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# Drug repositioning and repurposing for Alzheimer disease

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## Abstract

Drug repositioning and repurposing can enhance traditional drug development efforts and could accelerate the identification of new treatments for individuals with Alzheimer disease (AD) dementia and mild cognitive impairment. Transcriptional profiling offers a new and highly efficient approach to the identification of novel candidates for repositioning and repurposing. In the future, novel AD transcriptional signatures from cells isolated at early stages of disease, or from human neurons or microglia that carry mutations that increase the risk of AD, might be used as probes to identify additional candidate drugs. Phase II trials assessing repurposed agents must consider the best target population for a specific candidate therapy as well as the mechanism of action of the treatment. In this Review, we highlight promising compounds to prioritize for clinical trials in individuals with AD, and discuss the value of Delphi consensus methodology and evidence-based reviews to inform this prioritization process. We also describe emerging work, focusing on the potential value of transcript signatures as a cost-effective approach to the identification of novel candidates for repositioning.

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Author contributions

The authors contributed equally to all aspects of the article.

Review criteria

Searches were performed in EMBASE, PsycINFO, MEDLINE and Cochrane databases for papers published after 1960. Search terms were as follows: generic class OR specific drug names OR any known alternative name (obtained from the electronic Medicines Compendium and the British National Formulary) AND dement* OR Alzheim* OR mild cognitive impairmen* OR neuropsych* test* OR cognitive func*.

The growing global health challenge posed by dementia needs to be addressed. Currently, more than 40 million people have Alzheimer disease (AD) worldwide, and this number is expected to increase to more than 100 million by 2050 (REF.[1]). In addition, estimates indicate that at least 15% of people aged 60 years or above have mild cognitive impairment (MCI) and that 8–15% of these individuals will progress from MCI to dementia each year, most commonly to AD[2]. AD is a devastating, progressive neurodegenerative disease that has a massive personal and financial impact on individuals, families and society. The estimated annual cost of dementia worldwide is US$818 billion, which is predicted to increase to US $1 trillion within this decade[1]. In the past 20 years only two new pharmacological therapies have become available for the treatment of AD. One of the treatments, memantine, has been licensed for the treatment of AD globally, whereas the other, oligomannate, is only licensed in China. Importantly, no pharmacological treatments have been licensed for use in individuals with MCI.

The core pathological substrates of AD in the brain are amyloid plaques and neurofibrillary tangles; the latter involve the hyperphosphorylation of tau[3]. The importance of other potential mechanisms, including neuroinflammation, protein misfolding, mitochondrial dysfunction and clearance of abnormal proteins, in the pathophysiology of AD has become increasingly apparent[4]. Despite a number of controversies regarding the role of amyloid in the pathogenesis of AD, including the question of whether neuronal death is driven by amyloid plaques or soluble amyloid and oligomers[5], the vast majority of treatments evaluated in clinical trials have focused on amyloid-related targets. The past decade has seen a number of high profile unsuccessful randomized clinical trials (RCTs) of amyloid-focused treatments, for example the anti-amyloid immunotherapy solanezumab[6] and the β-secretase inhibitor verubecestat[7]. A recent review of the NIH clinical trial registry identified only 29 pharmacological or biological treatments in ongoing phase II or phase III trials for disease modification in AD or MCI[8]. This number is 40-fold less than the number of ongoing RCTs in cancer[8], and the number of RCTs of disease-modifying therapies for AD has not substantially increased since 2012 (REF.[9]). Despite the enormous potential value of an effective disease-modifying therapy for AD or MCI, this area of research is considered to be high risk by the pharmaceutical industry, particularly as a result of low clinical trial success rates, and a number of global pharmaceutical companies have withdrawn investment from this therapeutic area[10]. Multiple factors could be responsible for the failed trials of disease-modifying therapies for AD; for example, the use of suboptimal treatments and targets, a narrow range of targets and methodological issues with the trials (BOX 1). Furthermore, owing to the low sensitivity of clinical and neuropsychological outcome measures, nearly 500 participants per treatment arm are needed for adequately powered phase II trials in individuals with MCI, which means that many phase II trials in individuals with this condition are significantly under-powered and the results are difficult to interpret[11].

The results from trials of the amyloid-targeting antibody aducanumab are emerging. In one of the two completed phase III trials, participants receiving aducanumab showed a statistically significant improvement in cognition and function compared with participants receiving placebo, particularly in the groups of participants carrying *APOE* ε4 (REF.[12]). The data from the other phase III trial are less clear, although some indication of benefit in

participants exposed to higher doses was reported[12]. The results of these trials are not yet fully in the public domain and have not been subjected to peer review, so interpretation needs to be cautious. Therapies that focus on other key treatment targets such as tau and neuroinflammation are at an earlier stage of development than aducanumab, but the preclinical data are promising[13]. These encouraging results might have a positive impact on AD drug discovery, for example, by attracting increased investment from the pharmaceutical industry. However, complementing traditional drug discovery with a broader range of approaches, such as drug repositioning and repurposing, will maximize drug development efforts. We used a systematic review of the literature and a Delphi consensus approach to highlight existing compounds that we feel should be prioritized for clinical trials in individuals with AD. In this Review, we present the results of that Delphi consensus and describe the evidence underlying the consensus prioritization. We then describe emerging work, focusing on the potential value of transcript signatures as a cost-effective approach to identifying novel candidates for repositioning.

## Drug repositioning and repurposing

Drug repositioning occurs within the biopharma industry during drug development and refers to the development of an agent for an indication other than the indication it was originally intended for. This new indication is prioritized during the development process and before approval. By contrast, drug repurposing is defined as “the application of established drug compounds to new therapeutic indications”[14] and offers a route to drug development that is accessible to academic institutions, government and research council programmes, charities and not-for-profit organizations, thus complementing the work of pharmaceutical and biotechnology companies. Repositioning and repurposing offer an attractive way of enhancing traditional drug development and accelerating the arrival of new treatments for AD dementia and MCI in the clinic. Phase II trials assessing repurposed agents must consider the best target population for a specific candidate therapy as well as the mechanism of action of the treatment.

