JIM Review

doi: 10.1111/joim.12959

Current and emerging avenues for Alzheimer's disease drug targets

R. Loera-Valencia[1], A. Cedazo-Minguez[1], P.A. Kenigsberg[2], G. Page[3], A.I. Duarte[4,5], P. Giusti[6], M. Zusso[6], P. Robert[7], G. B. Frisoni[8], A. Cattaneo[8], M. Zille[9], J. Boltze[10], N. Cartier[11,12], L. Buee[13], G. Johansson[1] & B. Winblad[1,14]

From the [1]Division of Neurogeriatrics, Centre for Alzheimer Research, Department of Neurobiology, Care Sciences and Society, Karolinska Institutet, Solna, Sweden; [2]Fondation Médéric Alzheimer, Paris; [3]Neurovascular Unit and Cognitive impairments – EA3808, University of Poitiers, Poitiers, France; [4]CNC- Center for Neuroscience and Cell Biology; [5]Institute for Interdisciplinary Research (IIIUC), University of Coimbra, Coimbra, Portugal; [6]Dipartimento di Scienze del Farmaco, Università degli Studi di Padova, Padova, Italy; [7]CoBTeK - lab, CHU Nice University Côte d'Azur, Nice, France; [8]University Hospitals and University of Geneva, Geneva, Switzerland; [9]Institute of Experimental and Clinical Pharmacology and Toxicology, Lübeck, Germany; [10]School of Life Sciences, The University of Warwick, Coventry, UK; [11]Preclinical research platform, INSERM U1169/MIRCen Commissariat à l'énergie atomique, Fontenay aux Roses, France; [12]Université Paris-Sud, Orsay, France; [13]Alzheimer & Tauopathies, LabEx DISTALZ, CHU-Lille, Inserm, Univ. Lille, Lille, France; and [14]Theme Aging, Karolinska University Hospital, Stockholm, Sweden

Abstract. Loera-Valencia R, Cedazo-Minguez A, Kenigsberg P, Page G, Duarte AI, Giusti P, Zusso M, Robert P, Frisoni GB, Cattaneo A, Zille M, Boltze J, Cartier N, Buee L, Johansson G, Winblad B (Karolinska Institutet, Solna, Sweden; Fondation Médéric Alzheimer, Paris; University of Poitiers, Poitiers, France; University of Coimbra. Coimbra, Portugal; Università degli Studi di Padova, Padova, Italy; CHU Nice University Côte d'Azur, Nice, France; University Hospitals and University of Geneva, Geneva, Switzerland; Institute of Experimental and Clinical Pharmacology and Toxicology, Lübeck, Germany; The University of Warwick, Coventry, UK; INSERM U1169/MIRCen Commissariat à l'énergie atomique, Fontenay aux Roses; Université Paris-Sud, Orsay; Univ. Lille, Lille, France; Karolinska University Hospital. Stockholm, Sweden). Current and emerging avenues for Alzheimer's disease drug targets (Review). *J Intern Med* 2019; **286**: 398–437.

Alzheimer's disease (AD), the most frequent cause of dementia, is escalating as a global epidemic, and so far, there is neither cure nor treatment to alter its progression. The most important feature of the disease is neuronal death and loss of cognitive functions, caused probably from several pathological processes in the brain. The main neuropathological features of AD are widely described as amyloid beta (Aβ) plaques and neurofibrillary tangles of the aggregated protein tau, which contribute to the disease. Nevertheless, AD brains suffer from a variety of alterations in function, such as energy metabolism, inflammation and synaptic activity. The latest decades have seen an explosion of genes and molecules that can be employed as targets aiming to improve brain physiology, which can result in preventive strategies for AD. Moreover, therapeutics using these targets can help AD brains to sustain function during the development of AD pathology. Here, we review broadly recent information for potential targets that can modify AD through diverse pharmacological and nonpharmacological approaches including gene therapy. We propose that AD could be tackled not only using combination therapies including Aβ and tau, but also considering insulin and cholesterol metabolism, vascular function, synaptic plasticity, epigenetics, neurovascular junction and blood–brain barrier targets that have been studied recently. We also make a case for the role of gut microbiota in AD. Our hope is to promote the continuing research of diverse targets affecting AD and promote diverse targeting as a near-future strategy.

Keywords: AD gene therapy, AD molecular targets, AD therapeutics, alzheimer´s disease, amyloid beta therapies, Tau therapies.

Introduction

Alzheimer's disease is a steadily growing global epidemic. Estimates suggest more than 47 million people worldwide were affected in 2015 and a staggering 131 million is predicted 30 years from now [1–4]. AD is a neurodegenerative disorder characterized mainly by the loss of memory functions and accompanied by other symptoms in a wide range of classes from mood, verbalization to motor problems. The most striking outcome from this type of dementia is the incremental disability for performing everyday life routines and increasing dependence from others for care. Aging is the

main risk factor for developing AD [5, 6], and the risk of developing AD dementia becomes even higher as life expectancy increases and the world population becomes older [5, 7]. Other reviews have dealt extensively with the economic burden this disease represents for countries [1, 4], estimating it at 0.65% of the world gross domestic product, a cipher rarely seen for a single disease [8]. Moreover, it is likely that the economic burden for AD is largely underestimated since it is difficult to account for the expenditure from family members paying for nursing or stop working to take care of their relatives [7, 9, 10]. Thus, solving the AD puzzle should be hand in hand with increasing the lifespan of humans, in order to reach for healthy aging, one of the main goals for the World Health Organization (WHO) and for many states worldwide [11, 12].

Currently, AD has no treatment available to modify its progression. Pioneering efforts from scientists and clinicians led to discovery and development of cholinesterase inhibitors for AD, capable of improving symptoms such as mood swings or dyskinesia, but these treatments do not halt AD progression nor improve memory performance in patients, as revised by Schneider *et al.* [13] and by Mangialasche *et al.* [14]. Antibody therapies have been developed from the main pathological hallmarks of AD, Amyloid beta (Aβ) and Tau proteins, to normalize their levels in the brain. These therapies are based on the amyloid cascade hypothesis, proposing that Aβ and Tau accumulation in the brain mediates synapse loss and neuronal death, leading to diminished memory function [15]. Nevertheless, many clinical trials aimed at reducing amyloid levels have not reached significant improvement in memory performance, or caused secondary, often-adverse effects and have dropped out [16]. Moreover, some failed clinical trials also led to the scientific community to explore additional hypotheses for AD pathogenesis [17–21].

Therefore, it has become more important to generate novel strategies and targets that will effectively alter in any form of the progression and the underlying causes of memory loss in AD. Novel evidence behind alternative mechanisms of the disease and improvements in technology from imaging to gene editing has opened new lines of research that could help to explain the origin and progression of AD [22–25]. In addition, the field is moving increasingly towards earlier and more accurate diagnostic of the pathology, where technology can help us to better classify and even redefine AD [26]. This review summarizes pioneering efforts in mechanisms of disease and novel drug targets for Alzheimer's disease research. We would like to emphasize the importance of multidisciplinary research in finding new treatment avenues in what it is a complex disease with many challenges.

Different treatment approaches based on pathogenesis

Synaptic plasticity and AD

Neuroplasticity is a complex response of neurons to endogenous and exogenous stimuli; it is a continuous process that embraces learning and memory processes. Neuroplasticity comprises morphological and functional interchanges, including differences in synaptogenesis, remodeling of the synaptic, axon and dendritic structures, and generation of new neurons (neurogenesis). All brain tissues are associated to neuroplasticity but hippocampus, neocortical areas and cholinergic basal forebrain neurons, which are involved in the regulation of higher brain functions, such as learning, memory and cognition, maintain an elevated degree of plasticity during all life stages.

The adult central nervous system (CNS) has a limited, although effective, ability to restore synaptic circuitry and its impact on cognition remains controversial. Furthermore, mechanisms that regulate neuroplasticity seem to be involved in neurodegenerative diseases. It is of interest to note that brain regions with elevated neuronal plasticity develop more slowly during infancy and are the most vulnerable in the aging and in AD. A disproportion between synapse formation and elimination could be responsible for defective plasticity during ageing and disease. If defective mechanisms controlling developmental plasticity are reactivated in later life, they could contribute to inefficient plasticity processes [27].

Memory deficits in AD could be related to early events that come before neurodegeneration, such as synaptic loss and dysfunction. Synapse degeneration is believed to begin with dendritic spines and with decreased quantity of molecules that regulate spine signaling [28].

Insoluble Aβ fibrils are taken into consideration as the main responsible for spine pathology. On the other hand, in both transgenic mouse models of AD and human AD brain, synapse defects and memory loss correlate weakly with the presence of Aβ

plaques and could take place before the formation of plaques. Indeed, small neurotoxins comprised of soluble Aβ oligomers (Aβ-derived diffusible ligands, ADDLs), present in the brain and cerebrospinal fluid of AD patients, are ligands able to compromise synaptic plasticity, even at nanomolar concentrations, by binding to dendritic spines or by the interference of transcription factor activation, mediated by N-methyl-D-aspartic acid (NMDA) receptors [29–31] (See Fig. 1).

Bidirectional trafficking of proteins at postsynaptic level is a mechanism involved in synaptic plasticity. For example, synaptic activity and activation of AMPA/NMDA receptors control AMPA receptor sorting. Moreover, endocytosis and exocytosis are involved in long-term potentiation (LTP) and long-term depression (LTD) of hippocampal synapses. Induction of LTP and LTD are prevented by blocking exocytosis and endocytosis, respectively. Recycling endosomes located at the spine level regulates spine growth, suggesting that stimulation of endocytosis and dendritic spine could promote plasticity [32].

Kinases play a critical role in synapse formation and plasticity. For example, the mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (Erk) pathway mediates the synaptogenic action of neurotrophic factors. This intracellular pathway could contribute to long-term synaptic plasticity by coordinating the activity of transcription factors and their subsequent nuclear translocation. MAPKs are located and active in synaptic terminals, suggesting a role in subcellular compartments during short- and long-term plasticity by phosphorylation of synaptic targets. Cyclin-dependent kinase-5 shows many roles in spine formation, expression of proteins in postsynaptic neurons, as well as in the phosphorylation of numerous molecules important for synaptic plasticity [33].

The immunoglobulin and cadherin super families of cell adhesion molecules control cell migration, growth of axons and synapse formation. The neural cell adhesion molecule (NCAM), expressed in neuron and glia cell surface plasma membrane, regulates the consolidation of learning and memory processes. Enreptin, a peptide agonist of NCAM, enhances long-term memory and reduces neuronal death. Furthermore, the cell adhesion molecule N-cadherin regulates spine stability. Synaptic cell adhesion molecules interact with Aβ and also control its production by regulating the activity of enzymes involved in Aβ formation. Aβ-dependent reduction of synaptic adhesion alters function and integrity of synapses, indicating an important role of synaptic adhesion in the maintenance of neuronal integrity [34].

The family of neurotrophins regulates synapse formation and synaptic plasticity. Nerve growth factor (NGF), a member of the neurotrophin family, promotes the synaptic function of cholinergic basal forebrain neurons, which contribute to memory process. Considering the regenerative effect of NGF on cholinergic neurons, its targeted delivery has emerged as a potential therapy for AD. Recently, a small clinical trial inserting encapsulated NGF-producing cells in AD patients has shown safety and tolerability increasing cholinergic markers in CSF [35]. Long-term exposure of hippocampal neurons to brain-derived neurotrophic factor (BDNF, another neurotrophin with structural similarity to NGF) modulates synaptic transmission and plasticity and effects structural changes of dendrites, spines and presynaptic terminals. Moreover, BDNF exposure has effects on the synaptic proteome, by affecting protein synthesis or degradation [36].

Glial cells are involved in nervous system stability and synaptic plasticity. Glial processes ensheath synapses, support their development and functions and secrete proteins (e.g. thrombospondins) that promote CNS synaptogenesis. Complement C1q and C3, upregulated in neurons exposed to astrocytes, participate in synapse elimination. Patients with frontotemporal dementia have low levels of progranulin (a protein antagonist of tumor necrosis factor-alpha (TNF-α)), which results in defects of lysosomal functions and excessive activation of complement, causing synaptic pruning by microglia and behavioural defects rescued by blocking complement activation [37].

17β-estradiol (E2) supports dendrite growth, spine and synapse formation in both developing and adult CNS. In the hippocampus, E2 modulates synaptic plasticity slowly (genomically via classical nuclear receptors) and rapidly (nongenomically via extranuclear receptors) [38]. Nanomolar concentrations of E2 cause changes in hippocampal spine morphology. Activation of neuronal glutamate receptors, by glutamate released from astrocytes in response to PGE2, modulates dendritic spine density [39].

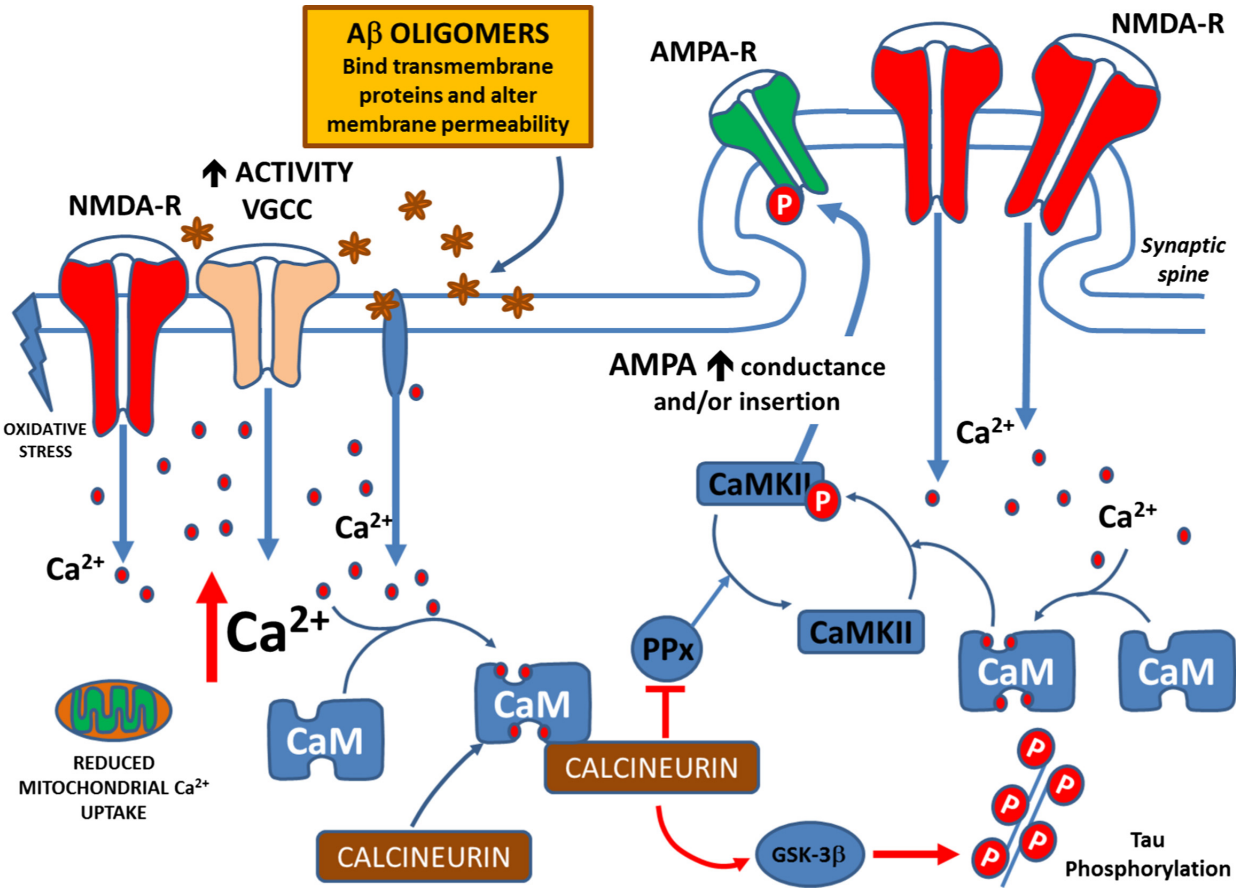

Fig. 1 *Dendritic spine and AD: mechanisms causing spine degeneration. An impaired synaptic plasticity is an early event in Alzheimer disease (AD). Synaptic plasticity is accompanied by morphological adaptations of dendritic spines, such as changes in the number and shape of spines (structural plasticity). A fundamental mechanism for modification of synaptic strength is insertion (activation) or removal (inactivation) of alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPARs) at the postsynaptic membrane. Such adaptation occurs within minutes, yet may also extend over longer times. Aβ 1–42 over-activates NMDAR/ calmodulin (CaM)/ calcineurin/ glycogen synthase kinase-3β (GSK-3β). Calcineurin, a calcium-sensitive phosphatase, regulates synaptic plasticity and is required for AMPARs internalization and long-term depression. Aβ oligomer-induced AMPARs endocytosis and spine loss are prevented by calcineurin inhibition. Therefore, inhibition of calcineurin could be a therapeutic strategy for combating early-stage AD impairment.*

The role of inflammation in AD is well recognized. Elevated levels of TNF-α, a pro-inflammatory cytokine responsible for the neuroinflammatory response, have been reported in brain and plasma of AD patients. TNF-α modifies synaptic transmission and strength. Synaptic scaling, which is a homeostatic mechanism that takes part in the synaptic dysfunction in AD may be the mechanism involved in these events. As TNF-α modulates synaptic scaling, alteration of synapsis mediated by elevated levels of TNF-α could contribute to cognitive and behavioural impairments in AD [40].

In summary, in the mammalian CNS, dendritic spines are essential for synaptic function and plasticity. AD and other CNS disorders have a strong relation with aberrant dendritic spines. Synaptic plasticity and spine alteration can be influenced by many factors, including Aβ, impaired glucose and lipid metabolism, steroids, kinase pathways, cell adhesion molecules, neurotrophic factors, glial cells and inflammation. A better knowledge of cellular and molecular molecules able to control the age and/or cognitive abilities may lead to effective treatments for age-associated

memory impairment and for other, more severe cognitive impairments, in particular AD.

