuable confidence

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Population Description</th>
<th>Duration</th>
<th>Result</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selegiline (monoamine oxidase inhibitor) and vitamin E (antioxidant)</td>
<td>Moderate AD</td>
<td>24 months</td>
<td>Selegiline, vitamin E, or both, delayed time to primary outcome$^a$</td>
<td>107</td>
</tr>
<tr>
<td>Prednisone (corticosteroid anti-inflammatory)</td>
<td>AD</td>
<td>12 months</td>
<td>No difference in cognitive decline from placebo</td>
<td>53</td>
</tr>
<tr>
<td>Oestrogen (hormone)</td>
<td>Mild to moderate AD</td>
<td>12 months</td>
<td>No slowing of disease progression, nor improvement of global, cognitive or functional outcomes</td>
<td>106</td>
</tr>
<tr>
<td>Celecoxib (COX-2 inhibitor)</td>
<td>AD</td>
<td>12 months</td>
<td>No slowing of disease progression, nor improvement of global or cognitive outcomes</td>
<td>54</td>
</tr>
<tr>
<td>Rivastigmine (acetylcholinesterase inhibitor)</td>
<td>Mild cognitive impairment</td>
<td>36 months</td>
<td>Ongoing</td>
<td>Scrip 15 March 1999</td>
</tr>
<tr>
<td>Naproxen (conventional NSAID$^b$), celecoxib (COX-2 inhibitor)</td>
<td>AD</td>
<td>12 months</td>
<td>Ongoing</td>
<td>ADCS web site$^c$</td>
</tr>
<tr>
<td>Vitamin E (antioxidant) and donepezil (acetylcholinesterase inhibitor)</td>
<td>Mild cognitive impairment</td>
<td>36 months</td>
<td>Ongoing</td>
<td>ADCS web site$^c$</td>
</tr>
<tr>
<td>Naproxen (conventional NSAID$^b$), celecoxib (COX-2 inhibitor)</td>
<td>High-risk of AD (&gt;70 years with close relative with serious age-related memory loss, dementia, senility or AD)</td>
<td>30 months</td>
<td>Ongoing</td>
<td>PRNewswire 30 Jan 2001</td>
</tr>
</tbody>
</table>

$^a$Primary outcomes were death, institutionalization, loss of the ability to perform basic activities of daily living or severe dementia (as defined by Clinical Dementia Rating of 3).

$^b$Non-steroidal anti-inflammatory drug.

$^c$ADCS: Alzheimer's Disease Cooperative Study (http://antimony.ucsd.edu/).
in novel approaches before the expensive clinical phase of development. Clinical trials of disease-modifying drugs still constitute a major challenge, but the improved identification of at-risk individuals will enable smaller and more cost-effective trials, which will in turn allow increased testing of new agents. Potential new measures of disease progression, such as volumetric MRI, might facilitate future trials. Thus, it is hoped that preclinical breakthroughs, such as the identification of secretases and the validation of an immunization approach, can translate into much-needed new therapies for AD.

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