Drug repurposing has enabled the identification of successful therapies for many diseases including cancer[15] and Parkinson disease (PD)[16]. One important advantage of this approach is that the safety of the candidate compound has already been established, which removes the need for further preclinical safety testing, chemical optimization or toxicology studies, and thus substantially reduces the time and cost involved in progressing the potential treatment into clinical trials. Marketed drugs are likely to have a reasonable safety database derived from previous registrational programmes, postmarketing experience and safety surveillance. In many cases, understanding this safety profile offers a solid ‘freedom to operate’ when repurposing the drug in a relatively fragile population, such as individuals with AD. Drug repurposing might also offer the further key advantage of bypassing the early preclinical, phase II and even phase IIa trials, all of which are time consuming and represent periods of relatively high drug attrition. In addition, many of the costs of drug development that are not always readily recognized, such as those associated with formulation optimization, manufacturing development and drug–drug interaction studies, have been addressed by the biopharmaceutical company that originally developed the drug. The estimated cost of developing a drug to the point of approval is US$5.6 billion[17], but these

extreme costs can be lower in programmes that focus on repurposed agents. Furthermore, for repurposed agents, clinical evidence of potential efficacy can be derived from existing pathophysiological observations, epidemiological cohort studies, open-treatment studies and preliminary clinical trials. This clinical information provides an important added dimension to the available evidence, particularly given the limitations of animal models.

Candidates for drug repurposing can be selected via a number of different routes, one of which is the use of large datasets to detect drug-associated patient outcomes that would otherwise have not been identified[18]. An alternative route is hypothesis-driven repurposing, which combines information about the disease of interest and the properties and targets of existing drugs for other conditions to identify potential candidates[9]. Similarly, high-throughput screening using in vitro models designed to assess the effects of compounds on known target mechanisms, such as amyloid toxicity, can be used[19]. A novel method is the use of disease-associated transcriptional signatures as a tool for identifying candidate therapies[20].

Another approach is to combine several of the above sources of information by manually reviewing the existing literature to identify candidates for repurposing. The challenge is that the kind of evidence available often varies among different compounds; for example, strong in vitro or in vivo evidence might exist for some candidates, whereas strong epidemiological evidence might exist for others. In addition, any identified treatment has to also be suitable for the target population, which for AD is older individuals with dementia. One way of addressing this challenge is to combine systematic review of the evidence with rigorous expert interpretation and consensus using methodologies such as the Delphi consensus approach, which is a standardized approach to achieving expert consensus based on a standardized review of the evidence and serial re-rating of priorities by a panel of experts.

## The Delphi consensus process

In writing this Review we combined available evidence from the repurposing routes described in the previous section with the aim of identifying the best candidate compounds for the treatment of AD or MCI. This process involved a comprehensive assessment of the published literature, a systematic evaluation of the evidence and a formal Delphi consensus process involving an expert panel. The Delphi panel had 12 members with expertise from the pharmaceutical industry, academia or drug development funding within the charity sector, including the authors of this Review (with the exception of G.W., P.D., A.C. and J.S.) and three additional panel members who represented patient organizations (see Acknowledgements section). Each panel member was asked to nominate up to ten candidate compounds for further consideration. A full systematic review of the literature was prepared for all five candidate compounds that were identified by at least three members of the panel. The members of the panel then ranked these five drug candidates in order of priority on the basis of the strength of evidence. The key factors used for this ranking included the mechanism and efficiency of brain penetration, the safety profile of the compound and whether or not the dosage of the drug used in preclinical studies was equivalent to the safe human dosage. The prioritization ratings of each panel member were shared with the panel at a face-to-face meeting and a second prioritization exercise was undertaken by e-mail. The

prioritization was then finalized at a further face-to-face meeting of the panel. This methodology was designed to update the systematic review and Delphi consensus published in 2012 in *Nature Reviews Drug Discovery*[9]. As the aim of this second Delphi consensus was to identify new candidate compounds, priority candidates from the 2012 census were excluded, but candidates not prioritized by the 2012 consensus were eligible if new evidence had emerged.

## Update on existing priority compounds

The 2012 Delphi consensus[9] prioritized five classes of compounds for repurposing as treatments for AD: tetracycline antibiotics, calcium channel blockers, angiotensin receptor blockers (ARBs), glucagon-like peptide 1 (GLP1) analogues and retinoid therapy. With the exception of retinoid therapy, all of the prioritized classes of compounds have now been taken into clinical trials. Trials of the tetracycline antibiotic minocycline[21], the calcium channel blocker nilvadipine[22] and the ARB losartan[23] have been completed and did not find any significant benefits of treatment on the cognition or function of individuals with AD.

### Tetracycline antibiotics

The RCT of minocycline[21] was a three-arm 24-month trial that compared the effects of either 400 mg minocycline per day, 200 mg minocycline per day or placebo, in a total of 554 participants with mild AD and a Mini Mental Status Examination (MMSE) score of ≥24. The two groups of participants who received the minocycline treatment were combined for the data analysis. In this combined group, the change in mean MMSE score, the primary outcome measure, over 24 months was only 0.1 points less than in the group that received placebo. No difference in the change in ability to perform activities of daily living over the 24 months was detected between the two groups. This was a pragmatic, but well-designed study, and provides a clear negative result, which suggests that further trials of minocycline for the treatment of AD are not warranted.

### Calcium channel blockers

Nilvadipine (8 mg per day) was evaluated in an 18-month double-blind RCT in 511 participants, of whom 253 received nilvadipine and 258 received placebo[22]. The participants were over the age of 50 years and had an MMSE score between 12 and 27, thus meeting the National Institute of Neurological and Communicative Disorders and Stroke — Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria for probable AD[24]. The primary outcome measure was a change in Alzheimer's Disease Assessment Scale – Cognitive Subscale (ADAS-Cog) score; however, only a 0.21-point non-significant difference in average ADAS-Cog score was observed between the two treatment groups over 18 months. For context, studies of cholinesterase inhibitors have found differences of >2 points in the ADAS-Cog score between groups of participants receiving treatment and groups of participants receiving placebo[25], and this would usually be regarded as the minimum clinically meaningful degree of change[26]. No benefit of treatment with nilvadipine was detected with the co-primary outcome measure (Clinical Dementia Rating — Sum of Boxes), or on any of the secondary or exploratory outcome measures. This trial was well designed and adequately powered and the absence of any significant differences between

groups is clearly a negative result, and plans for further studies of nilvadipine for the treatment of AD have not been reported.