Epigenetics and AD

Epigenetics involves heritable changes in gene function not caused by mutations in DNA sequence [41]. Such changes may relate to chromosomal ones that affect gene activity and expression, as well as heritable phenotypic changes that do not derive from genome modification. These effects on cellular and physiological phenotypic traits could be driven by external or environmental factors, or be part of a normal developmental programme. Numerous CNS physiological functions (neural stem cell fate determination, neural plasticity, and learning and memory) have significant epigenetic components. This is the case also for neurodegenerative diseases. For example, in Alzheimer disease (AD), both genetic and non genetic factors contribute to disease etiopathology. Whilst over 250 gene mutations have been related to familial AD, <5% of AD cases are gene-related.

At least three systems including DNA methylation, histone modification and noncoding RNA (ncRNA)-associated gene silencing can initiate and sustain epigenetic change. More than likely nongenetic factors, probably triggered by environmental factors, are causative factors of late-onset AD. Many CNS pathologies, including AD are associated with dysregulation of DNA methylation, histone modifications (deacetylation, phosphorylation, ubiquitylation and SUMOylation) and ncRNAs [42]. Histone phosphorylation, in particular, appears to be part of a complex interplay between other epigenetic markers, such as histone acetylation and methylation, and DNA methylation.

Indeed, histone phosphorylation increases pro-inflammatory gene activation [43]. A number of proteins involved in AD pathology (amyloid precursor protein (APP), Tau, β-site amyloid precursor protein cleaving enzyme 1 (BACE1), glycogen synthase kinase-3β and c-Jun N- terminal kinase) are SUMO (small ubiquitin-like modifier) targets [44]. Furthermore, AD patients have altered levels of SUMOylation and SUMO-related protein expression [45].

Amongst the classes of ncRNA, microRNAs (miRNAs) are highly expressed in CNS neurons, where they play a major role in neuron differentiation, synaptogenesis and plasticity. MicroRNAs impact higher cognitive functions, as their functional impairment is involved in the aetiology of neurological diseases, including AD [46]. A growing body of evidence points to alterations in the miRNA network as active contributors to AD disease processes [47]. Alterations in the miRNA network contribute to AD disease pathogenesis by (i) regulating expression of APP and other enzymes involved in Aβ processing, in particular BACE1; (ii) Neurofibrillary tangles in AD brain are composed mainly of hyperphosphorylated Tau, whose state of phosphorylation represents a fine balance between kinases and phosphatases, processes that may be regulated by miRNAs; (iii) regulation of lipid metabolism [48]; and (iv) neuroinflammation [49].

Understanding epigenetic dysregulation in AD could contribute to our view of the origin and progression of AD and, possibly, the development of efficacious therapeutics. However, one caveat with epigenetic studies is the issue of causality. Yet, given the failure of AD clinical trials to date, focus is now shifting to diagnose AD at as early a stage as possible, even before onset of cognitive decline. Despite the inherent difficulties, timely disease detection offers a multitude of benefits, not the least of which are opportunities for early intervention and better management of symptoms. miRNAs have emerged as potential candidates for reliable biomarkers of early-stage AD, being present in biofluids and displaying high stability in terms of storage/handling. Moreover, ncRNAs, miRNAs—and especially long ncRNAs—as therapeutic targets are only beginning to be considered. Even so, these transcripts represent potential targets for two reasons: (i) long ncRNA expression seems to be rather cell- and tissue-specific; (ii) the sequence-specific function of long ncRNA can be advantageous in designing specific therapies.

Blood–brain barrier targets

Blood–brain barrier (BBB) is a multicellular vascular structure that separates the central nervous system (CNS) from the peripheral blood circulation. The core anatomical element of the BBB is the cerebral blood vessel formed by endothelial cells (ECs). Mural cells represented by pericytes and astrocytes sit on the abluminal surface of the microvascular endothelial tube. Astrocytes interact with neurons and microglia. Both pericytes and astrocytes interact with ECs and maintain the sealing of interendothelial tight and adherent junctions, loss of leucocyte adhesion molecules

and inhibition of transcytosis [50–53]. The vascular tube is surrounded by two basement membranes, the endothelial vascular basement membrane corresponded to an extracellular matrix secreted by the ECs and pericytes, and the parenchymal basement membrane primarily secreted by astrocytic processes that extend towards the vasculature. The molecular components of these basement membranes also contribute to the complexity of the barrier [54].

Besides, these complex cellular interactions which correspond to the neurovascular unit instead of BBB, endothelial cells express different types of transporters and receptors amongst which some are involved in the efflux and influx of the amyloid peptide [55, 56]. Beyond barrier function, influx and efflux are actively regulated at the blood–brain interface. Moreover, recent research has uncovered different transcription factors involved in phenotype change (zonation) along the vessels of the BBB [57]. The BBB maintains an environment that allows neurons to function properly by tightly controlling the passage of molecules and ions, instantaneously delivering nutrients and oxygen according to current neuronal needs, and by protecting the brain from toxins and pathogens. We now know that the cellular and molecular complexity of the BBB explains that the dysfunction of a cellular or molecular actor can disrupt its dynamics, although the precise process is unclear [58].

Blood–brain barrier in AD

Several impairments of the neurovascular unit have been described in Alzheimer's disease (AD), but the time-point at which they occur during disease pathogenesis remains unclear because they are too often seen in *post-mortem* brains. However, for the past 3 years, medical imaging has demonstrated the early BBB disruption in the hippocampus even before the onset of hippocampal atrophy [59]. In addition, many studies indicated cerebral microbleeds (micro haemorrhages) in AD [60, 61]. Compared with controls, BBB P-glycoprotein activity was significantly lower in the parietotemporal, frontal and posterior cingulate cortices and hippocampus of mild AD subjects by PET-scan [62]. Besides, many morphological and functional changes in brain vasculature in AD were observed: thinning of microvessels, referred to as atrophic or string vessels; twisted or tortuous vessels and fragmented vessels [63]; thickening and vacuolization of the vascular basement membrane with increase of collagen IV [64]; leakage and accumulation of circulating plasma proteins with direct neurotoxic properties and erythrocyte-derived haemoglobin in brain [56]. Additional changes include also pericyte loss [65], astromicrogliosis, many molecular changes directly impacting the clearance of the amyloid peptide (decrease of GLUT-1, LRP-1, P-gp and increase RAGE) [60], hypoperfusion and permeability failure [66].

Chemokines as critical targets for diagnosis or therapeutic strategies

Amongst the peripheral molecular actors, we can target chemokines. Indeed, many articles have shown the involvement of chemokines in the pathophysiology of AD [67]. Of those that are deleterious, the pro-inflammatory chemokines CXCL10/CXCR3, CCL3, CCL4, CXCL8/CXCR8 and CX3CL1/CX3CR1 increase in AD, lead to inhibition of Aβ clearance, increased adhesion of PBMCs [68–73]. On the contrary, CCL5 is known as neuroprotective [74, 75]. Besides the too high or too low levels of some chemokines including CCL2 are unfavourable in AD because the physiological activation of the CCL2/CCR2 signalling pathway is crucial to limit the progression of the disease in AD experimental models [76–78].

In the light of these elements of the literature, we studied the impact of PBMCs issued from AD patients on the chemokines' signature at the level of a healthy BBB, given that the current data on chemokine levels are derived from isolated biological samples (Plasma, serum, brain and cell culture) whilst BBB displays a great cellular and molecular complexity, finely orchestrated to preserve the brain.

In a human BBB model comprising two cell lines, an endothelial cell line (hCMEC/D3) and U87 cell line (human glioblastoma), PBMCs from patients (control, mild and moderate AD patients) were added in the luminal medium. It should be noted that all analyses were also performed on isolated cultures of each cell type and on a BBB model without PBMCs. A previous work on a group of patients with AD at a moderate stage has already been published, and we also verified in this study with the 3 groups of patients the interest to go to an integrated model to take into account the cellular and molecular interactions in the neurovascular unit [79]. Results showed that PBMCs from moderate AD patients decreased CCL2 and

CCL5 levels in luminal and abluminal compartments (2-3-fold) and CXCL10 only in the abluminal compartment (3-4-fold) compared to PBMCs from mild AD patients. Levels of CCL2 and CCL5 also significantly decreased on PBMCs of moderate AD patients compared to PBMCs from mild AD patients. The CX3CL1 expression increased in luminal and abluminal compartments with PBMCs from mild AD patients compared to controls [80].

In a murine BBB model (French patent in August 2017 and PCT extension in August 2018), the impact of mouse PBMCs from transgenic mice (APPswePS1dE9) or their control littermates in the signature of chemokines in BBB prepared from mouse brains was studied at 3, 6 and 12 months. In this model, a healthy abluminal compartment is used, and PBMCs and luminal compartments came from AD or wild-type mice. Compared to results obtained in human BBB model, we also showed a decrease in CCL2 expression (about 6-fold) in the abluminal medium by PBMCs issued from AD mice at 12 months compared to WT mice. Furthermore, results showed an increase in CX3CL1 in the abluminal compartment (2.3-fold) and a decrease (4-5 fold) in cells used in luminal compartment in 12-month-old mice compared to 3-month-old mice [80].

In both BBB models, the PBMCs come from patients or mice with advanced disease (moderate and 12 months) and the abluminal compartment is healthy. Even if the luminal compartment is AD in the mouse model, we observed:

- a significant decrease in CCL2 in abluminal compartment with AD PBMCS (moderate stage or 12 months)
- an early increase in CX3CL1 (mild *versus* controls) in luminal and abluminal media and also an increase in abluminal compartment with mouse AD PBMCs (12 months *versus* 3 months).

It is known that the variations of these two chemokines are deleterious in AD, and it has been demonstrated that they are induced by PBMCs from AD patients or mice with advanced AD. Thus, the results join other publications highlighting an origin of peripheral blood in AD. The modulation of the blood–brain interface by targeting CCL2 and CX3CL1 could be a new therapeutic pathway.

Neurovascular junction damage and therapeutic targets: insights from preclinical research on vascular dementia and microbleeds

Vascular dementia and its most prominent subtype, subcortical atherosclerotic encephalopathy (M. Binswanger), represent the second most frequent and important form of dementia in the elderly after Alzheimer's disease. It represents about 15% of all dementia cases, whilst another 15% of cases are mixed forms occurring together with Alzheimer's disease. Vascular dementia has therefore rapidly gained attention as a growing medical and socioeconomic burden. Vascular dementia and, in particular, M. Binswanger are characterized by progressive white matter lesions that are strongly related to cognitive decline and believed to be an important pathophysiological hallmark of almost half of all dementias in the elderly (and even beyond 'pure' forms of vascular dementia [81]. Symptoms of vascular dementia are also observed in cases of disseminated cerebral microbleeds.

Despite its significant impact, relatively little is known about central pathogenic mechanisms, and no casual treatments are available so far. It is known, however, that hypertension plays an important role in vascular dementia and rigorously controlling blood pressure may slow down its progress. In turn, increased systolic BP progressively disrupts white matter integrity already in young adults and increases the risk for late-life dementia [82].

In human vascular dementia patients, microbleeds and lacunar infarcts typically occur in the basal ganglia whilst white matter hyperintensities preferentially develop in the centrum semiovale. Anatomical factors might explain these differing predilection sites: arterioles entering the deep white matter from the superficial cortex are coated by a single leptomeningeal layer rendering them more susceptible to hypertension-related vascular damage [83, 84]. Microbleeds preferably appear in the basal ganglia.

In this section, current findings will be outlined from preclinical research that may indicate such novel therapeutic targets for vascular dementia, which, at least in part, may also be relevant for Alzheimer's disease. Potential therapeutic approaches will also briefly be presented.

Preclinical research in vascular dementia: state of the art

Preclinical research in vascular dementia relies on a number of animal models, most of which separately mimic a selected aspect of human disease, predominantly lacunar infarcts, white matter damage and vessel dysfunction. An important animal model is stroke-prone spontaneously hypertensive rats (SHR-SP). They feature most of the cardinal histopathological signs of cerebral small vessel disease (cSVD) [85] likely as a consequence of chronically increased arterial blood pressure that causes vascular dysfunction [86]. However, the SHR-SP model is biased towards the bleeding facet of cSVD [87] which might be due to genetically fixed alterations of the endothelial tight junctions [88] and a massively increased blood pressure by far exceeding that observed in human patients. Recent research on SHR, which present high, but not extremely increased systolic blood pressure, revealed very similar behavioural and histological findings as seen in human vascular dementia patients [89] (Fig. 2). Moreover, a number of disease-driving alterations such as focal BBB breakdown, macro- and microglial activation, and immune alterations may also provide promising targets for early-stage AD (Fig. 2).

Behavioural changes in SHR, white matter and BBB breakdown

Middle-aged SHR showed a reduced discrimination capability between known and unknown objects, indicating a decline of the nonspatial working memory, primarily related to frontal-subcortical circuits [90]. Spatial memory is initially not affected. However, spatial memory deficits being typical in human vascular dementia patients [91] may develop over time since time-dependent loss of cornu ammonis 1 pyramidal neurons occurs in SHR [92].

Macro- and microglial activation

A sustained macro- and microglial activation in deep cortical regions can be observed in SHR. Although the number of Iba1-positive microglia in DCR is comparable results between SHR and normotensive Wistar Kyoto rats (WKY), single-cell morphological analysis increased cellular volumes

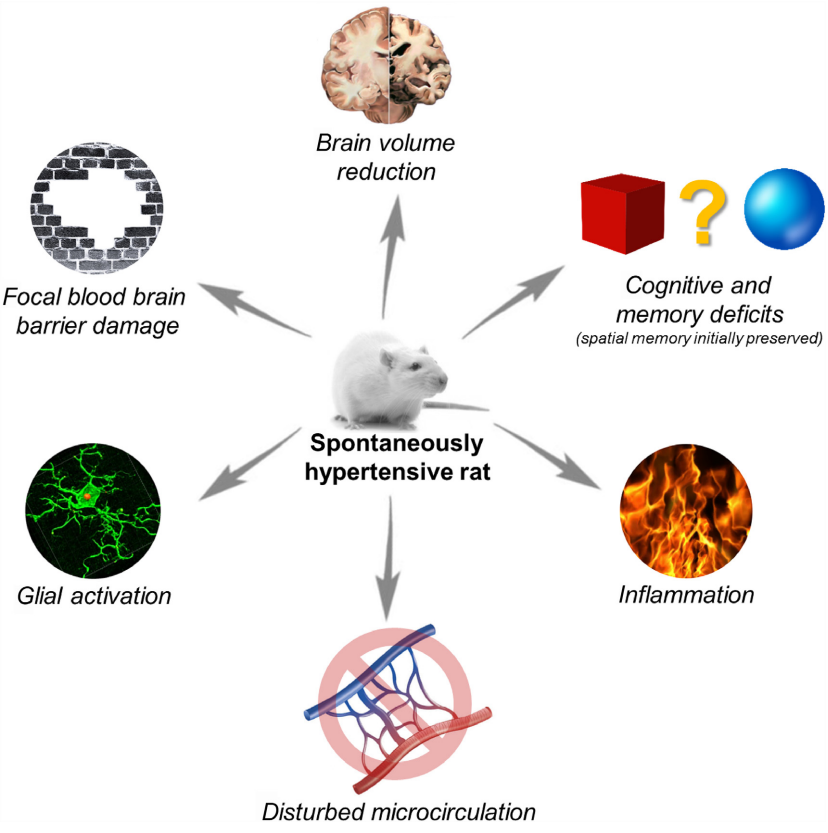

Fig. 2 *Spontaneously hypertensive rats as a model of vascular dementia. Spontaneously hypertensive rats develop cognitive deficits in their middle age that continue to aggravate with age. The animals also show an increasing loss of brain tissue with age, particular in in-deep cortical regions, subcortical areas and the corpus callosum. The primary cause of this cognitive decline could be chronic hypertension and the animals exhibit a number of pathophysiological hallmarks of vascular dementia such as blood–brain barrier damage, impaired microcirculation, glial response and chronic inflammation. Scheme drawing based on study of Kaiser* et al. *[89]*

being indicative of microglial hypertrophy. Microglial activation is further indicated by increased CD11b expression [89].

Immunological mechanisms potentially contributing to vascular dementia

There is increasing evidence that the immune system significantly contributes to the development and progression of vascular dementia. For instance, serum levels of soluble adhesion molecules were increased in patients with white matter lesions [93] and c-reactive protein (CRP) levels correlate with the existence and progression of white matter damage [91]. The association of inflammation and vascular dementia is not surprising since chronic inflammation also plays an important role in the pathophysiology of its primary risk factor hypertension [94, 95]. However, whether such inflammatory processes initiate vascular and tissue damage, promote its propagation or simply constitute a response to ongoing reorganization remains unclear. Similar relationships have been described for Alzheimer's disease.

Distribution of blood-borne leucocytes differs between SHR and WKY strains. In WKY, T cells were mostly localized within the meninges and the choroid plexus (CP), whilst the majority of T cells populated microvessels within the SHR brain parenchyma. The different T cell distribution patterns may be explained, for instance, by an upregulation of VCAM-1 in brain endothelial cells, which occurs as a consequence of an activated renin-angiotensin system during arterial hypertension in hypertensive rats [96] and vascular dementia patients [97]. The increased presence of T cells adhering to the luminal side of cerebral microvessels might indicate slowed vascular transit time of leucocytes due to pseudopod formation [98] or may be part of a systemic adaptive immune response against vascular neoantigens. Importantly, T cells directly promote endothelial dysfunction.

An interesting finding was the considerable decrease of T cells in the meningeal space and the choroid plexus of SHR. Meningeal T cells have a significant impact on learning behaviour, memory function and mood stabilization [99]. Moreover, higher amounts of natural killer (NK) cells were present in the SHR brain.

Besides their direct participation in endothelial dysfunction (as an indicator of BBB disintegration), T and NK cells may play an important role in cerebral arteriogenesis [100] owing to perfusion deficits and shear stress. Increased angiogenesis helps to restore the neurovascular junction in areas where blood vessels become rare due to continuous vascular damage. This may indicate repair processes, along with an increase of DCX-positive neural progenitors in the SHR subventricular zone. Neurogenesis might indeed be initiated by white matter injury in vascular dementia [101].

Preclinical research on post haemorrhage neuronal damage: novel insights

Recent clinical evidence suggests that the occurrence of microbleeds leads to a greater cognitive decline in vascular dementia and AD [102–104]. In the Rotterdam Study, higher levels of plasma Aβ were associated with increasing lacunar and microbleed counts [105]. However, microbleeds are often functionally asymptomatic in patients [106, 107] and are therefore difficult to detect clinically, except using modern imaging technology.

Furthermore, cognitive decline is particularly worsened when microbleeds occur in deep brain regions or simultaneously in lobar and deep structures [103]. Blood breakdown products may lead to axonal and white matter injury of fibres trespassing the lesion site resulting in delayed, distal cell death. There is evidence from larger brain haemorrhages in the basal ganglia that axonal degeneration occurs in the internal capsule due to its close proximity. For example, Wallerian degeneration is common in intracerebral haemorrhage (ICH) patients and occurs particularly in the corticospinal tract in deep ICH [108].

The underlying molecular mechanisms of how microbleeds promote cognitive decline and axonal degeneration/white matter damage remain incompletely understood. Blood breakdown products released from the bleed can cause neuronal cell death engaging nonapoptotic forms of regulated cell death [109, 110]. In addition, it is known that degeneration of axons, in general, occurs actively, but autonomously from neuronal cell body death, and via different molecular mechanisms [111]. Whilst neuronal cell bodies may die via the canonical caspase-3-dependent apoptotic pathway, blockade of this pathway does not prevent axonal degeneration [112]. Axon degeneration depends on the proapoptotic family member bax and requires caspase-6 [113].

Gut microbiota and AD

In AD subjects, higher levels of pro-inflammatory cytokines have been found, together with reactive microglial cells co-localizing with amyloid plaques. It has been proposed that high levels of inflammation are a consequence of Aβ signalling [114, 115]. However, recently this hypothesis has been revised since the induction of the pro-inflammatory state can promote the amyloid cascade. It is in this context that we look at the role of the microbiota.

The gut microbiota has been named our other brain for the functional connections between the two. The microbiota weighs as much as the brain itself (up to 1.5 kg [116]) and is made of bacteria, viruses and fungi. The number of bacteria in the gut exceeds the number of somatic cells by 10-fold and the number of microbial genes (the microbiome) exceeds the number of human genes by 100-fold [116, 117].

The human gut has bacteria with pro-inflammatory and others with anti-inflammatory properties, in dynamic homeostatic balance. Different stressors can lead to dysbiosis, that is an imbalance between pro- and anti-inflammatory bacteria that has been invoked to explain observations in patients with rheumatoid arthritis, atherosclerosis, obesity and other diseases (Fig. 3) [118].

The Gut microbiota and the immune system in AD

Differences in the gut microbiota composition have been described, suggesting a specific microbial signature typical of AD [120]. The question arises of the causality behind these intestinal changes and brain pathology. The immune system seems to play a crucial role in the gut–brain communication.

Higher levels of peripheral and central pro-inflammatory cytokines have been found in AD patients as compared to controls; reactive microglial cells co-localize with amyloid plaques, indicating that the pathology is accompanied by peripheral and neuroinflammation [114, 115]. Importantly, gut microbiota communicates with the immune system, for example by inducing T regulatory cells to turn off inflammatory processes [121], but alterations in its composition have been seen to be related to inflammatory pathologies. A dysbiotic flora, indeed, produces metabolites or release molecules, such as lipopolysaccharides, that can induce a peripheral inflammatory response, which, in turn, could reach the brain. In case of gut microbial dysregulation, both the intestinal barrier and the blood–brain barriers become more leaky, leading to an augmented passage of these molecules, from the gut into the circulation [122, 123].

In AD, inflammation has always been considered one of the downstream phenomena of amyloid deposition. However, recent findings showed that immune system activation and a pro-inflammatory state could promote amyloid deposition [114]. In this regard, preclinical studies indicated that Aβ exerts antimicrobial properties: temporal lobe homogenates from AD patients inhibit *Candida albicans* growth, in a dose-dependent manner, as compared to non-AD temporal lobe homogenates or to cerebellum homogenates from AD patients [124]. In a mouse and a nematode model of AD the presence of Aβ protected from *Salmonella* or *C.albicans* infection by creating a net that entrapped microbes and prevented their adhesion to the host [125]. As the immune system regularly produces amyloid nets to entrap uninvited guests [126], Aβ could represent a first immune response against a microbial invader in the brain. Interestingly, fungi and the bacterial component lipopolysaccharide have been found in post-mortem brains of AD patients, especially in the area where Aβ plaques are present[124, 125, 127, 128], raising new hypothesis of AD pathogenesis.

A theoretical framework integrating the current hypotheses in AD and microbiota-mediated inflammation would look as follows: intestinal lumen is sensible to signals coming from microorganisms and directly from the diet. These signals activate inflammatory mediators in the gut mucosa and submucosal layers, which can generate adaptive responses through antimicrobial and active peptides. The generated peptides can be secreted into the lumen to help maintain homeostasis. Simultaneously, effector cells will secrete chemokines to the bloodstream that are able to communicate to the central nervous system (CNS) by and/or through the BBB. In response to the gut signalling, inflammatory cells in the brain can activate the complement C1q, activate inflammatory receptors such as RAGE and modulate deposition of Aβ. Nevertheless, the presence of gut metabolites and microbiota-induced inflammation in the brain of man require more research for confirmation and further characterization (Fig. 3).

In conclusion, the microbiome is influenced by factors such as the environment, the diet and the

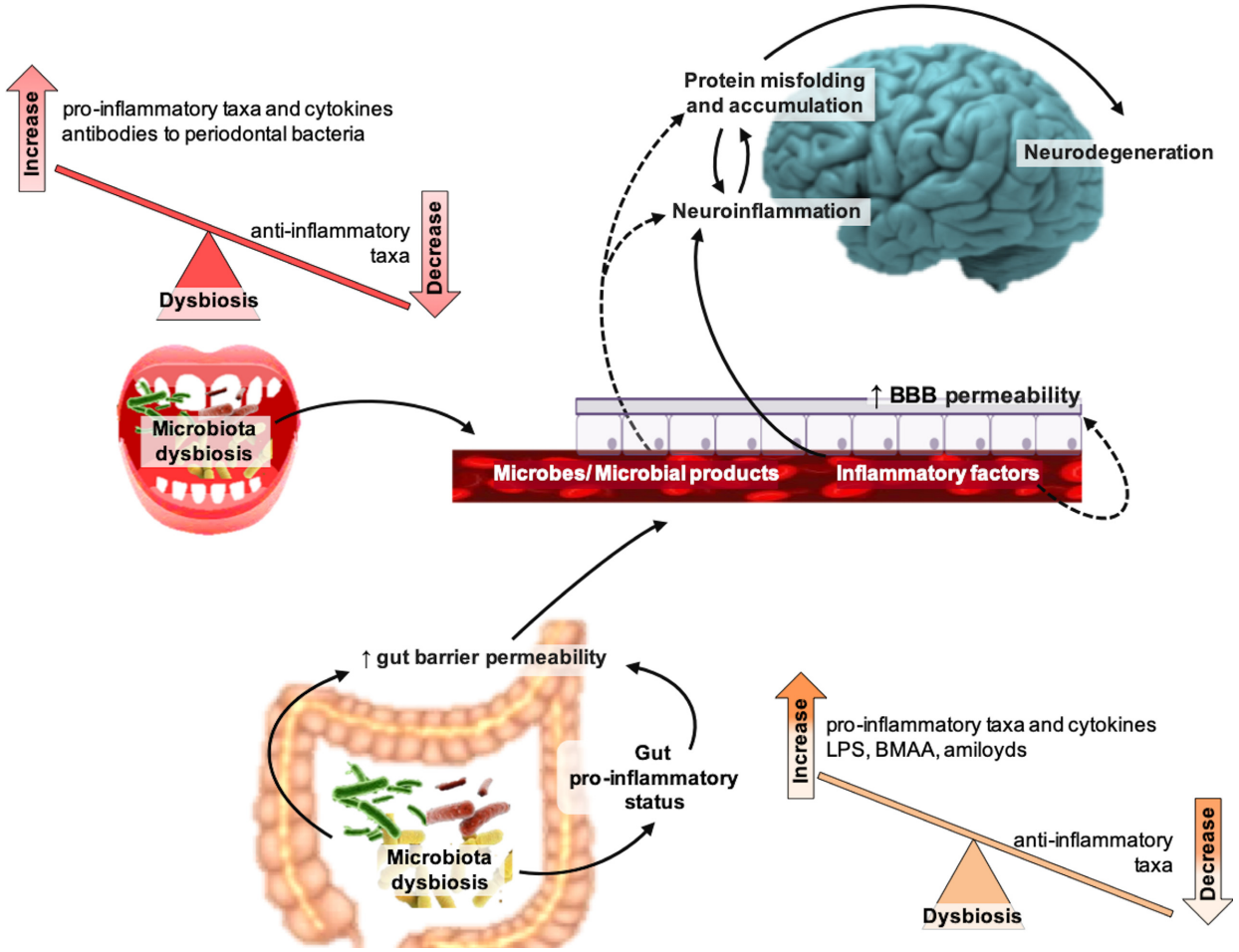

Fig. 3 *Possible pathophysiologic role of microbiota in Alzheimer's disease. BBB, blood–brain barrier; BMAA, b-Nmethylamino-L-alanine; HSV-1, herpes simplex virus type 1; LPS, lipopolysaccharide. From: Marizzoni* et al. *[119].*

season of the year. All these parameters can be used to improve the power of these studies to find more specific signatures. Moreover, stable microbiota signatures have been identified that can be as unique to individuals as fingerprints [129].

Regarding therapeutic opportunities, it is possible in theory to modify the composition of the gut microbiota to prevent or improve cognitive symptoms in AD. The most common strategy to induce gut microbiota modification is the dietary supplementation of probiotics—living microorganisms that provide health benefits when ingested. In the only intervention trial reported so far, a probiotic containing *Lactobacillus acidophilus, Lactobacillus casei, Bifidobacterium bifidum* and *Lactobacillus fermentum* has been tested in Iran on severely demented older patients in comparison to a placebo. The probiotic group showed an amelioration in cognitive functions from baseline (MMSE = 8.7) after 12 weeks of supplementation (MMSE = 10.6), whereas the placebo group showed a decrease in MMSE scores from 8.5 to 8.0 over 12 weeks. An impact was reported not only in MMSE scores but also in blood markers of insulin and lipid metabolism, suggesting that the probiotic formulation has several beneficial effects [130].

Despite the above intriguing results, it is still challenging to identify a universal AD gut microbiota signature and also a therapeutic composition of microorganisms as a potential therapy.

Therefore, further studies on novel mediators of gut microbiota-induced inflammation in blood and CSF are the potential keys to develop therapeutic strategies.

Restoring insulin action & glucose metabolism in AD: our short-term perspectives

Besides the known effects of amyloid-β (Aβ) and hyperphosphorylated Tau protein in the central nervous system (CNS) in AD, they may be also important at the periphery[131]. For instance, Aβ may compete with insulin and bind to its receptors at the periphery, impairing pancreatic β-cells and leading to insulin resistance and glucose dysmetabolism [132, 133]. This may in turn exacerbate Aβ deposition [134, 135], creating a vicious cycle of dysfunctional CNS insulin signalling, oxidative stress and neuroinflammation, culminating in cognitive deficits [136]. Despite controversial, this may also involve the hyperphosphorylated Tau-induced destabilization of microtubules in β-cells, blunting insulin secretion [137] and insulin-mediated trafficking of glucose transporter-4 (GLUT4)-containing vesicles to the plasma membrane. Hence, glucose uptake into skeletal muscle and adipocytes is inhibited and type 2 diabetes (T2D) may arise [138–140].

Insulin and its downstream signalling cascades play a crucial role against CNS damage and disease. Besides the known regulation of brain glucose/bioenergetic homeostasis [141–143], insulin signalling protects against oxidative stress, (neuro) inflammation and dysfunctional intracellular quality control mechanisms [144–147], rescuing synaptic/neuronal function [148, 149] and cognition [150]. This downregulation of bioenergetic metabolism in insulin-resistant brain may arise years before the onset of clinical symptoms (possibly during midlife), affecting Aβ or Tau homeostasis and rendering people (especially women) more prone to dementia and AD [151–155]. Thus, AD has been increasingly considered a metabolic disorder, also termed 'type 3 diabetes' [156].

Evidence for AD-related brain glucose hypometabolism includes the slowdown in cerebral blood flow due, for example, to brain vascular atrophy [157]. This, together with the lower levels of GLUT-3 and -4 in AD brain, may attenuate the glucose uptake across the blood–brain barrier (BBB) and its use by CNS [158, 159]. AD also inhibits brain enzymes from glycolysis and Krebs cycle (e.g. lactate dehydrogenase (LDH), aconitase, glutamine synthetase, creatine kinase, pyruvate dehydrogenase (PDH) and alpha-ketoglutarate dehydrogenase (α-KGDH)) [160, 161], depending on disease progression [162]. Besides the possible direct impact of PDH inhibition in lowering the levels of acetyl-coenzyme A, acetylcholine, cholesterol and neurosteroidal hormones (e.g. oestrogen) upon AD [163], these metabolic changes further associate with mitochondrial alterations along disease progression [164]. In this perspective, Aβ is widely known to deregulate mitochondrial proteins, blunting mitochondrial cytochrome c oxidase (or complex IV) activity and oxygen respiration rate, either centrally and/or peripherally (e.g. in platelets) [165–168]. This may be also due to a reduction in the neuronal expression of nuclear genes that code for mitochondrial electron transport chain subunits [169], or to a decrease in the number of neuronal mitochondria [155]. Importantly, the disruption between mitochondrial respiration and energy metabolism in AD was also associated with oxidative stress [156, 170], possibly due to activation of p38MAPK signalling and subsequent hippocampal glutamatergic synaptotoxicity/death, culminating in the AD cognitive deficits [170, 171]. Alternatively, disrupted mitochondrial dynamics (fission and fusion) and trafficking upon AD may hamper the development and maturation of synapses [164, 172, 173]. Moreover, the correlation between early deficits in synaptic mitochondria and synaptic loss in AD [162, 174] reinforce the notion that brain glucose (energy) hypometabolism may constitute an early event in disease pathogenesis, starting decades before its diagnosis (probably during midlife) [175, 176]. This may impair neuronal insulin signalling, creating a vicious cycle of Aβ-mediated and hyperphosphorylated Tau-mediated injury [177, 178].

Although this is not the aim herein, there are extensive differences (even at the level of gene expression) between male and female brain (metabolism) upon ageing and/or AD [153, 179] that may further condition the whole discovery/development of successful preventive and therapeutic strategies against the disease.

Opportunities in drug development in AD

The 'charm' of repurposing efficient anti-T2D drugs to recover brain insulin signalling and glucose metabolism in AD. The failures described above point to the urgent need to unveil the precise

aetiology and pathophysiological mechanisms of AD, as these will be also crucial to discover more accurate diagnostic and efficient therapeutic tools [157, 180]. They also emphasize the need of supporting Phase III clinical trials on strong and accurate preclinical data and to tackle multiple therapeutic targets [157]. Moreover, the refocus on preventive strategies and/or drugs targeting the prodromal or very early stages of AD (before the onset of dementia) will hopefully maintain a longer quality of life [157].

Amongst such promising therapeutic (and preventive?) strategies in AD, one tempting target is the rescue of brain insulin signalling and glucose metabolism [157]. Accordingly, an increasing attention has been given to the potential benefits of repositioning efficient, commercialized anti-T2D drugs to treat AD [181–184]. This hypothesis is supported by the molecular mechanisms shared by T2D and AD [183, 184]. This is also tempting due to the potential targeting of preclinical/prodromal AD, mild cognitive impairment (MCI), or at-risk conditions (prevention), rather than just its later stages [157, 183, 184].

The temptation of using biguanides (metformin) against AD: a friend or foe?. Metformin is the most efficient anti-T2D biguanide [183–185]. It is relatively inexpensive and with a low risk of hypoglycaemia [183]. Metformin inhibits insulin-mediated hepatic glucose production and promotes peripheral glucose disposal by activating liver and skeletal muscle AMPK signalling [183, 184]. Given its good tolerability, metformin can be used as mono- or multi-therapy at all stages of T2D [183]. Amongst its adverse effects are gastrointestinal distress, hepatic dysfunction, congestive heart failure, dehydration and alcoholism [183, 184]. Therefore, metformin must be used with caution in elderly patients.

Preclinical data suggest that metformin may be neuroprotective, probably by recovering brain insulin action and energy metabolism [183–185]. Metformin also increased markers for mitochondrial biogenesis and fusion (e.g. Mfn2 and OPA1), attenuated mitochondrial transition pore opening and oxidative stress, protecting against apoptosis and cognitive deficits [183–185]. It also modulated lipid and protein synthesis, fatty acid oxidation and promoted neurogenesis [183]. However, the rescue in hippocampal JNK signalling and synaptic markers achieved by metformin did not improve cognitive function in obese T2D mice [186] and even promoted hepatic mitochondrial dysfunction and cell death [187].

Concerning its role in ageing and AD, metformin decreased the risk for dementia in aged individuals and improved cognition in AD patients [188, 189]. This may involve the attenuation in neuronal insulin resistance and AD-like neuropathology, most likely via AMPK-related regulation of APP amyloidogenic processing; inhibition of mTOR and subsequent autophagic/lysosomal removal of Aβ; and/or the stimulation of PP2A activity and decreased Tau hyperphosphorylation [190, 191]. Given such promising data, according to *ClinicalTrials.gov*, two Phase II clinical trials on the effects of metformin administration in middle-aged and aged obese patients with amnestic MCI (NCT00620191), or in MCI and early AD patients (NCT01965756) were recently completed and results are awaited soon.

The potential of thiazolidinediones to tackle AD. The main thiazolidinediones (TZDs) used in T2D are Rosiglitazone, Pioglitazone and troglitazone [183]. Though TZDs are relatively expensive, they are very efficient in the long-term management of T2D [183, 184]. These drugs act as PPARγ agonists to promote the transcription of genes related to lipid and glucose metabolism [192, 193]. More specifically, TZDs increase insulin-induced glucose uptake (most likely via GLUT-1 and GLUT-4) and decrease lipid accumulation by skeletal muscle, stimulate triglyceride storage in adipocytes, hepatic fatty acid oxidation and inhibit hepatic gluconeogenesis [184]. Amongst their adverse effects are a possible weight gain and increased risk of myocardial infarction [184].