### Angiotensin receptor blockers

In a preliminary study, 20 participants with probable AD and essential hypertension were randomly assigned to receive either the ARB telmisartan (ten participants, 40–80 mg per day) or the calcium channel blocker amlodipine (ten participants, 5–10 mg per day) for 6 months[27]. The group of participants who received telmisartan had increased regional cerebral blood flow in the right supramarginal gyrus, superior parietal lobule, cuneus and lingual gyrus compared with the group of participants that received amlodipine. No differences in cognition were observed between the two groups, but the study was very under-powered for detecting neuropsychological outcomes. More recently, in an RCT of the ARB losartan, 211 participants with mild or moderate AD were randomly assigned to receive either 100 mg losartan or placebo once daily for 12 months[23]. Preliminary results from the trial were presented at the Clinical Trials on Alzheimer's Disease (CTAD) conference in 2019. No significant reduction in the rate of cortical atrophy, which was the primary outcome measure, was observed in the participants receiving losartan compared with those receiving placebo, and the other clinical and cognitive outcome measures showed no indication of improvement associated with losartan treatment. Although the trial was under-powered for detecting changes in clinical outcomes, the absence of any trends towards improvement in the treatment group was disappointing[23].

Despite these negative clinical trial results, a solid body of in vitro and in vivo work supports the potential utility of ARBs as a treatment for AD[28–40]. In vitro work has identified multiple effects of centrally acting angiotensin II, including vasoconstriction, mitochondrial dysfunction, inhibition of acetylcholine release, increased production of angiotensin IV and release of inflammatory mediators[28–30], that suggest ARBs could be suited to repurposing for AD. Many commonly used ARBs, such as candesartan and losartan, have known blood–brain barrier penetration properties and have been shown to attenuate the central effects of angiotensin II in animal studies[31]. For example, in one study treatment with the ARB valsartan was associated with reduced amyloid-β (Aβ) aggregation in vitro[32], and improvements in behavioural tests of cognitive performance and reductions in amyloid pathology in a mouse model of AD[32]. In other studies of mouse models of AD, animals treated with ARBs showed reduced brain levels of total amyloid or Aβ aggregation, improvements in cognition and reduced neuroinflammation compared with animals treated with saline[33–37]. Studies of ARBs in Sprague Dawley rats have produced contradictory results, with some studies finding an ARB-associated decrease in tau phosphorylation and some studies finding an ARB-associated increase in tau phosphorylation[38–40].

Some epidemiological evidence also supports the use of ARBs for the treatment of AD. A large 4-year study of the medical records of 800,000 adults aged over 65 years found an almost 50% reduction in incident AD in individuals receiving ARBs compared with individuals receiving other cardiovascular treatments. The ONTARGET trial included 16,000 participants with hypertension, and significantly fewer participants showed a decrease in MMSE score to <18 in the group receiving the ARB telmisartan than in the

group receiving the ACE inhibitor ramipril[41]. However, this finding was not replicated in the parallel TRANSCEND trial in 5,000 participants with hypertension, which compared telmisartan with placebo[41], nor in the SCOPE trial in nearly 5,000 participants with hypertension, which compared the ARB candesartan with placebo. However, a subgroup analysis in participants from the SCOPE trial with pretreatment MMSE scores of 24–28 showed a modest benefit of treatment on cognitive ability[42].

The overall evidence for the use of ARBs to treat AD is mixed, and the absence of any benefits in the RCT of losartan is disappointing. However, the evidence reviewed in this section focuses on specific treatment mechanisms that are related directly to actions on the renin–angiotensin system. These observations must be interpreted in the context of strong epidemiological evidence indicating that hypertension is a risk factor for AD dementia[43] and the results of the recent SPRINT MIND trial, which demonstrated a significant reduction in the risk of MCI and probable AD dementia in participants receiving intensive anti-hypertensive management (target systolic blood pressure <120 mmHg) compared with the usual anti-hypertensive management (target systolic blood pressure <140 mmHg)[44]. The potential overall benefits of blood pressure reduction for heart and brain health should also be considered. Indeed, RCTs of candesartan and telmisartan in individuals with or at risk of AD are ongoing, and we should not discount ARBs as a potential treatment until the results of these trials are reported[45–47].

## GLP1 analogues

The emerging evidence base for the use of GLP1 analogues to treat AD is more encouraging than that of the other compounds prioritized by the 2012 Delphi consensus[9]. GLP1 analogues were prioritized on the basis of several in vivo studies in mouse models of AD that demonstrated an effect of this treatment on amyloid and tau pathologies[48–51] as well as oxidative stress, apoptosis, synaptic plasticity and other core neuronal functions[49,51–57]. More recently, this work was extended by a study of the GLP1 analogue liraglutide[58]. In this study, treatment of amyloid precursor protein–presenilin 1 (APP–PS1) mice (which carry AD-associated mutations in *App* and *PSEN1*) with liraglutide from the age of 2 months attenuated the development of progressive AD-related pathological changes, such as synapse loss, synaptic plasticity and amyloid plaques. Indeed, treatment with liraglutide has consistently been associated with improvements in cognition and memory in animal models of AD[58–61].

Three randomized, double-blind, multicentre, placebo-controlled trials examining of the cardiovascular effects of liraglutide or semaglutide also included the development of dementia as an exploratory outcome. A total of 15,820 participants were included in the three trials, and the median follow-up period was 3.6 years. Across the three trials, 15 participants who received a GLP1 analogue and 32 participants who received placebo developed dementia, with an estimated hazard ratio of 0.47 (95% CI 0.25–0.86) in favour of the GLP1 analogue treatment (C.B., unpublished work). This analysis is exploratory, and the frequency of incident dementia was modest. A post hoc analysis of the data from a RCT of another GLP1 analogue, dulaglutide, for the prevention of adverse cardiovascular outcomes in people with diabetes, also showed a significant reduction in incident dementia in

participants treated with dulaglutide compared with participants receiving placebo[62]. The findings of these RCTs need to be interpreted cautiously as they are based on post hoc analyses, but are consistent with a role for GLP1 analogue treatment in preventing the development of dementia.