Some neuroprotective effects were described for TZDs, including a decrease in stroke-related damage and neurological deficits in T2D mice [194]. Others suggested that TZDs-mediated reduction in brain oxidative stress and rescue in STAT3/Wnt signalling pathways may promote neuronal progenitor cells proliferation and differentiation upon T2D [195]. This, together with a protection against amyloidogenic processing of APP, Tau hyperphosphorylation, neuroinflammation and Aβ-induced neuronal insulin resistance may account for the recovery in memory and cognitive performance in patients and rodent models [183, 184]. In line with this, in a randomized clinical trial, Rosiglitazone improved cognitive function in mild to moderate AD

patients [196], whereas in a Phase III study the drug did not show beneficial effects in AD patients, and the long-term use of thiazolidinediones did not attenuate the risk for AD [197].

According to *ClinicalTrials.gov*, a Phase III clinical trial is currently analysing the potential of Pioglitazone as a β-secretase inhibitor (*TOMMORROW*; NCT01931566) in people aged 65-83 years, at risk of MCI due to AD. A masked extension of this study (NCT02284906; phase III) is planned with 316 individuals with an MCI diagnosis due to AD that complete the *TOMMORROW* study.

Is it still worthy to evaluate (intranasal) insulin for AD treatment? The pros and cons…. Insulin has been increasingly used in T2D, not only for blood glucose management but also to prevent its chronic microvascular complications and death [198]. However, some controversy persists on its efficacy, which may be lost upon T2D progression.

Physiologically, brain insulin signalling promotes synaptic remodelling and memory formation [199, 200]. We also found that restoring insulin and IGF-1 signalling recovered both peripheral and brain glucose metabolism, and motor function *in vitro* and *in vivo* in Huntington's disease models [201–203]. Moreover, insulin decreased synaptic Aβ accumulation, oxidative damage and mitochondrial dysfunction [143, 204]. This was accompanied by a protection against Aβ-induced neuronal insulin resistance [205, 206]. However, associated with insulin administration is the high risk of recurrent hypoglycaemia, which has been increasingly related to neuronal dysfunction/death and cognitive deficits [207, 208]. But since restoring brain insulin signalling constitutes a promising approach against AD, an alternative could be the potential use of intranasal insulin herein.

Intranasal insulin promoted brain insulin signalling in AD, without affecting blood insulin or glucose levels [209]. Clinical trials involving MCI or early AD patients showed that intranasal insulin improved brain glucose metabolism and stabilized or even rescued their memory and cognitive deficits [199, 210, 211]. According to *ClinicalTrials.gov*, results are awaited from two recently completed Phase II/III and II clinical trials on insulin (*SNIFF*; NCT01767909) and glulisine (a rapid-action insulin analog that regulates glucose metabolism and counteracts Aβ) (NCT02503501), involving middle-aged and aged MCI or mild AD individuals. Possible limitations to the use of intranasal insulin for AD treatment could be the generalized increase in brain insulin levels and its possible adverse consequences on brain regions (like hypothalamus) that control, for example, water and food intake [212].

The increasing therapeutic potential of incretin drugs in AD. -Dipeptidyl peptidase-IV inhibitors—Sitagliptin, Saxagliptin, Linagliptin, Vildagliptin, Alogliptin, Tenegliptin, Dutogliptin and Gemigliptin are the main dipeptidyl peptidase-IV (DPP-IV) inhibitors used to treat T2D [183, 184, 213]. DPP-IV inhibitors are oral small molecules that blunt the degradation of native GLP-1 by the aminopeptidase DPP-IV, increasing its half-time and circulating levels, together with the attenuation of glucagon effects [213–215]. DPP-IV inhibitors are well tolerated and can be used either as mono- or multi-therapy [184, 213]. Apparently, these drugs do not affect gastric emptying, body weight or cardiovascular function and present a low risk of hypoglycaemia [184, 213]. However, their efficacy may be lost upon T2D progression [216].

Sitagliptin attenuated mouse hippocampal AD neuropathological hallmarks, improving also acetylcholine and adiponectin receptor levels in T2D rat brains [217, 218]. Sitagliptin and Vildagliptin also decreased peripheral T2D and oxidative stress markers and rescued learning and memory deficits in insulin-resistant and T2D rats [217, 219, 220]. Vildagliptin also decreased the levels of Aβ, hyperphosphorylated Tau and neuroinflammatory markers, and rescued memory deficits upon AD [221]. However, it is still debated whether DPP-IV inhibitors can cross the blood–brain barrier and exert direct effects in the brain or if their effects are mostly peripheral [184, 213]. Further research is needed before including DPP-IV inhibitors into clinical trials in AD.

-GLP-1 receptor agonists—Exendin-4, liraglutide and lixisenatide are the most used GLP-1 receptor (GLP-1R) agonists in T2D [183, 184, 213, 222]. They act as incretin mimetics, promoting insulin secretion in a glucose-dependent manner to overcome insulin resistance [183, 213, 222]. Besides their minimum risk of hypoglycaemia, GLP-1R agonists have also potent, long-lasting anti-obesogenic effects, possibly via a hypothalamic-regulated decrease in appetite and food intake [213]. They also showed benefits in blood pressure, cholesterol and triglycerides levels, as well as in

cardiac function upon T2D [213]. Though the mechanisms involved herein remain poorly known, they may rely on a decrement in markers for cardiovascular risk (as IL-6, TNFα), endothelial dysfunction, oxidative/endoplasmic reticulum (ER) stress and inflammatory pathways [213]. Interestingly, liraglutide promoted GLUT4 translocation in mouse skeletal muscle via cAMP signalling and may thus affect glucose uptake and metabolism [223].

GLP-1R agonists are known to readily cross the blood–brain barrier and directly affect the brain, where they may act like neurotrophic factors [213, 222]. Mounting evidence point towards a neuroprotective role of GLP-1R agonists against *in vitro* and *in vivo* AD [222, 224, 225]. Specifically, these drugs attenuated Aβ, APP and hyperphosphorylated Tau levels, neuroinflammation, oxidative/ER stress and neuronal death in AD [213, 222, 224, 225]. These were mirrored by increased insulin degrading enzyme and insulin signalling, which may recover glucose metabolism, synaptic transmission/plasticity, neurogenesis and, ultimately, memory and cognitive performance [178, 213, 222, 224, 225]. Similar results were recently reported with lixisenatide [226].

Results are awaited from the two recently completed clinical trials on exendin-4 (Phase II; NCT01255163; involving MCI or mild AD individuals, aged ≥ 60 years) and liraglutide (Small randomized; NCT01469351; involving early-onset AD patients), as well as from a recently started large-scale, phase II clinical trial on liraglutide in early AD patients, aged 50–85 years (ELAD, NCT01843075).

The novel and still unexplored anti-AD therapeutic potential of SGLT2 inhibitors. The main SGLT2 inhibitors used to treat T2D are empagliflozin and dapagliflozin. Although they exert their glucose-lowering effects mainly through a novel, insulin-independent mechanism (via increased renal glycosuria), one cannot exclude the increase in peripheral insulin sensitivity, GLP-1 levels and/or β-cell function [227–232]. This may be accompanied by decreased leptin levels, endothelial dysfunction, oxidative stress and inflammation markers, ultimately reducing blood pressure and body weight [233]. Thus, SGLT2 inhibitors may optimally reduce the long-term complications associated with T2D, with a low risk of hypoglycaemia and hypotension [230, 234]. Although little is known on its neuroprotective role, empagliflozin may protect obese T2D mice against brain oxidative stress and DNA damage probably via the recovery in serum insulin levels and vascular function, ultimately rescuing their learning and memory function [235, 236]. Additionally, dapagliflozin-mediated attenuation of retinal capillary hyperperfusion, arteriole wall thickening and microvascular remodelling in T2D were followed by a decrease in brain markers for oxidative stress, inflammation and apoptosis [235, 237, 238]. This was further accompanied by an improved insulin action, mitochondrial function, synaptic density/plasticity, neurogenesis and in learning and memory in T2D patients and animal models [235–238].

To our knowledge, there are no current clinical trials on the use of SGLT2 inhibitors to tackle AD. In conclusion, one can hypothesize that either by ameliorating peripheral insulin action and glucose homeostasis and/or by crossing the blood–brain barrier and exerting similar effects in the central nervous system, anti-T2D drugs from the different classes may represent promising therapeutic approaches to tackle AD (Fig. 4).

Restoring brain cholesterol metabolism by CYP46A1 gene therapy

Increasing evidence demonstrates the role of brain cholesterol in the physiopathology of neurodegenerative disease particularly in Alzheimer's disease and Huntington's disease[239, 240]. Brain contains a particularly high portion of total body cholesterol, since our brain represents 2% of our body weight, but contains 25% of total cholesterol. Besides the important (70%) myelin fraction, brain cholesterol is a major constituent of neuronal membranes and plays crucial role in synaptic function and neuronal survival.

Increasing arguments link brain cholesterol metabolism and AD. Tangles of Tau are observed In Niemann–Pick-C, a genetic disease of cholesterol metabolism, confirming the direct connection between dysfunction of cholesterol in the brain and the tangles of Tau. The role of ApoE, the main cholesterol transporter in the brain, and of the ApoE4 allele has been long recognized as the main risk factor (after age) for Alzheimer's disease [6]. More recently, GWAS analysis has identified several genes of lipid metabolism, like SORL, ABCA7 and CLU in association with AD [241]. Cholesterol increased concentration has been evidenced in the brain of AD patients. The role of statins (inhibitors of HMGCoA reductase key enzyme of cholesterol

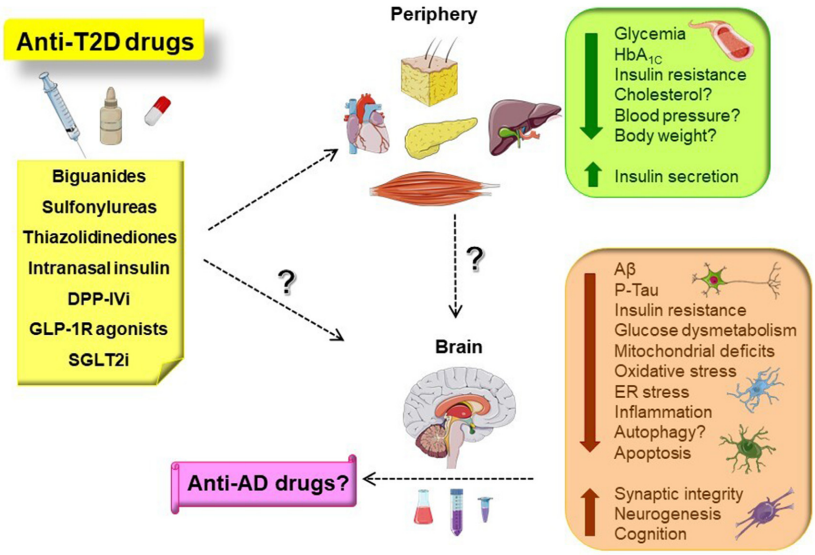

Fig. 4 *Type 2 diabetes drugs for treating AD. Strategies to improve insulin actions in AD brains could act by ameliorating peripheral insulin action or cross directly into the CNS to restore glucose homeostasis.*

synthesis) is still debated. However, a recent retrospective study on 400 000 patient receiving long-term treatments by statins evidenced a link between a decreased frequency of Alzheimer's disease to chronic administration of statins, a link varying upon sex, ethnicity and molecules [242]. Yet, the discussion on statins and cognitive decline in AD remains open [243].

Cholesterol is directly associated with plaques and tangles. *In vitro* and *in vivo* studies have shown that increased cholesterol content in membranes is associated with increased A-beta production. Conversely, decreased cholesterol in the membranes favours the nonamyloidogenic pathway of APP processing [244].

Cholesterol cannot cross the blood–brain barrier (BBB), and brain cholesterol is produced *in situ*, mostly by astrocytes in adults. It is then transported to neurons by APOE, which are the major consumers of the generated cholesterol. To some extent, cholesterol is also produced by synthesis in neurons and this is an important part of the brain cholesterol homeostasis. Cholesterol is excreted from the brain mostly after transformation into 24-hydroxycholesterol (24-OH), that can freely cross the BBB and is metabolized in the liver. 24-OH is produced by CYP46A1, a cytochrome enzyme specifically expressed in the brain [245, 246]. CYP46A1 is a key enzyme of brain cholesterol metabolism. Not only CYP46A1 allows most cholesterol efflux from the brain, it also activates the whole pathway of cholesterol metabolism, the so-called mevalonate pathway (Fig. 5) and represents an important stress response factor to noxious stimuli like ageing, toxic protein aggregates, disease conditions like AD [247, 248]. CYP46A1 was shown in response to stress, to induce the relocation of Trkb in plasma membranes, leading to its activation and to postsynaptic stress response signalling, a pathway that could be associated with improved cognition and synaptic plasticity [247].

The decrease in CYP46A1 function in normal mouse hippocampus, using AAV- CYP46A1 shRNA delivery is associated with cholesterol accumulation in cell membranes and strong neuronal toxicity leading to severe endoplasmic reticulum stress and neuronal death with hippocampal atrophy. Interestingly a reduction of only 30 to 50% in CYP46 levels induces amyloid beta accumulation and hyperphosphorylation of Tau protein in the hippocampus, a phenotype resembling Alzheimer's disease. CYP46A1 inhibition in AD mice with amyloid pathology leads to accelerated toxicity with major amyloid accumulation, rapid neuronal death and seizures, evidencing the toxic loop between cholesterol metabolism impairment and amyloid production [249, 250].

On the contrary, CYP46 overexpression in AD models improves cognition and decreases pathology in the brain. Injection of an AAV vector coding for the enzyme CYP46A1 restores cholesterol

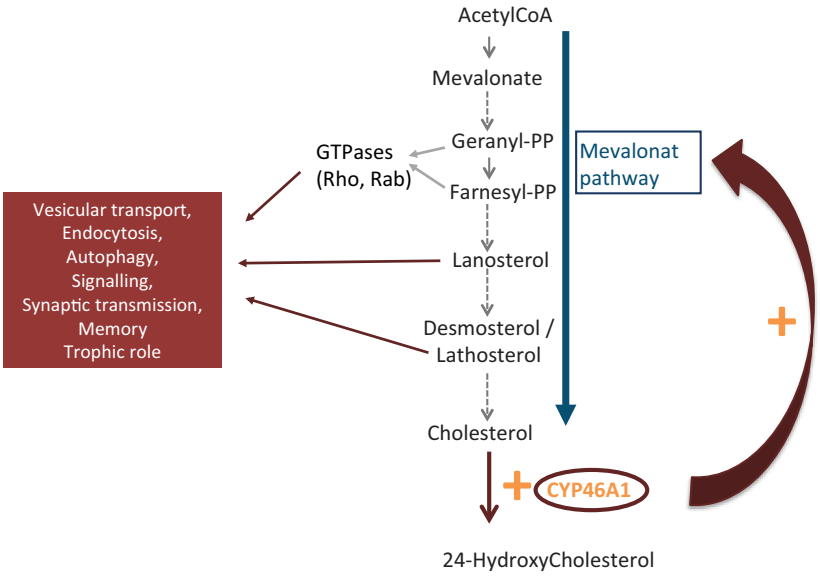

Fig. 5 *The mevalonate pathway of cholesterol metabolism: roles in neuronal functions.*

metabolism, decreases amyloid beta accumulation and plaque formation in the different AD models [249, 251] (Fig. 6). Importantly, this beneficial effect of AAV-CYP46A1 delivery is demonstrated not only in three different amyloid models but also in Tau22 mice [252]. Dendritic spine density are restored, together with electrophysiological parameters (LTC), contributing to the correction of memory deficits in these mice. In parallel, results from studies in Huntington mouse model demonstrated that overexpressing CYP46A1 restores deficient cholesterol metabolism, behaviour deficits and neuropathological hallmarks confirming the link between brain cholesterol impairment and neuronal function[253].

A gene therapy approach based on AAV-CYP46A1 brain delivery is thus a potentially powerful strategy, acting both on the amyloid and the Tau hallmarks of the disease. Feasibility and safety of the procedure were demonstrated in monkey brain (unpublished results). Improvement of AAV vectors able to efficiently target brain neurons after intrathecal or intravenous injection should help the development of such therapeutic approaches. Increased 24-OH cholesterol in CSF could be evaluated as a biomarker of mechanism to evidence the efficacy of the therapy. AAV vectors have been approved for human use and several clinical trials using AAV vector delivery to the brain have been performed or are ongoing [254]. These vectors allow long-lasting expression with only one injection.

A first application in human patients could be in severe familial forms of Alzheimer's disease. Patients can be diagnosed and treated at very early stages of the disease, when a therapeutic benefit could be expected.

Molecular mechanisms behind glucose and cholesterol metabolism for developing AD

Several reports have looked at patients with altered cholesterol metabolism in the brain and their susceptibility to developing AD [255–259], and in the last two decades, BBB-permeable cholesterol metabolites (known collectively as oxysterols) have been identified as possible mediators of cholesterol effects in the brain [248, 260–264]. The main oxysterol exchange between the brain and the circulation is between 24-hydroxycholesterol (24-OH), which is originated in the brain as discussed before, and 27-hydroxycholesterol (27-OH) which is generated by the activity of the enzyme CYP27A1 in the periphery [248, 261, 263, 264].

Excessive 27-OH has been related to AD [265, 266] and to PD [267–269]. It's also associated to breast cancer [270] and to a genetic disease called hereditary spastic paraplegia of the fifth type (SPG5) where a mutation causes a loss of function of CYP7B1, an enzyme that degrades 27-OH, so these patients have ten times more 27-OH than normal individuals [271, 272]. Nevertheless, individuals without this mutation can also show

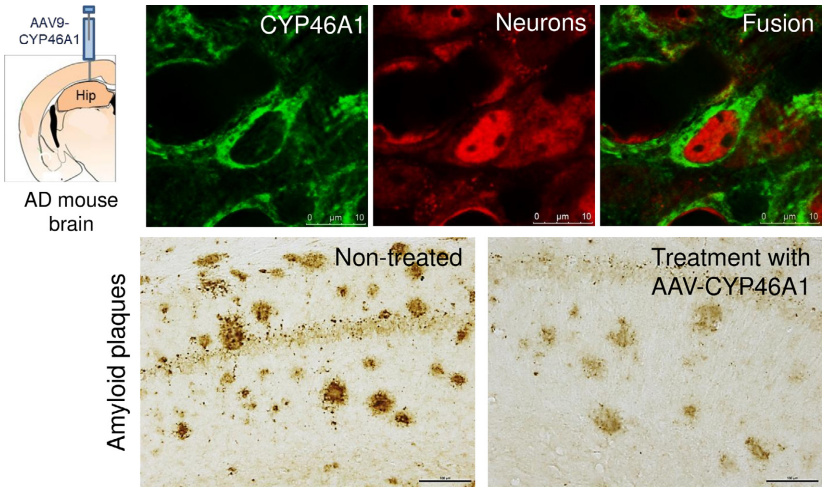

Fig. 6 *Gene therapy on CYP46A1. The first panel shows the site of injection with the viral vector carrying an overexpressing copy of CYP46A1 for gene therapy. Neuronal transfection of CYP46A1 can be detected (green) in neurons in the hippocampus (red) as shown by the upper pannels. After gene therapy treatment with CYP46A1, amyloid plaques in the hippocampus are reduced.*

elevated levels of 27-OH in the blood, which correlate to 27-OH levels in the CSF [273]. In these patients, elevated 27-OH levels correlated negatively with glucose uptake in the hippocampus, posterior cingulate and cerebellum as measured by ^{18}F-FDG PET. The patients could be stratified in two groups: the first one would have more than 1 ng/mL 27OH in CSF and a global decrease in brain glucose uptake, whilst the second group with less than 1 ng/mL 27-OH in CSF with decreased glucose uptake only in the hippocampus and posterior cingulate.

Preclinical models have already provided information on the mechanisms of elevated 27-OH influences glucose metabolism in the brain, contributing to cognitive decline in AD. High-fat/high-cholesterol diet (HFD) in aged mice led to decreased ARC protein levels in the hippocampus as well as reduced NMDA receptor activity [274]. As mentioned previously, cholesterol does not cross the blood–brain barrier and these studies pointed to 27-OH as a mediator of the negative effects of high-fat diets in brain function markers. Moreover, HFD leads to cognitive impairment in mice and knocking out Cyp27A1, the enzyme converting cholesterol to 27-OH, protects mice against HFD-induced cognitive deficit [275].

Alterations of the renin-angiotensin system in the brain in AD

In an effort to identify the mechanisms by which high levels of 27-OH produce neuronal damage, we reported high levels of 27-OH increase the renin-angiotensin system activity in HFD fed mice (Fig. 8) [276]. These results were proven translatable when found that patients with MCI and AD also present increased angiotensin (AGT) and angiotensin-converting enzyme (ACE) in the brain [277]. Going back to the animal models to clarify the mechanisms of action of 27-OH in the brain, the Cyp27TG mice were used, a transgenic mouse model overexpressing CYP27A1 to produce 5 times more 27-OH systemically. These animals also show cognitive impairment at 12 months old together with reduced glucose uptake in the brain [273, 278]. The mechanisms leading to reduced glucose uptake in these mice is mediated by an over-activation of the RAS system, leading to an imbalance between the angiotensin isoforms AngIII and IV [273]. The balance between these forms is also modified by the catabolism of AngIV by aminopeptidases, which are modulated importantly by 27-OH, particularly aminopeptidase-A (AP-A) and aminopeptidase-N (AP-N). In CYP27TG brains, elevated 27-OH increases AP-N, which cleaves AngIV thus decreasing its levels. AngIV under physiological conditions downregulates the abundance of insulin-regulated aminopeptidase (IRAP) in the brain [279], but under high 27-OH levels, AP-N degrades AngIV allowing increased IRAP activity [273].

Elevated 27-OH levels not only increase IRAP activity but also decrease the levels of the glucose transporter GLUT4, which is regulated negatively by AngIII. In CYP27TG brains, Ang III is elevated due to increased AP-A activity (converting AngII to AngIII), which in turn downregulates GLUT4 [273].

Together with increased IRAP activity, GLUT4 downregulation leads to reduced glucose uptake by neurons (Fig. 7). These mechanisms have been confirmed *in vitro* by knock-down experiments and they explain the alterations observed in patients with altered RAS markers in the CSF.

Taken together, these results point CYP27A1 as a druggable target to prevent the effects of peripheral hypercholesterolaemia in the blood, in opposition to statins or ACE inhibitors. A recent clinical trial with atorvastatin in SPG5 patients did not decrease 27-OH levels in patient's CSF, whilst effectively decreasing them in the blood together with cholesterol, meaning that statins cannot effectively normalize 27-OH in the brain in the short term [272]. CYP27A1-specific inhibitors, such as anastrozole, have been proposed as therapeutics for AD [280]; however, to our knowledge, no clinical trial is testing the effect of these compounds in AD risk or cognition.

Insulin aggregation in AD

The reduced glucose uptake mediated by high 27-OH levels might pose a link between cholesterol and glucose metabolism alterations as important players in the development of AD. We recently reported that insulin aggregates and accumulates in neurons with hyperphosphorylated Tau from humans [151]. The finding was not only exclusive of AD but also of other tauopathies, having insulin resistance as a common denominator for most cases. Moreover, neurons with aggregated insulin show decreased insulin receptor levels and neuroblastoma cells overexpressing Tau show decreased GLUT4 expression levels (Fig. 7). This is in line with other papers suggesting similar relationships between insulin resistance and Tau [281, 282]. Whilst some reports exist linking high cholesterol with hyperphosphorylated Tau [267, 283, 284], the mechanistic link between glucose and cholesterol metabolism underlining neurodegeneration needs further research. Yet, it is highly possible that such a link exists and plays a major role not only in AD but also in other tauopathies and in PD.

This underlines the importance of redirecting research efforts to classify AD patients according to specific biochemical pathway imbalances they might present in order to better design clinical trials for treating or modifying AD progression. Further research should focus on topics of insulin metabolism, cholesterol and lipid dynamics in the brain and their relationship with parallel pathologies such as diabetes that could lead to earlier hallmarks of AD manifestation, new targets for drug development and new therapeutic strategies to treat neurodegenerative diseases.

Amyloid beta in Alzheimer's disease: an overview

Aβ load in the brain correlates well with the degree of dementia, where it becomes more neurotoxic after polymerization [285, 286]. Most familial cases show increased Aβ isoforms with 42 to 43 amino acids in the CSF [287–290]. Persons with Down's syndrome also present an over-production of Aβ and an early onset of AD [287]. Transgenic mice overexpressing Aβ are available for the investigation of the AD pathogenic mechanisms. Many of these models develop AD-like lesions and show impaired memory [291–294]. Nevertheless, overexpression of APP in mice has received criticism due to the difference from physiological levels observed in human brains [295, 296]. Because of this, novel models closer to human brain biochemistry aim to uncover mechanisms with more translational profiles [297–299].

In the amyloid cascade model, Aβ42 aggregates in the presence of binding proteins such as ApoE, as well as with metal ions, leading to plaque formation [15, 241, 300]. Downstream, the cascade leads to Tau aggregation, inflammation, oxidative stress and ultimately neuronal death [301]. These pathways are rather clear at least for the familial cases. Now with the genome-wide association studies (GWAS), we have several new prospects coming up, but still, it is a scientific consensus that Aβ processing and Tau hyperphosphorylation are key components of AD pathology [302].

Upstream, we have more new pathways that have been elucidated, mainly by the GWAS [241]. These are the glucose metabolism, the cholesterol metabolism, inflammation, intracellular membrane, and vesicle recycling and oxidative stress [4, 241, 303, 304]. These pathways and targets will be useful in the future and could potentially lead to a combined therapeutic strategy.

Positron emission tomography (PET), biomarkers and early diagnosis in AD

Another important part is the biomarkers. Amyloid accumulation comes early in the disease, long before clinical diagnosis [305]. With PET scanning, it is possible to see the amyloid assemblies in the

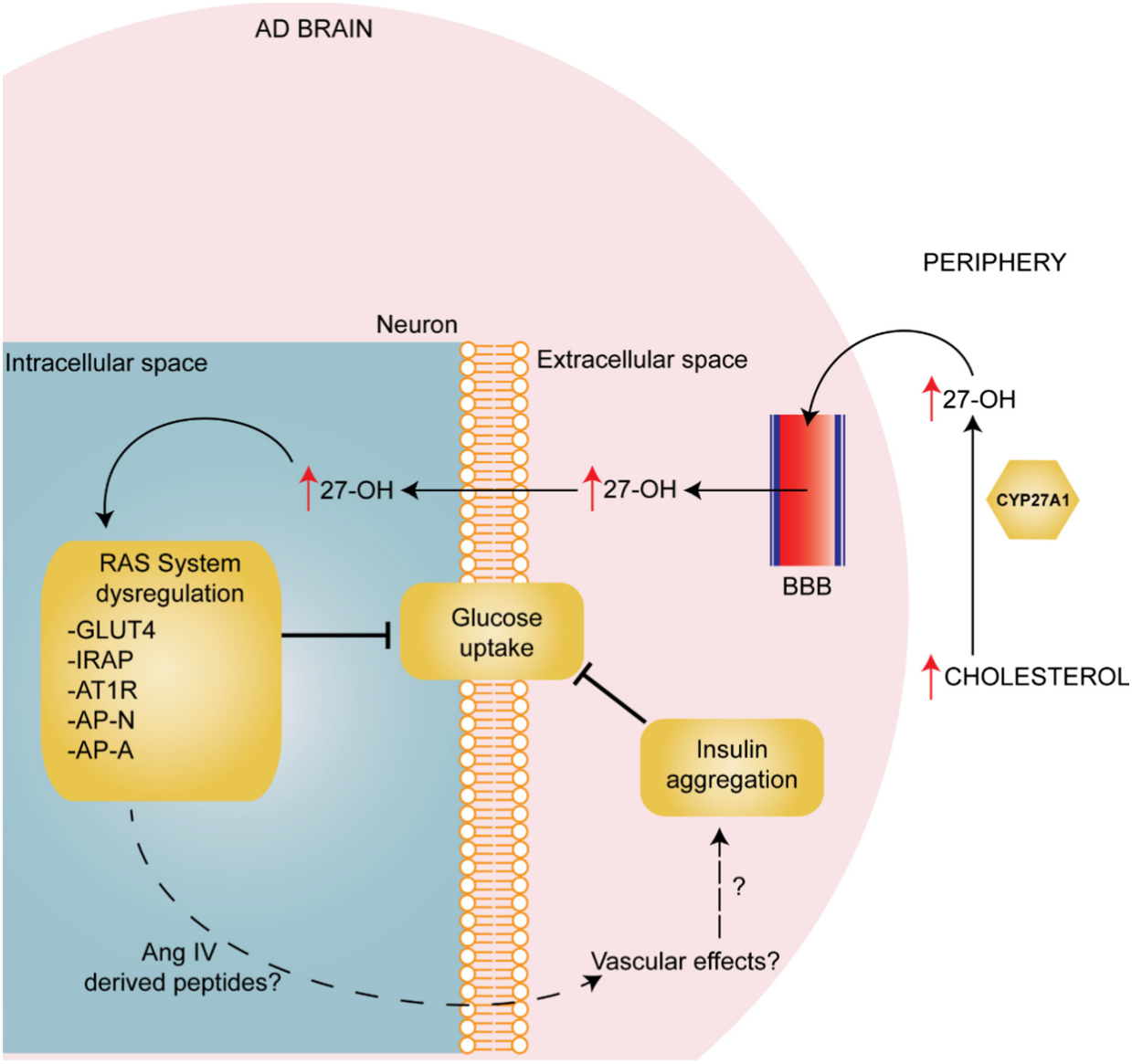

Fig. 7 *Metabolic effects of elevated 27-OH in the brain. Elevated cholesterol in the periphery cannot cross the BBB. CYP27A1 converts peripheral cholesterol to 27-OH, which is able to cross the BBB into the brain and inside cells. In neurons, increased levels of 27-OH dysregulate the renin-angiotensin system (RAS), by acting directly or indirectly over the glucose transporter GLUT4 and the insulin-regulated adaptor protein (IRAP). This leads to a decreased glucose uptake and reduces the metabolic activity of neurons, contributing to cognitive decline. Moreover, insulin aggregation also decreases glucose uptake. Insulin aggregation reduces dramatically its signalling and these effects are not fully understood. A link between RAS system and insulin aggregation also remains unknown, but it could involve IRAP signalling, AngIV derived peptides (produced by AP-N and AP-A activity), or through reduction of blood flow in brain vessels.*

brain [306]. Today other markers are known, such as inflammatory markers in PET imaging coming up years before the diagnosis [307–309]. Today with the help of biomarkers, we can put the diagnosis 10-15 years earlier in patients. Recently, a group from the National Institute on Aging and Alzheimer's Association (NIA-AA) have proposed a framework of preclinical AD based on biomarkers

capable to determine the state of the pathology [310].

Many research efforts aim to find specific patterns between Aβ accumulation in the human brain and AD progression. Patients with MCI have shown that converting to AD implies PIB retention in the frontal cortex 2–3 years before the symptoms appear [311]. Additionally, stable PIB retention after 2 years in human brains can be used to pinpoint the stage before the onset of cognitive decline in AD [305]. Yet, resolution limits on PET scanning do not allow identifying small changes in Aβ accumulation in the preclinical stage that might be important for preventive or intervention strategies. Because of this, more studies are required to complement imaging data to accurately diagnose early stages of AD.

Clinical trials and immunotherapy in AD

There are clinical trials ongoing with molecules affecting the Aβ metabolism by either decreasing their production, inhibiting their aggregation or increasing the clearance [13, 14]. Ongoing studies in the field of amyloid-related approaches are clearing out the Aβ monomers, forming dimers, pentamers, oligomers, fibrils, diffuse plaques and senile plaques using antibody therapies. Some antibodies such as aducanumab, even reduce the senile plaques [312].

Since 2013, many trials have been withdrawn due to lack of effects, most notably the anti-amyloid antibody solanezumab [313] and the BACE1 inhibitor verubecestat [314]. The negative outcome in these trials has led to an extensive revision of the amyloid cascade hypothesis [16, 17, 315, 316]. These clinical trials have provided data supporting previous hypotheses proposing dementia as a product of the cellular phase of AD [19], which comprise pathological processes involving targets in microglia, astrocytes, oligodendrocytes and vasculature before clinical manifestations. Still, several aspects of the amyloid cascade hypothesis remain to be tested, such as the therapeutic relevance of clearing insoluble amyloid versus preventing its production and accumulation [17].

There is great interest in the immunotherapy approach as a therapeutic option for AD, which comprises the active immunotherapy, also called vaccination [317], and the passive immunotherapy [318]. For active immunotherapy, the immune system is stimulated to produce antibodies against Aβ [319]. For passive immunotherapy, preformed antibodies are humanized and injected into the individuals with advanced AD [320]. Passive immunization protocols require a repeated infusion of the stimulus for the remaining lifespan of the patients. The produced antibodies pass in minute fractions into the brain and bind to fibrils, oligomers and plaques preventing aggregation and/or improving clearance. A promising immunotherapy for AD therapeutics is AADvac1, a vaccine against pathological Tau. For this vaccine, the phase I results of the FUNDAMANT study (NCT02031198) have been published [321, 322]. The latest report showed slower atrophy in the hippocampus in patients with high titers of antibody response and less cognitive decline.

Many clinical trials aiming at controlling the amyloid cascade propose diverse strategies and targets, but so far, there is no consensus about a universal target or approach for any form of AD.

Studies performed in Sweden, working with Novartis as the sponsor yielded an active immunotherapy against Aβ using the CAD106, a small six-residue Aβ fragment recognized by B cells, administered together with an adjuvant of viral origin [337, 340]. In order to have a clinical effect, a specific concentration of the IGG A-beta titer is required. After three or four injections, produced a significant antibody titer; however, it was not sustained long enough to induce a therapeutic antibody response. A recent phase II study with CAD106 showed target engagement and tolerability; however, no improvement in MMSE scores was obtained, possibly because of the small size of the control cohort (14 patients) [332].

Swiss researchers created aducanumab, an interesting antibody under BIOGEN development. Aducanumab is an antibody derived from healthy elderly subjects with no decline in cognition and from cognitively impaired elderly subjects with an unusually slow decline rate. From these subjects, memory B cells were isolated from lymphocyte libraries and their produced antibody screened for their ability to bind to Aβ, in a process known as reverse translational medicine [341]. In a very late phase I study with its highest doses, aducanumab could reduce Aβ levels by 90%, as shown by PET imaging [342]. Currently, aducanuab is in phase III clinical trials for early AD with the ENGAGE (NTC02484547) and EMERGE (NCT02484547)

Table 1. *Advantages and disadvantages of passive and active immunotherapies for AD*

	Advantages	Disadvantages	Cost
Passive immunotherapies			
Aducanumab [312] Solanezumab [313] BAN2401[323]	Increased control of epitope binding affinity [324]	Require continuous reinforcement of the immunization [325]	High Due to the need to design, generate and humanize antibodies [326]
	Possible to stop therapy if secondary effects are found [327]	Increased risk of oedema/ micro bleeds due to repeated infusion [328]	
	Modification of antibodies is possible to improve efficacy [329]	Longer time is required to reach effective antibody titers [323, 252]	
	Better for targeting Tau oligomer isoforms [330]		
	Does not require the host immune system. Elderly patients may benefit [331]		
Active immunotherapies			
CAD106 [332] AADvac1 [321]	Slightly different epitopes can be recognized for each individual, tailoring antibody response [320, 333, 334]	Reduced control over epitope choice and specificity [324]	Lower than passive immunotherapies The host produces own antibodies [332, 335–337]
	Fewer treatments needed to achieve therapeutic effect [338]	Difficult to reverse immune response if secondary effects are found [325]	
	Reduced risk for allergic reactions to foreign molecules [338]	Elderly patients may have hypo-responsiveness to immunization [339]	
	A better candidate for early prophylactic approach		

studies. Discussion from previous clinical trials has pointed out that the main challenges to overcome for aducanumab are to inhibit amyloid aggregation avoiding cerebral angiopathy, and achieve improvement in cognition [343, 344].

Recently, EISAI published positive results with the antibody from BIOARCTIC, used in the clinical trial phase IIb for BAN2401, a protofibril selective amyloid beta antibody [323, 252]. The results for BAN2401 came after a trial at 18 months in early Alzheimer's disease in a cohort of 856 patients. The analysis of the results showed slowing in clinical decline, which was dose dependent, together with the reduction of Aβ [345].