Several more recent studies of GLP1 analogues in individuals with AD are underway or have been completed. A preliminary randomized, placebo-controlled clinical $^{18}$F-fluorodeoxyglucose (FDG)-PET study in 38 individuals with AD demonstrated that, compared with placebo, 6 months of treatment with liraglutide at a dose of 1.8 mg per day by subcutaneous injection prevented a decline in glucose metabolism in the brain[63]. Glucose metabolism is used as a marker of brain activity, and a lack of decline in glucose metabolism is usually taken to indicate preservation of biological brain function. Further analysis indicated that the underlying mechanism for this effect was an increase in blood–brain glucose transfer capacity and that, in the group of participants who received liraglutide, transfer capacity was the same as in healthy controls. A larger phase II RCT involving 204 participants with AD was completed in 2019 (REF.[64]). The results of an 18-month pilot double-blind placebo-controlled RCT of exenatide have been reported[65]. The study, which included only 21 participants, found that exenatide was well-tolerated, although an expected increase in nausea and decreased appetite was observed in the group that received the drug compared with the group that received placebo. The study found no significant difference in clinical, cognitive, neuroimaging or cerebrospinal fluid (CSF) measures between the two groups; however, given the very limited power of this study, these observations cannot be meaningfully interpreted. The levels of $A\beta_{42}$ in plasma extracellular neuronal vesicles were lower in participants receiving exenatide than in participants receiving placebo, which is an interesting result[65].

The results of these studies of GLP1 analogues are promising and provide increasing evidence that these drugs might prevent incident dementia in people with diabetes. A broader question is the potential utility of GLP1 analogues for the treatment of MCI due to AD or AD outside the context of diabetes. The preclinical studies in this area are encouraging, but further trials are needed and the results of the ongoing Evaluating Liraglutide in Alzheimer’s Disease (ELAD) trial are eagerly awaited.

## New priority compounds

During the 2018–2019 Delphi process a total of five compounds (or classes of compounds) were nominated for further consideration by at least three members of the panel. These compounds were ACE inhibitors, antiviral drugs, disease-modifying antirheumatic drugs (DMARDs), fasudil and phenserine (TABLE 1). Following several rounds of prioritization, the panel came to a clear consensus that the three highest priority candidates for repurposing in AD were fasudil, antiviral drugs and phenserine. Each of these identified candidates achieved the same prioritization rating, and there was no specific prioritization among the three candidates.

## Fasudil

Fasudil, a selective inhibitor of rho kinase (ROCK) 1 and 2, is a potent vasodilator, particularly of the cerebral vasculature[66], and is approved in Japan and China for the treatment of cerebral vasospasm following subarachnoid haemorrhage[67]. Fasudil was first suggested as a potential treatment for AD in 2009 when a study found that administration of the compound was associated with protection against age-related memory impairment in rats[68]. In a subsequent study, fasudil was mixed into artificial CSF administered directly into the brain in the APP–PS1 mouse model of AD. The aberrant dendritic arborization phenotype of this mouse model was reduced in mice receiving fasudil compared with mice receiving artificial CSF alone[69]. Fasudil administration was also associated with protection against hippocampal neurodegeneration induced by intracerebroventricular injection of $A\beta_{1–42}$ in rats. The authors reported increased IL-1β, increased tumour necrosis factor (TNF) production and increased activation of NF-κB in rats receiving fasudil treatment compared with rats receiving placebo and postulated that the protection against amyloid might be related to suppression of inflammatory responses[70]. More recent work using cell culture and several different transgenic mouse models of AD suggests that fasudil can protect against synaptic loss and cognitive impairment mediated by Aβ through the Dkk1-driven Wnt–PCP pathway[67,71]. Fasudil, delivered intraperitoneally, was also associated with reduced brain amyloid burden in the 3×AD-TG mouse model of AD[72].

Killick et al. identified 14 randomized placebo-controlled trials of fasudil in the literature[73]. These trials included a combined total of >500 participants with a range of indications from coronary heart disease to pulmonary hypertension. Fasudil was administered at doses of 60–240 mg per day, and most trials found good tolerability with no significant safety concerns. However, one double-blind, placebo-controlled clinical trial of a new extended release formulation of fasudil for pulmonary arterial hypertension did highlight several safety concerns[74]. In this trial, of 12 patients in the active treatment group, treatment was discontinued in one because of renal impairment and one patient died from heart failure. One small 2-month RCT conducted in China investigated the efficacy of fasudil for the treatment of AD[75]. In this trial, 106 male participants with MCI treated with nimodopine were randomly assigned to receive either 30 mg intravenous fasudil (once per day) or placebo for 2 months. Preliminary results indicated that fasudil was well tolerated, and the group treated with fasudil had significantly higher MMSE scores than the group that received placebo. This efficacy data should be interpreted cautiously, but good tolerability in individuals with MCI is important.

Overall, there is high concordance in the results of different preclinical studies, which suggest that fasudil targets classic AD neuropathology[76] by reducing amyloid burden and also targets other pathological mechanisms that contribute to AD, for example, by protecting against inflammation and synaptic damage[76,77]. These biochemical and physiological benefits have consistently translated into cognitive improvement in in vivo AD models[70,76,77].

## Phenserine

Phenserine was initially developed and evaluated as a cholinesterase inhibitor[78]. However, several mechanisms exist by which phenserine might reduce neuronal and synaptic loss[79]. which are important pathways in AD, traumatic brain injury and other neurodegenerative diseases. The results of a range of preclinical studies indicate that phenserine suppresses production of IL-1β, reduces glutamate-induced excitotoxicity, protects against $H_2O_2$-induced oxidative toxicity, reduces levels of Aβ, improves neural precursor cell viability, elevates brain-derived neurotrophic factor and inhibits APP and α-synuclein synthesis[79–82]. In particular, the results of several preclinical studies indicate that phenserine can reduce APP levels in vitro and in vivo[83–87]. Although these potential actions are of interest, more importantly, recent work has suggested that phenserine might confer significant neuroprotection by inhibiting apoptosis via actions on a pre-programmed cell death pathway[82]. This hypothesis has been evaluated in several rodent models of neuronal loss, including the APP–PSEN1 mouse model of AD, a rat model of post-stroke reperfusion injury and a weight drop mouse model of traumatic brain injury[80–82]. In all of these animal studies, treatment with phenserine was associated with significant reductions in the severity of neurodegenerative lesions and decreases in the neuroinflammatory response (via suppression of the IBA1 and TNF pathways) in the hippocampus and/or cortex[79,81,82]. Phenserine treatment was also associated with protection against reductions in synaptic density and levels of synaptophysin in animal models of AD and traumatic brain injury[79–82]. The multifaceted pharmacological action of phenserine as a neuroprotective agent was an important factor in the prioritization of this compound by the panel. In addition, administration of phenserine was associated with improved cognition in rats with NMDA receptor antagonist-induced impairments in learning[88].