Chaperones and mitochondria: new and old options for AD therapeutics

Another approach to AD treatment is to inhibit protein aggregation using chaperone proteins. Evidence shows the intraneuronal amyloid is also important enough to affect the parenchymatous amyloid inside the brain [297, 346]. Chaperones transport amyloid into the mitochondria, inducing neurotoxicity [347] and if we could inhibit that transport, it would be possible to preserve

mitochondrial function and save the synapses in these nerve cells. On the other hand, chaperones can also help increase the solubility of Aβ. BRICHOS is a conserved domain in proteins with proposed chaperone activity and it has been related to amyloidosis and dementia in the British and Danish familial cases [348]. Expression of BRICHOS in the brain prevents toxicity in *Drosophila* Aβ models [349] and in APP mice [350]. In prematurely born children with a collapse of the lungs, BRICHOS is used as lung surfactant and applied in form of aerosol [351, 352]. We have difficulties in preclinical models, as the peptides forming the chaperones do not cross the blood–brain barrier. A promising solution to solve this problem is the focused ultrasound therapy, which employs controlled high-frequency ultrasound pulses to shortly disrupt the BBB and allow passage of bigger molecules into the brain [353]. Other approaches make use of native BBB transporters such as transferrin-1, which can actively pass molecules from the plasma into the brain [354, 355].

Considerable research in AD is focusing on synaptic function, where mitochondria might underlie one of the molecular pathways for neurodegeneration. Dimebon is a small molecule approved for human use as an antihistamine [356]. Reports of neuroprotective effects in models for AD led to a first randomized clinical trial that was successful even in terms of MMSE score improvement, daily life activity and global cognition [357]. However, the following phase 3 clinical trial (CONNECTION, NCT00675623) for dimebon terminated early due to lack of efficacy. One of the mechanisms of action of dimebon is to inhibit mitochondrial pore opening, increasing the membrane potential of the mitochondria and improving ATP synthesis [358]. Since mitochondrial dysfunction is strongly associated with ageing and AD, it is logical that small molecules restoring mitochondrial function will serve largely as part of future AD therapies.

In summary, several different dementia disorders involve different protein aggregation profiles. Our latest data support the claim that oligomers are more toxic than fibrils for neurons. Although it is very possible that the amyloid beta-peptide is somehow involved in the different stages, we will need a combination therapy. Many trials are ongoing for lowering Aβ levels in either the production or the aggregation or increasing its clearance. Moreover, similar approaches will give rise to therapies for amyloid diseases in other organs such as the heart and kidney, as well as in the peripheral nervous system [359]. There is still a lot to do, but what the scientific community and society must do first is to re-stimulate public and private interests in going back to basic science if we are to have a pharmacological treatment strategy as early as possible.

Tauopathies

In late stages of AD, we have an end-stage picture of the disease where amyloid deposits are formed, together with neurofibrillary degeneration, meaning extracellular Aβ and intracellular Tau. However, as mentioned before, early in the process, intracellular Aβ might be more toxic than the extracellular one. We also know that Tau can be secreted in the extracellular space.

With our current knowledge, the amyloid cascade is likely to be more complicated than initially described. The linear cascade of events leading to the formation of Aβ oligomers, aggregation, and maybe propagation may not be so simple. Does Tau pathology fuel this amyloid cascade? Does the amyloid pathology facilitate Tau secretion and propagation? Finally, in the amyloid cascade hypothesis, inflammation arrives at the end. However, since there are already early extracellular proteins aggregating, inflammation is likely to be present at the beginning of AD as suggested by several studies. Our understanding of the sequence of events has been hampered by the fact that there are not truly faithful animal models of AD for research. Current models mimic either amyloid pathways or Tau pathways. Some studies have attempted to show a link between amyloid and Tau pathways [360]. This work shows the possible interaction of both systems and also including the idea of prion-like propagation and seeding. Still, we do not know the real links between amyloid and Tau pathways. For example, in several GWAS [361–364], there are a number of genes identified like PICALM, BIN1, PPK2B. These genes are involved in Tau-mediated synaptic dysfunctions but also in APP metabolism and Aβ clearance, meaning that some of these genes are really at the border between amyloid and Tau pathologies, and thus research has to focus on them.

According to Braak stages, it is well considered that neurofibrillary tangle pathology starts within the hippocampal formation. Then it goes to the temporal area, then to polymodal association

areas, unimodal association and finally the entire cerebral cortex [386]. Recently, researchers proposed a mechanism of prion-like propagation, meaning that these Tau aggregates behave like the prion proteins. In this way, Tau tangles induce normal proteins into conformational changes leading to aggregation. More research focused on this mechanism has shown incremental evidence supporting this hypothesis.

It is logical to propose targeting Tau and Aβ together with other targets, since Tau is the main component of aggregates leading to neurodegeneration. Nevertheless, these features represent the end stage of the disease. Does it mean there are other players in the initial process? For Aβ, it is likely because a mutation in APP is sufficient to cause AD. For Tau, it is more complicated. The presence of a mutation in Tau implies neurofibrillary degeneration without amyloid beta. Therefore, it means that by itself, aggregated Tau is toxic but does not lead to amyloid aggregation. In fact, Tau protein does not only aggregate in AD but also in other neurodegenerative disorders.

Tau biology is complex due to the presence of six Tau isoforms in the human brain. They are generated by alternative splicing from a unique gene MAPT located on chromosome 17. In the human brain, these six isoforms are in equal ratio of isoforms having three (3R) and four microtubule-binding domains (4R). Nevertheless, this ratio is modified amongst tauopathies showing different aggregation profiles (Table 2). In addition, these proteins go through posttranslational modification such as glycosylation, phosphorylation, oxidation, acetylation, and truncation. These posttranslational modifications and truncation may facilitate Tau aggregation even if the mechanisms are still unclear.

Thus, Tau is not only found in Alzheimer's disease, it is also found in progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), argyrophilic grain disease and some patients presenting with frontotemporal lobar degeneration. However, Tau aggregates display different regional and laminar distributions, different morphologies and different molecular characteristics. In fact, amongst these disorders, aetiologies are also different. Head trauma is a risk factor since Tau pathology is also found in traumatic brain injury like *dementia pugilistica*. It has also been reported in autistic children with self-injury behaviour. Therefore, Tau aggregation is not only related to ageing but to other factors that might differ from those belonging to AD. Another aetiology may be infectious agents like virus. Measles and Spanish flu viruses have been described to lead to cerebral Tau aggregation in subacute sclerosing panencephalitis and postencephalitic parkinsonism respectively. Therefore, infections may also trigger Tau aggregation. Other metabolism dysfunctions, as encountered in Niemann–Pick's disease type C (hereditary disorders with lysosomal lipidosis) and PSP (caused either by industrial waste or by mitochondrial toxins), also show Tau aggregation. Finally, Tau alternative splicing is also altered through genetic mechanisms either direct or indirect. For instance, some mutations have been on MAPT that lead to mis-splicing of exon 10 and overexpression of four microtubule-binding domain Tau isoforms. Tau aggregation can also show up indirectly, for instance in myotonic dystrophy type 1, a neuromuscular disorder. In this disease, the mRNA of this protein DMBK have CUG expansion triplets, causing sequestration of splicing factors that can change alternative splicing of many genes (APP, MAPT, Chloride channel, insulin receptor, troponin T, etc.). For MAPT, Tau splicing leads to the formation of the shortest Tau isoform which is found aggregated in some patients presenting with myotonic dystrophy. In the same way, some mutations on the Tau gene, MAPT, change the alternative splicing of Tau. For example, in AD, the six isoforms of Tau co-aggregate, in contrast to pathologies like PSP, in which only the 4R-Tau isoforms aggregate, and Pick's disease, where the aggregates are only comprised of 3R-Tau. Adding additional complexity, autosomal dominant mutations in MAPT have been reported to promote Tau aggregation and lead to frontotemporal lobar degeneration.

In AD, there is a large therapeutic time window for Tau treatments since neurofibrillary tangles last for decades. This is evident in neurons with pretangles that are still integrated into neuronal networks and still functional at early stages of AD [387]. Preclinical studies are possible because of the large number of animal models available [388–390]. Currently, PET ligands, peripheral biomarkers, and different therapeutic strategies are available for Tau therapeutic intervention. In addition, prevention and environmental factors may have an effect on Tau pathology and interventions can be used in combination with other treatments (Fig. 8).

Table 2. *Diversity of tauopathies in humans and their main traits*

	Aetiology	Tau lesions	Isoforms	Refs
Alzheimer's disease Down syndrome	Aß, ageing, genetics…	Neurofibrillary tangles (NFTs) & dystrophic neurites	3R + 4R	[365]
Argyrophilic grain disease	Ageing	Argyrophilic grains in limbic areas	4R ≫ 3R	[366]
Autism with self-injury behaviour Chronic traumatic encephalopathies Dementia pugilistica Traumatic brain injury	Repeated head trauma	NFTs & dystrophic neurites Astrocytic tangles	3R + 4R	[367–369]
Postencephalitic parkinsonism Subacute sclerosing panencephalitis	Virus	NFTs & dystrophic neurites Astrocytic tangles	3R + 4R ?	[370, 371]
Progressive supranuclear palsy • French Caribbean islands • Northern France Amyotrophic lateral sclerosis/parkinsonism dementia syndrome of Guam	Unknown Food toxins Industrial waste Unknown environment (food, heavy metals…)	NFTs & dystrophic neurites Tufted astrocytes	4R ≫ 3R 3R + 4R	[372–374]
Corticobasal degeneration	Unknown MAPT haplotypes	NFTs & neurites Astrocytic plaques	4R ≫ 3R	[375, 376]
Pick's disease	Unknown	Pick bodies	3R ≫ 4R	[375, 377, 378]
Niemann–Pick disease type C	Metabolism, genetics	NFTs & neurites	3R + 4R	[379, 380]
Myotonic dystrophy type 1	Indirect genetics: DMPK mutations (consequences on alternative splicing)	NFTs & neurites	0N3R	[381, 382]
Frontotemporal lobar degeneration (FTDP-17)	Direct genetics: MAPT mutations	NFTs & dystrophic neurites Astrocytic tangles Pick bodies… (highly dependent of mutations)	3R + 4R or 4R ≫ 3R	[383]
Progressive supranuclear palsy	MAPT haplotypes miR-132	NFTs & dystrophic neurites Tufted astrocytes	4R ≫ 3R	[384, 385]

There are many hypotheses of Tau aggregation. As for APP, Tau has chromosome micro-duplications and deletions important for tauopathies. In addition, specific haplotypes increase the amount of Tau, alternative splicing, conformation, posttranslational modification and change in degradation. All these hypotheses open up new therapeutic strategies. New therapies implicate for example to decrease posttranslational modifications like phosphorylation. Enhancing Tau dephosphorylation with sodium selenite is another possibility. Other approaches are the modulation of Tau

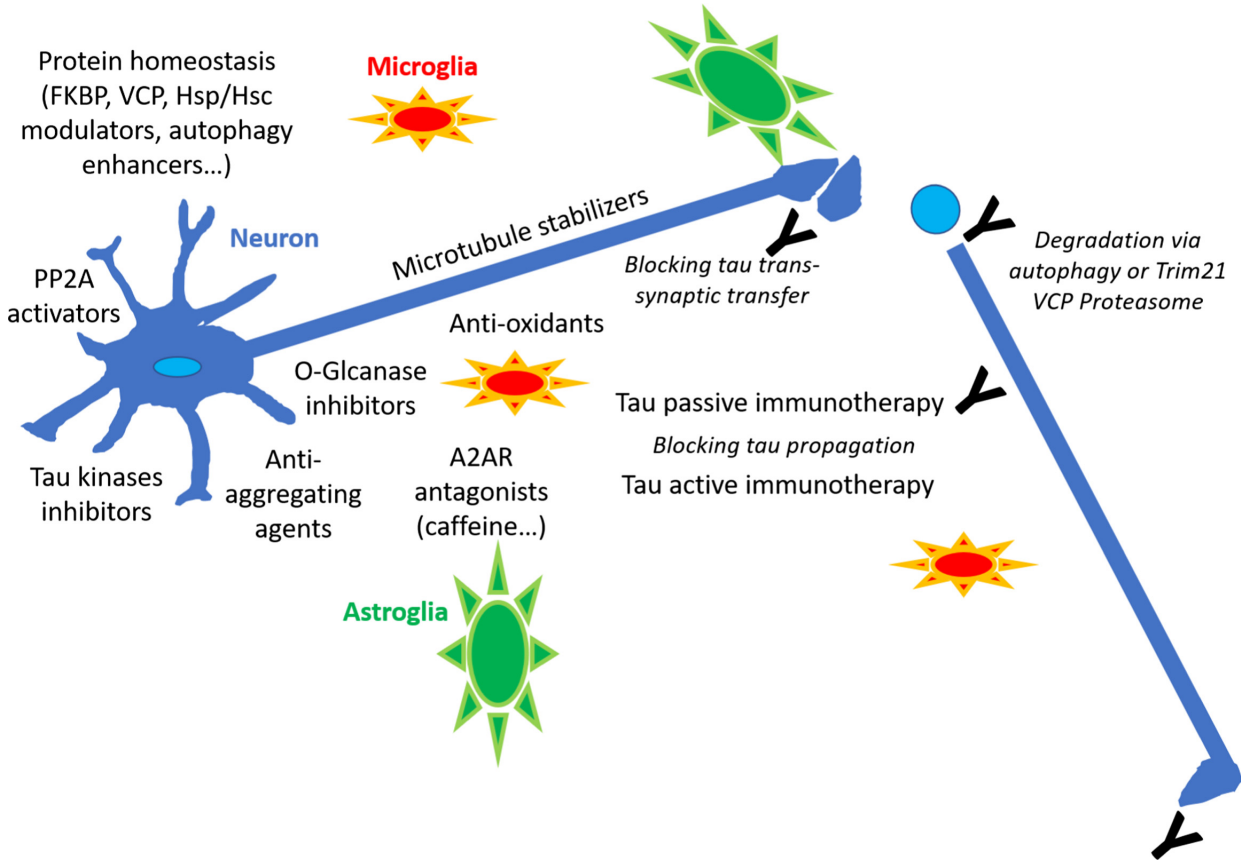

Fig. 8 *Key targets and therapeutic strategies for Tau treatments. Schematic representation of strategies for preventing Tau aggregation and spreading. Modulation of Tau metabolism can occur by regulating transcription, phosphorylation and degradation (e.g. through autophagy in pre- and postsynaptic neurons). Blockade of Tau trans-synaptic transfer through immunotherapy is also a strategy under test in the clinic.*

glycosylation, modulation of Tau aggregation (methylene blue derivatives failed in phase III), stabilization of microtubules and enhancement of Tau proteolysis. Immunotherapy is another very plausible approach. Modulation of alternative splicing may be an approach through gene therapy.

Finally, it is important to mention that Tau is not only a microtubule-associated protein, and its role in cells is not completely understood. Tau is bound to the plasma membrane [391], it is involved in transduction signalling, especially in brain insulin resistance [281]. It is also present in the nucleus, where it binds DNA, RNA, and is able to change the organization of the chromatin [392], thus influencing gene expression. It is also involved in synaptic plasticity through interaction with SH3 domains [394] and mediation of NMDA receptor phosphorylation [394].

The links between Aβ and Tau are still not fully defined. This is one of the main problems for Alzheimer's disease. For instance, for immunotherapy against Tau, decreasing its amount will improve the symptoms in the mouse models but does not cure the pathology. Thus, the real problem is that we do not have the right model for AD. Takomi Saido in Japan developed APP single copy knock-in mice, which contains the human APP gene without artefacts related to overexpression [297]. New Tau knock-in models are currently in development.

The presence of Amyloid beta and Tau cannot predict cognitive decline, possibly because of the

cognitive reserve. This point out the fact that we have too many therapeutic strategies without knowing the functions of Tau. In order to better target Tau in AD, research needs to focus on understanding brain homeostasis, microbial infections, hormones, about glucose uptake, microglia, insulin, leptin, ghrelin, and other mechanisms not really explored in the field yet. Even for immunotherapy, the mechanisms leading the antibodies to clearing, or to block extracellular amyloid propagation are not understood, simply because we do not know how Tau is transferred from one neuron to another.

Discussion and perspectives

Currently, in the clinic, the diagnosis of AD is based on symptomatology and, to some extent, on biomarkers. Memory tests help the neurologist or geriatrician to first identify dementia and then screen for Aβ via CSF tests, using PET imaging or both. The symptom-based classification leads to the staging of AD, as we know it, with MCI in the early phases and AD with its different stages later on [395]. Yet, from a neurobiological perspective, AD leads to synapse loss, neuronal death and decrease of cognitive function for reasons still unknown, and progresses in a continuum. Therefore, it is difficult to divide into defined stages. For these reasons, the NIA-AA has released a research framework suggesting a biological definition of AD based largely on biomarkers from living patients combined with cognitive tests [310]. These guidelines represent an effort to help a more accurate characterization of the aetiology of AD and help intervention studies targeting specific pathways involved in AD. Nevertheless, a clear stratification of AD requires much more research into the newly discovered mechanisms of disease progression.

In this work, we have revised the new risk factors that involve different pathways influencing AD development. However, screenings for all of these pathways are challenging for the clinic, sometimes requiring advanced techniques such as mass spectroscopy or special tracers [273]. Many of these methods require considerable technical knowledge or are too expensive for basic diagnostic laboratories. Still, the importance of novel biomarker detection lies in the possibility for intervention [101, 396, 397], rather than a prediction of AD progression and thus, represent valuable lines of research. These new genes and molecules hold the potential to allow us categorization of AD subtypes based on the clinical history of the patient [398, 399], improving, in turn, the predictions about the evolution of the disease and eventually, choosing a more personalized therapeutic strategy.

Clinical trials for NGF gene delivery in the hippocampus require complex procedures involving invasive injections in specific nuclei in the brain. It is possible that small deviations in the injection site can render the therapy ineffective in terms of improving cognition in subjects [400], although NGF cell therapy remains promising and under clinical research [35]. The unknown origin of the disease, mixed with complex genetic and environmental factors contributing to pathogenesis, have challenged clinical trials worldwide [401]. It follows that investment in AD research from the private sectors decrease and is virtually inexistent for promising mechanisms such as vesicular trafficking or autophagy [402].