Phenserine has been evaluated in two phase II placebo-controlled trials in individuals with mild to moderate AD[78,89]. The results of a phase II, 12-week RCT in 164 participants with AD indicated that (−)-phenserine (10–15 mg twice per day) had a favourable safety profile and the group of participants receiving the drug showed significantly better cognitive function than the group of participants receiving placebo[78]. A trend towards improvement in global outcome was observed in participants who received the higher dose of phenserine[78,90], with Cohen's *D* effect sizes of 0.3–0.4 for symptomatic benefits, which is similar to the effect sizes seen with other cholinesterase inhibitors[91]. A second, smaller RCT randomized 20 participants with mild AD to receive either phenserine (15 mg twice per day) or placebo for 3 months[89]. Over the subsequent 3 months, the patients allocated to phenserine continued to receive phenserine treatment while the placebo group then received donepezil in an open design. At the end of the first 3 months, the group of participants receiving phenserine had significantly better cognitive function (measured with a composite neuropsychological test) than the group of participants receiving placebo, and this significant difference between the two groups was maintained after the group receiving placebo had switched to donepezil for 3 months[89]. Although these results are encouraging, they must be interpreted cautiously given the small sample size of the study. Furthermore, a phase III trial of phenserine was discontinued early for commercial reasons and did not demonstrate a significant beneficial effect on the primary outcome measures, which were ADAS-Cog score and clinician's interview-based impression of change with care-giver input

(CIBIC+)[92]. The results of this phase III trial have not been published in full, but a press release described non-significant trends towards improvement with the doses of 10 mg and 15 mg[92]. These results are difficult to interpret on the basis of the preliminary reports, especially as the study was significantly under-powered to detect changes in cognitive and functional outcomes, with only 284 participants randomized in a 2:2:1 design. In addition, the dosing regimen was probably sub-therapeutic as the compound has a half-life of 5–6 hours, but was only administered twice per day, which led to criticism of the trial design[93].

Overall, the preclinical evidence that phenserine has biological effects that are relevant to the treatment of AD and other neurodegenerative conditions is strong. These effects include a newly identified influence on apoptosis. Phenserine also has a good clinical safety profile. Although the results from phase II studies are encouraging, they need to be interpreted cautiously given the small sample sizes and short trial durations. Trials of at least 12 months would be needed to identify disease-modifying effects. The potential of phenserine to combine the symptomatic benefits of a cholinesterase inhibitor with additional disease-modifying actions is, however, an exciting prospect.

## Antiviral drugs

The potential role of herpes simplex virus (HSV) as a risk factor or mediating factor in the development of AD emerged as a hypothesis in 1991, when HSV-1 was found in an active form in the brain of a large number of older people[94]. In 1991, a case–control post-mortem study found an association between HSV-1 infection and an increased risk of AD[95]. Little progress was made until the 2000s and 2010s, when further studies identified HSV-1 DNA within amyloid plaques in individuals with AD[96], and provided evidence for a role of HSV-1 in promoting the accumulation of Aβ[97–99] and the abnormal phosphorylation of tau[100–102]. In 2011, the authors of one study used quantitative immunocytochemistry in a kidney cell in vitro model to demonstrate that the changes in Aβ and phospho-tau production did not occur on initial entry of the virus into the cell, but were related to subsequent viral replication[103]. In vitro, the antiviral drugs aciclovir (the active form of the prodrug valaciclovir), penciclovir (the active form of the prodrug famciclovir) and foscarnet were associated with reductions in Aβ and phospho-tau accumulation, as well as levels of HSV-1. However, foscarnet had a more modest effect than the other two drugs. The accumulation of phospho-tau was dependent on HSV-1 DNA replication, whereas the accumulation of Aβ was not. This work is important in highlighting mechanisms that could link HSV-1 to the development of AD pathologies and in identifying candidate therapies.

More recently, the results of several epidemiological studies have supported the potential value of antiviral therapies in the treatment of AD. The authors of one study used Taiwan's National Health Insurance Research Database (NHIRD) to evaluate the records of 33,448 individuals and identified 8,362 individuals with a newly diagnosed HSV infection as well as 25,086 randomly selected sex-matched and age-matched controls without HSV infection[104]. The adjusted hazard ratio for the development of dementia in the participants with HSV-1 relative to the control participants was 2.6 ($P < 0.001$). Participants with HSV-1 who were treated with anti-herpetic medication had a significantly lower risk of developing dementia than participants with HSV-1 who were not treated with this medication. The risk

of dementia was lower among participants who used anti-herpetic medication for ≥30 days than in participants who used this medication for a shorter duration. Using the same database, a larger study of the records of 78,410 individuals identified a significant but more modest increase in the risk of dementia in participants with varicella zoster virus infection than in participants without the infection. This study also found that treatment with antiviral therapy significantly reduced the risk of developing dementia following the diagnosis of herpes zoster[105].

Overall, the evidence from in vitro and post-mortem studies suggests that HSV infection and possibly varicella zoster virus infection are risk factors for AD. Although the absence of substantive in vivo studies is a concern, emerging evidence from large-scale epidemiological studies confirms the association between risk of cognitive decline and HSV or varicella zoster virus infections. The results of these epidemiological studies also suggest that this risk can be mitigated by antiviral therapy. Therefore, strong arguments exist for exploring the potential benefit of antiviral drugs in individuals with AD. An ongoing phase II study of valaciclovir aims to recruit 130 participants with mild AD[106]. The existing evidence suggests that antiviral compounds might be more effective at diminishing the risk of AD or delaying the onset of AD in people with MCI, than as a treatment for individuals who have already developed AD.

In summary, three main classes of compound have emerged from the Delphi consensus process in 2018–2019: fasudil, phenserine and antiviral drugs. GLP analogues were prioritized by the 2012 Delphi consensus process and remain a high priority candidate for repurposing. The prioritization of these compounds is supported by strong packages of preclinical data, most of which include evidence from a number of different preclinical models. The preclinical data also suggest that each of these compounds can have an effect on multiple AD-related therapeutic targets in addition to amyloid. One advantage of repurposed compounds as opposed to newly developed therapeutic agents is that additional data can be gained from epidemiological studies, clinical cohort studies and clinical trials designed to measure a different outcome. For GLP analogues and antiviral drugs, clinical information from epidemiological studies or clinical trials with different primary outcomes support the potential utility of these drugs for the treatment of AD. However, information from clinical trials of any of the prioritized compounds in individuals with MCI or AD is much more limited. As discussed earlier, several clinical trials of phenserine have been performed, and two phase II trials suggested that in individuals with AD phenserine treatment is associated with improved cognition. However, these results are difficult to interpret because the studies used a suboptimal dose of the compound, were of short duration and had limited statistical power. Almost 500 participants per group are needed to provide reasonable power to detect changes in standard neuropsychology measures in an RCT in individuals with mild to moderate AD[11]. For GLP analogues, only very small preliminary studies have been performed, although the results of these studies are encouraging. The only reported study of fasudil in individuals with MCI or AD showed good tolerability of the compound, but was too small to allow conclusions to be drawn about the effect of the treatment on cognition. No RCTs of antiviral drugs in individuals with MCI or AD were identified in our literature searches. Therefore, the prioritization of these

candidates was predominantly based on the preclinical evidence, but with support from clinical information for most of the compounds.

### Compounds not short-listed

**Disease-modifying agents for rheumatoid arthritis.—**Although the anti-inflammatory action of DMARDs could theoretically reduce neuroinflammation in individuals with AD, the preclinical evidence supporting their usefulness was very limited[107]. The main evidence in favour of DMARDs was from an epidemiological population-based study that found a reduction in dementia risk in individuals receiving DMARDs compared with individuals not receiving DMARDs; however, the reported survival curves showed that the reduction in incidence of new-onset dementia among DMARD users compared with non-DMARD users was very small[18]. The study did not assess the effect of any single drug within the DMARD class, which is a limitation as these drugs vary widely in terms of pharmacological action, efficacy and tolerability. Furthermore, a placebo-controlled RCT of DMARDs in individuals with AD had negative findings[108]. On the basis of this evidence, the Delphi consensus panel concluded that DMARDs should not be prioritized as candidates for clinical trials in individuals with AD.

**ACE inhibitors.—**Some evidence from preclinical studies suggests that ACE inhibitors can protect against AD pathology; for example, in a transgenic mouse model of AD treatment with perindopril was associated with significantly reduced amyloid and tau burdens and levels of oxidative stress[109]. The clinical evidence in favour of ACE inhibitors was very weak. An open-label study in 113 individuals with AD[110] showed no significant benefits of perindopril treatment. A 4-month double-blind, placebo-controlled, pilot RCT of ramipril in 14 individuals with hypertension at risk of AD found that compared with placebo, treatment with ramipril was not associated with an improvement in cognition or a reduction in CSF levels of $A\beta_{1–42}$[111]. These poor preliminary clinical results led the panel to conclude that ACE inhibitors are not high-priority agents for repurposing for AD treatment, although the cardiovascular and cerebrovascular benefits of these drugs might indirectly reduce the risk of AD.

## Transcriptional approaches

Above, we have prioritized drugs on the basis of their established mechanisms of action. Strategies for identifying novel compounds for preclinical testing and clinical trials include transcriptional profiling, which could also be applied to the identification of drugs for repurposing. Disease or injury can perturb gene expression in a characteristic manner in a specific tissue, creating a ‘transcriptional signature’. If a drug perturbs gene expression in an opposing manner to the disease or injury, it might have therapeutic effects. Therefore, assessing the transcriptional changes induced by libraries of compounds could provide an important way of identifying novel candidates for repurposing. The Broad Institute Connectivity Map (CMAP) collated the transcriptional signatures induced by 1,300 drug-like compounds when applied to three cancer cell lines; importantly the CMAP data reflect responses specific to the known targets of the compounds as well as off-target responses[112]. The CMAP has been complemented by the LINCS L1000 project, which profiled the

changes in 1,000 ‘landmark’ transcripts induced by different compounds and used algorithms to predict the likely changes in expression levels of the non-measured transcripts to generate a full transcriptional signature[113]. The LINCS L1000 program has generated a database of transcriptional signatures for ~20,000 compounds, ~300 biological agents, and short hairpin RNA and/or cDNA against ~5,000 genes in ~100 human cell lines, including induced pluripotent stem cell (iPSC)-derived cortical neurons. The same approach could be applied to other compound libraries.

Transcriptional profiles are widely available for early, middle and late stages of AD and other dementias[114] and for almost all of the interventions, including genetic modifications, that are used to generate animal models of these diseases[114,115]. However, these data come from a variety of platforms and are hosted in different databases. The searchable, platform-independent expression database (SPIED) was developed to facilitate meta-analysis, with the aim of identifying disease-associated transcriptional perturbations that are common to multiple datasets, including data from post-mortem samples from individuals with AD[116,117]. This approach has identified shared transcriptional changes within multiple, independent AD-associated transcriptional signatures and the transcriptional signatures associated with other neurodegenerative diseases[114]. When the AD transcriptional signature was probed in CMAP, 153 drugs that perturb the cancer cell transcriptome in an opposing manner were identified[114]. Importantly, transcriptional changes that oppose those comprising the AD transcriptional signature were also observed when many of these drugs were applied to human iPSC-derived cortical neurons[20]. In a further study, transcriptional signatures for early and mild AD were used to probe both the CMAP and LINCS L1000 data, and 78 drugs with a transcriptomic signature that was significantly inversely correlated with the AD transcriptomic signature were identified and screened using six independent in vitro assays that were designed to mimic various aspects of AD pathology[118]. Of these 78 agents, 19 significantly reduced the AD-associated changes in at least two assays, and eight of these 19 agents were novel candidates known or likely to penetrate the brain. Some interesting candidates identified by this study included the adrenergic α1 receptor antagonist doxazosin, the antibiotic thiostrepton, which is known to have proteasome inhibitor properties, and the histamine H2-receptor antagonist famotidine. In addition to the identification of novel candidates for repositioning, the work supports the hypothesis that transcriptional profiling could be an effective way of identifying or triaging compounds for in vitro screening. For example, other hits included drugs already considered to be repositioning candidates in AD, such as metformin, nabumetone and several flavonoids[118].

## Future directions

The global transcriptional signatures discussed in the previous section were generated without considering the functions of the individual transcripts or the known mechanisms of drug action. Therefore, this process is a ‘black-box’ approach that operates independently of any mechanism-based hypothesis. Almost 30 risk genes for AD have now been detected[119], and the identification of drugs that alter the expression of some of these genes, or the expression of another gene with known therapeutic potential, would enable a hypothesis-driven approach to drug repositioning. There are no well-developed examples of this

approach in the AD field, but we briefly discuss three examples from related diseases that highlight the promise of this ‘targeted’ repurposing approach.