Still, there are some hopes for drugs tested in previous trials that have failed in phase II stages. The FDA grants accelerated approval of drugs that comply with guidelines classified as a determinant for a disease. With the aforementioned NIA-AA new definitions on AD based on biomarkers, these drugs could still get FDA approval should they show changes in any of the important biomarkers in CSF at early stages. Indeed, the preclinical stage is the go-to stage for testing some of these agents for improving the predictors of AD progression in any way [401, 403]. Gene editing and control of gene expression in the brain are technologies in very early stages for clinical application [404], however they are good future approaches in modifying mechanisms such as synaptic plasticity and epigenetics, as discussed earlier in this work. We have revised many approaches directed towards restoring insulin balance in the brain, or restore microbiota communities to anti-inflammatory states. Moreover, combination of the therapeutic approaches revised here and others remain largely unexplored and have great potential to alter AD progression.

The establishment of new biomarkers also corresponds to the discovery of new disease pathways related to increased risk of developing AD. Now several studies aim to evaluate the effect of statins in AD from a mechanistic focus, evaluating new biomarker molecules such as neprilysin [405, 406]. It is now evident that the field of AD research is starting to integrate knowledge from diverse

disciplines. For example, micro RNAs have shown important roles regulating pathways during AD [47, 48, 407]. Also, recent works have discovered miR exosomes mediating neuron-astrocyte communication [408] and network-specific glial functions [409], whose role in neurodegeneration remains completely unexplored. New ventures in research from systems biology and omics approaches aims to uncover master regulators of disease-specific genes as novel targets for AD therapeutics [410, 411]. In this way, these targets will also help to uncover previously unknown pathways of disease and can connect previously unrelated cascades underlying neurodegeneration, similar to what happened between inflammation, diabetes and obesity [412]. It is worth to mention there is a novel nonpharmacological approach aiming to re-establish gamma oscillations in brains with AD. In order to do so, 40 Hz stimulation pulses are applied using sound or light signals. So far, the therapy has been successful at restoring memory function in AD mouse models and a clinical trial is planned with human subjects. Nevertheless, whether the therapeutic effect is long-lasting in humans remain to be studied, as well as the basic mechanisms behind the healing effect [413].

The direct consequence of these discoveries is that future therapies are likely to involve multiple targets and/or multiple strategies to exert an effect on AD-induced neurodegeneration. Similar strategies have become the norm for other conditions such as AIDS, cancer or even infectious diseases, producing health schemes that are virtually equal to a cure in many cases [414–417]. AD therapeutics faces great challenges in terms of drug delivery, toxicity, and maintenance of cognition in patients [4, 17]. These topics will lead the future of AD basic research in the long term.

In the short term, the most important strategies to improve the chances for treating AD are the targeting of factors increasing the risk of developing AD. We must underline that many risk factors for AD are modifiable [418]. In this work, we have described clinical trials and strategies involving already approved drugs to alter specific pathways in the brain at early stages of AD. Some of these studies have reported improvement in cognition of patients and have a solid molecular and biochemical background behind them. This evidence call for larger studies, more thorough stratification of cohorts, and in-depth research into the mechanisms behind the risk factors involved. Some of these pathways can tremendously increase the possibilities for treatment. For example, vascular dynamics in the brain are susceptible to regulation from an 'out of the BBB' approach [419] and could consequently improve parallel dysfunctions such as insulin/glucose availability or amyloid clearing.

As pointed out by leading experts in AD, researchers should learn from the previous experiences in therapeutics and keep developing basic knowledge about this disease.

Only then, we will crystalize the possibility for treating AD in our lifetime.

Acknowledgements

This review was supported by the Fondation Médéric Alzheimer (Paris, France), including scientific coordination (BW) and travel costs for all the experts. AID was financially supported by European funds from FEDER, *via* Programa Operacional Factores de Competitividade – COMPETE 2020; by Portuguese funds from FCT - Fundação para a Ciência e a Tecnologia (PTDC/SAU-TOX/117481/2010 and Strategic Project POCI-01-0145-FEDER-007440), and by European Social Fund (Fellowship *SFRH/BPD/84473/2012* to A. I. Duarte).

ACM, BW and RLV were financially supported by Margaretha af Ugglas foundation. ACM and RLV were also partially funded by Olle Enqvist Foundation grant no. 2014/778 and Lindhés Advokatbyrå Foundation. BW is supported by the Swedish Research Council.

Conflict of interest

ACM holds a position at SANOFI, Paris, but he declares no conflict of interest. All of the authors declare that this review was written in the absence of commercial or financial relationships that could constitute a conflict of interest.

References

1 Brookmeyer R, Johnson E, Ziegler-Graham K, Arrighi HM. Forecasting the global burden of Alzheimer's disease. *Alzheimers Dement* 2007; **3:** 186–91.
2 Brookmeyer R, Evans DA, Hebert L *et al.* National estimates of the prevalence of Alzheimer's disease in the United States. *Alzheimers Dement* 2011; **7:** 61–73.
3 Ferri CP, Prince M, Brayne C *et al.* Global prevalence of dementia: a Delphi consensus study. *Lancet* 2005; **366:** 2112–7.

4 Winblad B, Amouyel P, Andrieu S *et al.* Defeating Alzheimer's disease and other dementias: a priority for European science and society. *Lancet Neurol* 2016; **15:** 455–532.

5 Lopez AD, Mathers CD, Ezzati M, Jamison DT, Murray CJ. Global and regional burden of disease and risk factors, 2001: systematic analysis of population health data. *Lancet* 2006; **367:** 1747–57.

6 Farrer LA, Cupples LA, Haines JL *et al.* Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer disease meta analysis consortium. *JAMA* 1997; **278:** 1349–56.

7 Prince M, Bryce R, Albanese E, Wimo A, Ribeiro W, Ferri CP. The global prevalence of dementia: a systematic review and metaanalysis. *Alzheimers Dement* 2013; **9:** 63–75 e2.

8 Wimo A, Guerchet M, Ali GC *et al.* The worldwide costs of dementia 2015 and comparisons with 2010. *Alzheimers Dement* 2017; **13:** 1–7.

9 Prince M, Ali GC, Guerchet M, Prina AM, Albanese E, Wu YT. Recent global trends in the prevalence and incidence of dementia, and survival with dementia. *Alzheimers Res Ther* 2016; **8:** 23.

10 Wimo A, Jonsson L, Bond J, Prince M, Winblad B, Alzheimer Disease I. The worldwide economic impact of dementia 2010. *Alzheimers Dement* 2013; **9:** 1–11 e3.

11 Clark BF. Healthy human ageing. *N Biotechnol* 2008; **25:** 13–5.

12 Daskalopoulou C, Stubbs B, Kralj C, Koukounari A, Prince M, Prina AM. Associations of smoking and alcohol consumption with healthy ageing: a systematic review and meta-analysis of longitudinal studies. *BMJ Open* 2018; **8:** e019540.

13 Schneider LS, Mangialasche F, Andreasen N *et al.* Clinical trials and late-stage drug development for Alzheimer's disease: an appraisal from 1984 to 2014. *J Intern Med* 2014; **275:** 251–83.

14 Mangialasche F, Solomon A, Winblad B, Mecocci P, Kivipelto M. Alzheimer's disease: clinical trials and drug development. *Lancet Neurol* 2010; **9:** 702–16.

15 Hardy JA, Higgins GA. Alzheimer's disease: the amyloid cascade hypothesis. *Science* 1992; **256:** 184–5.

16 Karran E, De Strooper B. The amyloid cascade hypothesis: are we poised for success or failure? *J Neurochem* 2016; **139 (Suppl. 2):** 237–52.

17 Hardy J, De Strooper B. Alzheimer's disease: where next for anti-amyloid therapies? *Brain* 2017; **140:** 853–5.

18 Pugazhenthi S. Metabolic syndrome and the cellular phase of Alzheimer's disease. *Prog Mol Biol Transl Sci* 2017; **146:** 243–58.

19 De Strooper B, Karran E. The cellular phase of Alzheimer's disease. *Cell* 2016; **164:** 603–15.

20 McGeer PL, McGeer EG. The amyloid cascade-inflammatory hypothesis of Alzheimer disease: implications for therapy. *Acta Neuropathol* 2013; **126:** 479–97.

21 Sarazin M, Dorothee G, de Souza LC, Aucouturier P. Immunotherapy in Alzheimer's disease: do we have all the pieces of the puzzle? *Biol Psychiatry* 2013; **74:** 329–32.

22 Loera-Valencia R, Piras A, Ismail MAM *et al.* Targeting Alzheimer's disease with gene and cell therapies. *J Intern Med* 2018; **284:** 2–36.

23 Kemppainen N, Johansson J, Teuho J *et al.* Brain amyloid load and its associations with cognition and vascular risk factors in FINGER study. *Neurology* 2018; **90:** e206–13.

24 Worker A, Dima D, Combes A *et al.* Test-retest reliability and longitudinal analysis of automated hippocampal subregion volumes in healthy ageing and Alzheimer's disease populations. *Hum Brain Mapp* 2018; **39:** 1743–54.

25 Fraser MA, Shaw ME, Cherbuin N. A systematic review and meta-analysis of longitudinal hippocampal atrophy in healthy human ageing. *NeuroImage* 2015; **112:** 364–74.

26 Vos SJB, Gordon BA, Su Y *et al.* NIA-AA staging of preclinical Alzheimer disease: discordance and concordance of CSF and imaging biomarkers. *Neurobiol Aging* 2016; **44:** 1–8.

27 Braak H, Braak E. Development of Alzheimer-related neurofibrillary changes in the neocortex inversely recapitulates cortical myelogenesis. *Acta Neuropathol* 1996; **92:** 197–201.

28 Scheff SW, Price DA, Schmitt FA, Mufson EJ. Hippocampal synaptic loss in early Alzheimer's disease and mild cognitive impairment. *Neurobiol Aging* 2006; **27:** 1372–84.

29 Klein WL. Synaptic targeting by Aβ oligomers (ADDLS) as a basis for memory loss in early Alzheimer's disease. *Alzheimers Dement* 2006; **2:** 43–55.

30 Lacor PN, Buniel MC, Furlow PW *et al.* A oligomer-induced aberrations in synapse composition, shape, and density provide a molecular basis for loss of connectivity in Alzheimer's disease. *J Neurosci* 2007; **27:** 796–807.

31 Wang H-W, Pasternak JF, Kuo H *et al.* Soluble oligomers of β amyloid (1-42) inhibit long-term potentiation but not long-term depression in rat dentate gyrus. *Brain Res* 2002; **924:** 133–40.

32 Esteves da Silva M, Adrian M, Schätzle P *et al.* Positioning of AMPA receptor-containing endosomes regulates synapse architecture. *Cell Rep* 2015; **13:** 933–43.

33 Angelo M, Plattner F, Giese KP. Cyclin-dependent kinase 5 in synaptic plasticity, learning and memory. *J Neurochem* 2006; **99:** 353–70.

34 Leshchyns'ka I, Sytnyk V. Synaptic cell adhesion molecules in Alzheimer's disease. *Neural Plast* 2016; **2016:** 1–9.

35 Eyjolfsdottir H, Eriksdotter M, Linderoth B *et al.* Targeted delivery of nerve growth factor to the cholinergic basal forebrain of Alzheimer's disease patients: application of a second-generation encapsulated cell biodelivery device. *Alzheimers Res Ther* 2016; **8:** 30.

36 Bothwell M. NFG, BDNF, NT3, and NT4. In: Lewin GR, Carter BD, eds. *Neurotrophic Factors*. Berlin and Heidelberg: Springer, 2014; 3–15.

37 Miyamoto A, Wake H, Ishikawa AW *et al.* Microglia contact induces synapse formation in developing somatosensory cortex. *Nat Commun* 2016; **7:** 12540.

38 Haraguchi S, Sasahara K, Shikimi H, Honda S-i, Harada N, Tsutsui K. Estradiol promotes purkinje dendritic growth, spinogenesis, and synaptogenesis during neonatal life by inducing the expression of BDNF. *Cerebellum* 2011; **11:** 416–7.

39 Dave KA, Platel J-C, Huang F, Tian D, Stamboulian-Platel S, Bordey A. Prostaglandin E2 induces glutamate release from subventricular zone astrocytes. *Neuron Glia Biol* 2010; **6:** 201–7.

40 Batti L, O'Connor JJ. Tumor necrosis factor-α impairs the recovery of synaptic transmission from hypoxia in rat hippocampal slices. *J Neuroimmunol* 2010; **218:** 21–7.

41 Dupont C, Armant D, Brenner C. Epigenetics: definition, mechanisms and clinical perspective. *Semin Reprod Med* 2009; **27:** 351–7.
42 Rogers J, Mastroeni D, Grover A, Delvaux E, Whiteside C, Coleman PD. The epigenetics of Alzheimer's disease — additional considerations. *Neurobiol Aging* 2011; **32:** 1196–7.
43 Neal M, Richardson JR. Epigenetic regulation of astrocyte function in neuroinflammation and neurodegeneration. *Biochim Biophys Acta* 1864, 2018,; 432–43.
44 Feligioni M, Nisticò R. SUMO: a (oxidative) stressed protein. *NeuroMol Med* 2013; **15:** 707–19.
45 Li T, Santockyte R, Shen R-F *et al.* Expression of SUMO-2/3 induced senescence through p53- and pRB-mediated pathways. *J Biol Chem* 2006; **281:** 36221–7.
46 Fiore R, Khudayberdiev S, Saba R. Schratt G. microRNA function in the nervous system. *Prog Mol Biol Transl Sci* 2011; 47–100.
47 Putteeraj M, Fairuz YM, Teoh SL. MicroRNA dysregulation in Alzheimer's disease. *CNS Neurol Disord Drug Targets* 2018; 16.
48 Goedeke L, Fernández-Hernando C. microRNAs: a connection between cholesterol metabolism and neurodegeneration. *Neurobiol Dis* 2014; **72:** 48–53.
49 Heneka MT, Carson MJ, Khoury JE *et al.* Neuroinflammation in Alzheimer's disease. *Lancet Neurol* 2015; **14:** 388–405.
50 Obermeier B, Verma A, Ransohoff RM. The blood–brain barrier. *Handbook Clin Neurol* 2016; 39–59.
51 Daneman R, Prat A. The blood-brain barrier. *Cold Spring Harbor Perspec Bio* 2015; **7:** a020412.
52 Sweeney MD, Ayyadurai S, Zlokovic BV. Pericytes of the neurovascular unit: key functions and signaling pathways. *Nat Neurosci* 2016; **19:** 771–83.
53 Cheslow L, Alvarez JI. Glial-endothelial crosstalk regulates blood–brain barrier function. *Curr Opin Pharmacol* 2016; **26:** 39–46.
54 Zhao Z, Nelson AR, Betsholtz C, Zlokovic BV. Establishment and dysfunction of the blood-brain barrier. *Cell* 2015; **163:** 1064–78.
55 Mahringer A, Fricker G. ABC transporters at the blood–brain barrier. *Expert Opin Drug Metab Toxicol* 2016; **12:** 499–508.
56 Zlokovic BV. Neurovascular pathways to neurodegeneration in Alzheimer's disease and other disorders. *Nat Rev Neurosci* 2011; **12:** 723–38.
57 Vanlandewijck M, He L, Mae MA *et al.* A molecular atlas of cell types and zonation in the brain vasculature. *Nature* 2018; **554:** 475–80.
58 Erdő F, Denes L, de Lange E. Age-associated physiological and pathological changes at the blood–brain barrier: a review. *J Cereb Blood Flow Metab* 2016; **37:** 4–24.
59 van de Haar HJ, Burgmans S, Jansen JFA *et al.* Blood-brain barrier leakage in patients with early Alzheimer disease. *Radiology* 2016; **281:** 527–35.
60 Sweeney MD, Sagare AP, Zlokovic BV. Blood–brain barrier breakdown in Alzheimer disease and other neurodegenerative disorders. *Nat Rev Neurol* 2018; **14:** 133–50.
61 Poliakova T, Levin O, Arablinskiy A, Vasenina E, Zerr I. Cerebral microbleeds in early Alzheimer's disease. *J Neurol* 2016; **263:** 1961–8.
62 Deo AK, Borson S, Link JM *et al.* Activity of P-glycoprotein, a -amyloid transporter at the blood-brain barrier, is compromised in patients with mild Alzheimer disease. *J Nucl Med* 2014; **55:** 1106–11.
63 Bailey TL, Rivara CB, Rocher AB, Hof PR. The nature and effects of cortical microvascular pathology in aging and Alzheimer's disease. *Neurol Res* 2004; **26:** 573–8.
64 Lepelletier FX, Mann DMA, Robinson AC, Pinteaux E, Boutin H. Early changes in extracellular matrix in Alzheimer's disease. *Neuropathol Appl Neurobiol* 2015; **43:** 167–82.
65 Halliday MR, Rege SV, Ma Q *et al.* Accelerated pericyte degeneration and blood–brain barrier breakdown in apolipoprotein E4 carriers with Alzheimer's disease. *J Cereb Blood Flow Metab* 2015; **36:** 216–27.
66 Nelson AR, Sweeney MD, Sagare AP, Zlokovic BV. Neurovascular dysfunction and neurodegeneration in dementia and Alzheimer's disease. *Biochim Biophys Acta* 2016; **1862:** 887–900.
67 Bagyinszky E, Giau VV, Shim K, Suk K, An SSA, Kim S. Role of inflammatory molecules in the Alzheimer's disease progression and diagnosis. *J Neurol Sci* 2017; **376:** 242–54.
68 Laurent C, Dorothée G, Hunot S *et al.* Hippocampal T cell infiltration promotes neuroinflammation and cognitive decline in a mouse model of tauopathy. *Brain* 2016; **140:** 184–200.
69 Krauthausen M, Kummer MP, Zimmermann J *et al.* CXCR69 promotes plaque formation and behavioral deficits in an Alzheimer's disease model. *J Clin Invest* 2014; **125:** 365–78.
70 Torres KCL, Santos RR, de Lima GSF *et al.* Decreased expression of CCL3 in monocytes and CCR70 in lymphocytes from frontotemporal dementia as compared with Alzheimer's disease patients. *J Neuropsy Clin Neurosci* 2012; **24:** E11–2.
71 Zhu M, Allard JS, Zhang Y *et al.* Age-related brain expression and regulation of the chemokine CCL4/MIP-1β in APP/PS1 double-transgenic mice. *J Neuropathol Exp Neurol* 2014; **73:** 362–74.
72 Franciosi S, Choi HB, Kim SU, McLarnon JG. IL-8 enhancement of amyloid-beta (Aβ1-42)-induced expression and production of pro-inflammatory cytokines and COX-2 in cultured human microglia. *J Neuroimmunol* 2005; **159:** 66–74.
73 Fuhrmann M, Bittner T, Jung CKE *et al.* Microglial Cx3cr1 knockout prevents neuron loss in a mouse model of Alzheimer's disease. *Nat Neurosci* 2010; **13:** 411–3.
74 Tripathy D, Thirumangalakudi L, Grammas P. RANTES upregulation in the Alzheimer's disease brain: a possible neuroprotective role. *Neurobiol Aging* 2010; **31:** 8–16.
75 Lee JK, Schuchman EH, Jin HK, Bae J-S. Soluble CCL5 derived from bone marrow-derived mesenchymal stem cells and activated by amyloid β ameliorates Alzheimer's disease in mice by recruiting bone marrow-induced microglia immune responses. *Stem Cells* 2012; **30:** 1544–55.
76 Naert G, Rivest S. CC chemokine receptor 2 deficiency aggravates cognitive impairments and amyloid pathology in a transgenic mouse model of Alzheimer's disease. *J Neurosci* 2011; **31:** 6208–20.
77 Kiyota T, Yamamoto M, Xiong H *et al.* CCL2 accelerates microglia-mediated Aβ oligomer formation and progression of neurocognitive dysfunction. *PLoS ONE* 2009; **4:** e6197.
78 Yamamoto M, Horiba M, Buescher JL *et al.* Overexpression of monocyte chemotactic protein-1/CCL2 in β-amyloid precursor protein transgenic mice show accelerated diffuse β-amyloid deposition. *Am J Pathol* 2005; **166:** 1475–85.