Accumulation of glutamate at synapses results in neuronal loss via ‘excitotoxicity’, and this process has been implicated as a causative mechanism in both acute brain injury and chronic neurodegenerative diseases such as AD[120]. Glutamate accumulation can result from the loss or failure of transporters that recycle this neurotransmitter, and reduced levels of the astrocyte glutamate transporter GLT1 (as known as EAAT2) is a characteristic feature of amyotrophic lateral sclerosis (ALS)[121]. In a milestone paper, Rothstein et al. postulated that drugs that increase the expression of GLT1 would be neuroprotective in a range of conditions, including ALS[122]. To test this hypothesis, the authors used neuronal cultures to screen 1,040 FDA-approved drugs and nutritionals and identified agents that increased levels of GLT1. The surprising finding was that the application of β-lactam antibiotics to neuronal cultures at concentrations similar to those in the brains of individuals being treated with these antibiotics increased GLT1 levels via a transcriptional mechanism. Moreover, treatment with the β-lactam ceftriaxone was associated with delayed neuronal loss and increased lifespan in a mouse model of ALS[123]. Beneficial effects of ceftriaxone have been reported in a wide range of non-clinical studies of pathologies that involve excitotoxicity, including models of AD[123]. Only one phase III clinical trial has tested the effects of ceftriaxone in neurodegenerative disease. The study cohort consisted of individuals with ALS and no significant differences in survival or functional decline (both primary end points) between the group of participants receiving ceftriaxone and the group of participants receiving placebo were detected[124]. Nonetheless, these findings are a useful example of a targeted repurposing approach and suggest that a trial of ceftriaxone or a related drug in individuals with AD could have positive results.

As opposed to increasing the expression of a protective gene, other researchers have sought to identify drugs that can reduce the expression of a risk gene. This strategy was recently applied to the search for PD therapies. Reducing α-synuclein transcription might be protective against PD[125], and a biological screen of FDA-approved drugs showed that α2-adrenergic agonists, such as salbutamol, suppress α-synuclein transcription[126]. Moreover, in a preclinical rodent model of PD, salbutamol was associated with some protection against pathology and motor deficits, and analysis of clinical records showed that the risk of developing PD was lower in individuals treated with salbutamol than in individuals not treated with the drug[126]. This association was confirmed in an independent patient cohort[127]; however, other researchers have suggested that the association might in part arise from the use of salbutamol to treat smoking-related pulmonary disease, which means that the cohort treated with salbutamol were likely to already have had a reduced risk of developing PD as a result of nicotine exposure[128]. Future clinical trials are needed to establish the effects of salbutamol on PD, but nonetheless similar approaches could be used to identify compounds that reduce the expression of AD risk genes.

Boosting levels of endogenous growth factors is another potential therapeutic approach that has been poorly explored in AD, but might be feasible, as shown by several studies in the field of PD[129–133]. Recombinant human fibroblast growth factor 20 (FGF20) can limit neuronal loss in preclinical models of PD[129,130]; however, delivery and target engagement of

growth factors remains a challenge in the clinical setting[131]. Endogenous FGF20 is enriched in the nigrostriatal pathway[132], and a simple in silico interrogation of CMAP identified 50 FDA-approved drugs that increase FGF20 transcript levels in cancer cell lines, 16 of which had transcriptional profiles that suggest they might be beneficial in PD[133]. Salbutamol and triflusal were in included in these 16 promising candidates and were then tested in vivo. In the 6-hydroxydopamine rat model of PD, treatment with either salbutamol or triflusal was associated with elevated levels of endogenous FGF20 in the nigrostriatal tract and a degree of neuroprotection. Evidence for salbutamol protecting humans against PD was discussed in the previous paragraph. Triflusal is a trifluoromethyl derivative of acetylsalicylic acid that inhibits platelet aggregation and, thereby, reduces the risk of stroke[134]. The drug also has anti-inflammatory, anti-excitotoxicity and anti-$Zn^{2+}$ toxicity effects that might limit ischaemic brain damage[135].

Limitations of the targeted repurposing approach include the fact that a drug is likely to alter the expression of perhaps hundreds of transcripts. For example, whether salbutamol is neuroprotective because it reduces α-synuclein expression, increases FGF20 expression, acts via a third unknown mechanism, or acts via a combination of multiple mechanisms is not clear. Likewise, triflusal could be neuroprotective in PD because it elevates FGF20 and/or because it has antioxidant and anti-inflammatory properties and/or because it acts via other unknown mechanisms. Similarly, although the parsimonious explanation for the neuroprotective properties of β-lactam antibiotics is an increase in glutamate uptake[122], these drugs also have antioxidant and metal-chelating properties that might explain or contribute to their efficacy as neuroprotective drugs[123]. This targeted repurposing approach is still in its infancy — transcriptional profiles have been successful in predicting some effects of compounds in vitro and in vivo, but it will be several years before we have any proof-of-concept clinical trials or examples of clinically available treatments. Nonetheless, the hypothesis-driven nature of targeted repurposing facilitates the design of experiments to directly test postulated mechanism of action of a specific compound.

## Conclusions

Drug repositioning or repurposing offers an attractive and cost-effective approach that can complement traditional drug development. We used a Delphi consensus process to identify promising classes of compound for repurposing that we feel merit evaluation in clinical trials. GLP1 analogues were identified as priority compounds in a Delphi consensus in 2012 (REF.[9]), but in this Review we discussed further supportive evidence that has subsequently emerged. We also presented and discussed three new compounds or classes of compound that were prioritized by the new Delphi consensus process. These compounds include the ROCK2 inhibitor fasudil, the cholinesterase inhibitor phenserine, which also has novel anti-apoptotic properties, and the antiviral drugs aciclovir, valaciclovir and famciclovir. We also reviewed the evidence for a novel transcriptomic approach to drug repurposing that could substantially increase the scale of identification of candidate compounds.

The potential advantages of complementing traditional drug discovery approaches with drug repositioning or repurposing include reduced costs and faster approval. However, several challenges to the expansion of this field remain, including the need for novel methodologies

to identify and screen new candidates, for example, transcriptomic approaches. Creating and expanding funding streams to prioritize this work and providing better commercial incentives for repurposing, perhaps through better protection by use patents, will also be important.

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Competing interests

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**Box 1 |**

## Potential reasons for failed RCTs of AD therapies

**Therapeutics and targets**

- The vast majority of trials have focused on amyloid targets, resulting in a lack of breadth.
- There is uncertainty regarding the specific disease mechanisms related to different amyloid species.
- Some therapeutics show poor brain penetration.
- Reducing amyloid deposition alone might not be sufficient to induce disease-modifying changes.
- There has been only limited use of target engagement biomarkers in phase II studies to inform phase III studies.

**Trial design**

- Many trials might have been performed in individuals with Alzheimer disease (AD) that has progressed too far for therapies to have a disease-modifying effect; an increased focus on preclinical AD and at-risk groups has been seen in more recent trials.
- The results of phase II trials have been interpreted in an overly optimistic manner, leading to the progression of some compounds to larger trials that might not have been warranted.
- Populations that are appropriately enriched for core AD pathologies have only been included in more recent trials.
- The neuropsychology measures used in trials can have a poor sensitivity to change; this insensitivity is a particular issue in phase II trials, which have usually been under-powered to detect changes in neuropsychology and clinical outcomes.

RCT, randomized clinical trial.

**Key points**

- Drug repositioning and repurposing offers a valuable alternative route for the identification of effective disease-modifying treatments for Alzheimer disease (AD).
- The Delphi method can be used to bring together the opinion of multiple experts to suggest candidates for repurposing.
- An expert Delphi consensus published in 2012 prioritized five compounds for repurposing as treatments for AD, of which glucagon-like peptide analogues remain high priority candidates.
- A Delphi consensus involving the authors of this Review was conducted in 2018–2019 and identified the ROCK inhibitor fasudil, the cholinesterase inhibitor phenserine and antiviral treatments such as valaciclovir as high priority candidates for trials in individuals with AD.
- The prioritization of these compounds was supported by strong packages of preclinical data, most of which include evidence from a number of different preclinical models.
- Transcriptional screening approaches offer a novel means of identifying potential treatment candidates by targeting AD-associated transcriptional profiles.

**Table 1 |**

Priority candidates from the 2018–2019 Delphi consensus

| Drug classes | Proposed candidates | Proposed mechanism of action | Summary of evidence | Remaining work required |
|---|---|---|---|---|
| ***Shortlisted candidates*** | | | | |
| ROCK inhibitors | Fasudil | Reduction in Aβ levels in vitro through the Dkk1-driven Wnt–PCP pathway[72]; reduction in inflammation[70]; prevention of synaptic damage[71] and impaired dendritic arborization[69] | Strong and consistent evidence of synaptic protection, reduction in amyloid and cognitive benefits in a range of in vivo animal models of AD[67,71,72]; several studies have shown acceptable safety in people with pulmonary hypertension and ischaemic heart disease[74,136,137]; only one very small study in MCI and AD, which found better scores on the verbal fluency test, Mini-Mental State Examination and activities of daily living with fasudil treatment than with nimodipine; the full study has not been published in English[75] | A well-powered RCT among participants with AD or MCI is needed to evaluate the effect of fasudil on cognitive function |
| AChE inhibitors | Phenserine | Suppression of IL-1β production; reduction in glutamate-induced excitotoxicity; protection against oxidative stress; reduction in Aβ levels; increase in production of BDNF; inhibition of APP and α-synuclein synthesis; and anti-apoptosis action on pre-programmed cell death pathway[19] | Several preclinical studies showed that phenserine reduces APP levels in cultured cells and in the brain of animal models[83–87]; phase II studies of phenserine showed good tolerability and demonstrated some indication of cognitive benefit, although the study was under-powered to properly examine cognitive function[78] | Further studies are needed to verify the potential mechanism of action in humans; these studies need to have adequate power to measure cognitive benefits |
| Antiviral drugs | Acidovir, pencidovir, valaciclovir and foscarnet | In vitro evidence suggests that HSV can accelerate the accumulation of amyloid[97–99] and promote abnormal tau phosphorylation[100–102]; antiviral drugs might mitigate these effects | A post-mortem case–control study in carriers of *APOE* ε4 found that AD was more common among individuals who had HSV than in those who did not[95]; an epidemiological study also showed that a cohort of persons with HSV had a higher risk of developing dementia than those without HSV[104]; recent large-scale studies suggest that the association between HSV and dementia is mitigated or reversed by antiviral therapy[94,104] | At least two small RCTs in a combined total of 163 individuals with AD are in progress[138,139], but a well-powered RCT is needed |
| ***Non-shortlisted compounds*** | | | | |
| DMARDs | Methotrexate, chloroquine phosphate, proguanil hydrochloride, cyclosporine, cyclophosphamide, hydroxychloroquine sulfate and sodium aurothiomalate | The potent anti-inflammatory actions of this class of agents might be a potential mechanism of action, but this has not been clarified in preclinical studies | A population-based retrospective cohort study found that participants using DMARDs had a modestly reduced risk of dementia compared with participants not using DMARDs[18]; a double-blind RCT in 168 individuals with mild AD over 18 months showed that hydroxychloroquine did not prevent cognitive decline[107] compared with placebo; an open-label trial in ten individuals with AD treated with hydroxychloroquine showed that CSF levels of Aβ did not change after treatment[140] (reviewed elsewhere[107]) | More robust preclinical studies are needed to establish mechanism of action; high-powered RCTs are also needed to confirm findings from observational studies |
| ACE inhibitors | Captopril, ramipril, lisinopril and perindopril | Reduction in amyloid deposition and tau hyperphosphrylation[141]; protection against oxidative stress[109,142]; reduction in blood pressure | Evidence of benefit inconsistent across studies[143] | Although there is some supportive preclinical evidence, the epidemiological evidence is fairly weak; RCTs, several of which are already ongoing, are needed to distinguish between the effect of hypertension control and the specific effects of ACE inhibitors |

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Aβ, amyloid-β; ACE, angiotensin-converting enzyme; AChE, acetylcholinesterase; AD, Alzheimer disease; APP, amyloid precursor protein; BDNF, brain-derived neurotrophic factor; CSF, cerebrospinal fluid; DMARDs, disease-modifying antirheumatic drugs; HSV, herpes simplex virus; MCI, mild cognitive impairment; RCT, randomized clinical trial; ROCK, rho kinase.