79 Verite J, Janet T, Julian A, Chassaing D, Page G, Paccalin M. Peripheral blood mononuclear cells of Alzheimer's disease patients control CCL4 and CXCL10 levels in a human blood brain barrier model. *Curr Alzheimer Res* 2017; **14.**

80 Verite J, Janet T, Chassaing D, Fauconneau B, Rabeony H, Page G. Longitudinal chemokine profile expression in a blood-brain barrier model from Alzheimer transgenic versus wild-type mice. *J Neuroinflamm* 2018; **15:** 182.

81 Gorelick PB, Scuteri A, Black SE *et al.* Vascular contributions to cognitive impairment and dementia: a statement for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke* 2011; **42:** 2672–713.

82 Maillard P, Seshadri S, Beiser A *et al.* Effects of systolic blood pressure on white-matter integrity in young adults in the Framingham Heart Study: a cross-sectional study. *Lancet Neurol* 2012; **11:** 1039–47.

83 Wardlaw JM, Smith C, Dichgans M. Mechanisms of sporadic cerebral small vessel disease: insights from neuroimaging. *Lancet Neurol* 2013; **12:** 483–97.

84 Yoshino H, Sakurai T, Oizumi XS *et al.* Dilation of perforating arteries in rat brain in response to systemic hypotension is more sensitive and pronounced than that of pial arterioles: simultaneous visualization of perforating and cortical vessels by in-vivo microangiography. *Microvasc Res* 2009; **77:** 230–3.

85 Hainsworth AH, Markus HS. Do in vivo experimental models reflect human cerebral small vessel disease? a systematic review. *J Cereb Blood Flow Metab* 2008; **28:** 1877–91.

86 Schreiber S, Bueche CZ, Garz C *et al.* Kidney pathology precedes and predicts the pathological cascade of cerebrovascular lesions in stroke prone rats. *PLoS ONE* 2011; **6:** e26287.

87 Schreiber S, Bueche CZ, Garz C *et al.* The pathologic cascade of cerebrovascular lesions in SHRSP: is erythrocyte accumulation an early phase? *J Cereb Blood Flow Metab* 2012; **32:** 278–90.

88 Bailey EL, Wardlaw JM, Graham D, Dominiczak AF, Sudlow CLM, Smith C. Cerebral small vessel endothelial structural changes predate hypertension in stroke-prone spontaneously hypertensive rats: a blinded, controlled immunohistochemical study of 5- to 21-week-old rats. *Neuropathol Appl Neurobiol* 2011; **37:** 711–26.

89 Kaiser D, Weise G, Möller K *et al.* Spontaneous white matter damage, cognitive decline and neuroinflammation in middle-aged hypertensive rats: an animal model of early-stage cerebral small vessel disease. *Acta Neuropathol Commun* 2014; 2.

90 Ennaceur A, Delacour J. A new one-trial test for neurobiological studies of memory in rats. 1: behavioral data. *Behav Brain Res* 1988; **31:** 47–59.

91 den Heijer T, Launer LJ, Prins ND *et al.* Association between blood pressure, white matter lesions, and atrophy of the medial temporal lobe. *Neurology* 2005; **64:** 263–7.

92 Sabbatini M, Catalani A, Consoli C, Marletta N, Tomassoni D, Avola R. The hippocampus in spontaneously hypertensive rats: an animal model of vascular dementia? *Mech Ageing Dev* 2002; **123:** 547–59.

93 Leeuw FE, Kleine M, Frijns CJM, Fijnheer R, Gijn J, Kappelle LJ. Endothelial cell activation is associated with cerebral white matter lesions in patients with cerebrovascular disease. *Ann N Y Acad Sci* 2002; **977:** 306–14.

94 Schiffrin EL. Immune mechanisms in hypertension and vascular injury. *Clin Sci (Lond)* 2014; **126:** 267–74.

95 Moller K, Posel C, Kranz A *et al.* Arterial hypertension aggravates innate immune responses after experimental stroke. *Front Cell Neurosci* 2015; **9:** 461.

96 Tummala PE, Chen XL, Sundell CL *et al.* Angiotensin II induces vascular cell adhesion molecule-1 expression in rat vasculature: a potential link between the renin-angiotensin system and atherosclerosis. *Circulation* 1999; **100:** 1223–9.

97 Rouhl RP, Damoiseaux JG, Lodder J *et al.* Vascular inflammation in cerebral small vessel disease. *Neurobiol Aging* 2012; **33:** 1800–6.

98 Harrison DG, Marvar PJ, Titze JM. Vascular inflammatory cells in hypertension. *Front Physiol* 2012; **3.**

99 Derecki NC, Cardani AN, Yang CH *et al.* Regulation of learning and memory by meningeal immunity: a key role for IL-4. *J Exp Med* 2010; **207:** 1067–80.

100 van Weel V, Toes RE, Seghers L *et al.* Natural killer cells and CD4+ T-cells modulate collateral artery development. *Arterioscler Thromb Vasc Biol* 2007; **27:** 2310–8.

101 Rosenberg A, Ngandu T, Rusanen M *et al.* Multidomain lifestyle intervention benefits a large elderly population at risk for cognitive decline and dementia regardless of baseline characteristics: the FINGER trial. *Alzheimers Dement* 2018; **14:** 263–70.

102 Martinez-Ramirez S, Greenberg SM, Viswanathan A. Cerebral microbleeds: overview and implications in cognitive impairment. *Alzheimers Res Ther* 2014; **6:** 33.

103 Ding J, Sigurðsson S, Jónsson PV *et al.* Space and location of cerebral microbleeds, cognitive decline, and dementia in the community. *Neurology* 2017; **88:** 2089–97.

104 Matsuyama H, Ii Y, Maeda M *et al.* Background and distribution of lobar microbleeds in cognitive dysfunction. *Brain Behav* 2017; **7:** e00856.

105 Hilal S, Akoudad S, van Duijn CM *et al.* Plasma amyloid-β levels, cerebral small vessel disease, and cognition: the Rotterdam study. *J Alzheimers Dis* 2017; **60:** 977–87.

106 Stehling C, Wersching H, Kloska SP *et al.* Detection of asymptomatic cerebral microbleeds: a comparative study at 1.5 and 3.0 T. *Acad Radiol* 2008; **15:** 895–900.

107 Vernooij MW, van der Lugt A, Ikram MA *et al.* Prevalence and risk factors of cerebral microbleeds: the Rotterdam scan study. *Neurology* 2008; **70:** 1208–14.

108 Kumar P, Yadav AK, Misra S, Kumar A, Chakravarty K, Prasad K. Prediction of upper extremity motor recovery after subacute intracerebral hemorrhage through diffusion tensor imaging: a systematic review and meta-analysis. *Neuroradiology* 2016; **58:** 1043–50.

109 Karuppagounder SS, Alim I, Khim SJ *et al.* Therapeutic targeting of oxygen-sensing prolyl hydroxylases abrogates ATF4-dependent neuronal death and improves outcomes after brain hemorrhage in several rodent models. *Sci Transl Med* 2016; **8:** 328ra29-ra29.

110 Zille M, Karuppagounder SS, Chen Y *et al.* Neuronal death after hemorrhagic stroke in vitro and in vivo shares features of ferroptosis and necroptosis. *Stroke* 2017; **48:** 1033–43.

111 Raff MC, Whitmore AV, Finn JT. Axonal self-destruction and neurodegeneration. *Science* 2002; **296:** 868–71.

112 Sagot Y, Dubois-Dauphin M, Tan SA *et al.* Bcl-2 overexpression prevents motoneuron cell body loss but not axonal degeneration in a mouse model of a neurodegenerative disease. *J Neurosci* 1995; **15:** 7727–33.

113 Nikolaev A, McLaughlin T, O'Leary DD, Tessier-Lavigne M. APP binds DR6 to trigger axon pruning and neuron death via distinct caspases. *Nature* 2009; **457:** 981–9.

114 Heppner FL, Ransohoff RM, Becher B. Immune attack: the role of inflammation in Alzheimer disease. *Nat Rev Neurosci* 2015; **16:** 358–72.

115 Zotova E, Nicoll JA, Kalaria R, Holmes C, Boche D. Inflammation in Alzheimer's disease: relevance to pathogenesis and therapy. *Alzheimers Res Ther* 2010; **2:** 1.

116 Sommer F, Backhed F. The gut microbiota–masters of host development and physiology. *Nat Rev Microbiol* 2013; **11:** 227–38.

117 Tremaroli V, Backhed F. Functional interactions between the gut microbiota and host metabolism. *Nature* 2012; **489:** 242–9.

118 Claesson MJ, Jeffery IB, Conde S *et al.* Gut microbiota composition correlates with diet and health in the elderly. *Nature* 2012; **488:** 178–84.

119 Marizzoni M, Provasi S, Cattaneo A, Frisoni GB. Microbiota and neurodegenerative diseases. *Curr Opin Neurol* 2017; **30:** 630–8.

120 Cattaneo A, Cattane N, Galluzzi S *et al.* Association of brain amyloidosis with pro-inflammatory gut bacterial taxa and peripheral inflammation markers in cognitively impaired elderly. *Neurobiol Aging* 2017; **49:** 60–8.

121 Russler-Germain EV, Rengarajan S, Hsieh CS. Antigen-specific regulatory T-cell responses to intestinal microbiota. *Mucosal Immunol* 2017; **10:** 1375–86.

122 Braniste V, Al-Asmakh M, Kowal C *et al.* The gut microbiota influences blood-brain barrier permeability in mice. *Sci Transl Med* 2014; **6:** 263ra158.

123 Dantzer R. Neuroimmune interactions: from the brain to the immune system and vice versa. *Physiol Rev* 2018; **98:** 477–504.

124 Soscia SJ, Kirby JE, Washicosky KJ *et al.* The Alzheimer's disease-associated amyloid beta-protein is an antimicrobial peptide. *PLoS ONE* 2010; **5:** e9505.

125 Kumar DK, Choi SH, Washicosky KJ *et al.* Amyloid-beta peptide protects against microbial infection in mouse and worm models of Alzheimer's disease. *Sci Transl Med* 2016; **8:** 340ra72.

126 Pulze L, Bassani B, Gini E *et al.* NET amyloidogenic backbone in human activated neutrophils. *Clin Exp Immunol* 2016; **183:** 469–79.

127 Pisa D, Alonso R, Rabano A, Rodal I, Carrasco L. Different brain regions are infected with fungi in Alzheimer's disease. *Sci Rep* 2015; **5:** 15015.

128 Zhao Y, Cong L, Lukiw WJ. Lipopolysaccharide (LPS) accumulates in neocortical neurons of Alzheimer's disease (AD) brain and impairs transcription in human neuronal-glial primary co-cultures. *Front Aging Neurosci* 2017; **9:** 407.

129 Franzosa EA, Huang K, Meadow JF *et al.* Identifying personal microbiomes using metagenomic codes. *Proc Natl Acad Sci USA* 2015; **112:** E2930–8.

130 Akbari E, Asemi Z, Daneshvar Kakhaki R *et al.* Effect of probiotic supplementation on cognitive function and metabolic status in Alzheimer's disease: a randomized, double-blind and controlled trial. *Front Aging Neurosci* 2016; **8:** 256.

131 Jarosz-Griffiths HH, Noble E, Rushworth JV, Hooper NM. Amyloid-β receptors: the good, the bad, and the prion protein. *J Biol Chem* 2015; **291:** 3174–83.

132 Miklossy J, Qing H, Radenovic A *et al.* Beta amyloid and hyperphosphorylated tau deposits in the pancreas in type 2 diabetes. *Neurobiol Aging* 2010; **31:** 1503–15.

133 Roher AE, Cribbs DH, Kim RC *et al.* Bapineuzumab alters Abeta composition: implications for the amyloid cascade hypothesis and anti-amyloid immunotherapy. *PLoS ONE* 2013; **8:** e59735.

134 Yang Y, Wu Y, Zhang S, Song W. High glucose promotes Aβ production by inhibiting APP degradation. *PLoS ONE* 2013; **8:** e69824.

135 Takeda S, Sato N, Uchio-Yamada K *et al.* Diabetes-accelerated memory dysfunction via cerebrovascular inflammation and Abeta deposition in an Alzheimer mouse model with diabetes. *Proc Natl Acad Sci USA* 2010; **107:** 7036–41.

136 Bharadwaj P, Solomon T, Malajczuk CJ *et al.* Role of the cell membrane interface in modulating production and uptake of Alzheimer's beta amyloid protein. *Biochem Biophys Acta* 2018.

137 Balczon R. The identification, purification, and characterization of a pancreatic beta-cell form of the microtubule adenosine triphosphatase kinesin. *Endocrinology* 1992; **131:** 331–6.

138 Alvim RO, Cheuhen MR, Machado SR, Sousa AGP, Santos PCJL. General aspects of muscle glucose uptake. *An Acad Bras Ciênc* 2015; **87:** 351–68.

139 Emoto M, Langille SE, Czech MP. A role for kinesin in insulin-stimulated GLUT4 glucose transporter translocation in 3T3-L1 adipocytes. *J Biol Chem* 2001; **276:** 10677–82.

140 Olson AL, Trumbly AR, Gibson GV. Insulin-mediated GLUT4 translocation is dependent on the microtubule network. *J Biol Chem* 2001; **276:** 10706–14.

141 Porte D, Baskin DG, Schwartz MW. Insulin signaling in the central nervous system: a critical role in metabolic homeostasis and disease from C. elegans to humans. *Diabetes* 2005; **54:** 1264–76.

142 Yin F, Yao J, Sancheti H *et al.* The perimenopausal aging transition in the female rat brain: decline in bioenergetic systems and synaptic plasticity. *Neurobiol Aging* 2015; **36:** 2282–95.

143 Moreira PI, Santos MS, Sena C, Seica R, Oliveira CR. Insulin protects against amyloid beta-peptide toxicity in brain mitochondria of diabetic rats. *Neurobiol Dis* 2005; **18:** 628–37.

144 Duarte AI, Proenca T, Oliveira CR, Santos MS, Rego AC. Insulin restores metabolic function in cultured cortical neurons subjected to oxidative stress. *Diabetes* 2006; **55:** 2863–70.

145 Santos RX, Correia SC, Alves MG *et al.* Insulin therapy modulates mitochondrial dynamics and biogenesis, autophagy and tau protein phosphorylation in the brain of type 1 diabetic rats. *Biochim Biophys Acta* 2014; **1842:** 1154–66.

146 Duarte AI, Santos MS, Oliveira CR, Rego AC. Insulin neuroprotection against oxidative stress in cortical neurons–involvement of uric acid and glutathione antioxidant defenses. *Free Radic Biol Med* 2005; **39:** 876–89.

147 Duarte AI, Santos P, Oliveira CR, Santos MS, Rego AC. Insulin neuroprotection against oxidative stress is mediated by Akt and GSK-3beta signaling pathways and changes in protein expression. *Biochim Biophys Acta* 2008; **1783:** 994–1002.

148 Duarte AI, Santos MS, Seica R, Oliveira CR. Oxidative stress affects synaptosomal-aminobutyric acid and glutamate transport in diabetic rats: the role of insulin. *Diabetes* 2004; **53:** 2110–6.
149 Duarte AI, Santos MS. Seiça R, oliveira CRd. Insulin affects synaptosomal GABA and glutamate transport under oxidative stress conditions. *Brain Res* 2003; **977:** 23–30.
150 De Felice FG, Benedict C. A key role of insulin receptors in memory: figure 1. *Diabetes* 2015; **64:** 3653–5.
151 Rodriguez-Rodriguez P, Sandebring-Matton A, Merino-Serrais P *et al.* Tau hyperphosphorylation induces oligomeric insulin accumulation and insulin resistance in neurons. *Brain* 2017; **140:** 3269–85.
152 Kullmann S, Heni M, Hallschmid M, Fritsche A, Preissl H, Häring H-U. Brain insulin resistance at the crossroads of metabolic and cognitive disorders in humans. *Physiol Rev* 2016; **96:** 1169–209.
153 Zhao L, Mao Z, Woody SK, Brinton RD. Sex differences in metabolic aging of the brain: insights into female susceptibility to Alzheimer's disease. *Neurobiol Aging* 2016; **42:** 69–79.
154 Brinton RD, Yao J, Yin F, Mack WJ, Cadenas E. Perimenopause as a neurological transition state. *Nat Rev Endocrinol* 2015; **11:** 393–405.
155 Swerdlow RH, Burns JM, Khan SM. The Alzheimer's disease mitochondrial cascade hypothesis: progress and perspectives. *Biochim Biophys Acta* 1842, 2014,; 1219–31.
156 Kandimalla R, Thirumala V, Reddy PH. Is Alzheimer's disease a type 3 diabetes? A critical appraisal. *Biochim Biophys Acta* 1863, 2017,; 1078–89.
157 Winblad B, Qiu C, Ballard C, Johansson G. Evidence-based prevention and treatment of dementia – Authors' reply. *Lancet Neurol* 2016; **15:** 1007–8.
158 Pearson-Leary J, Jahagirdar V, Sage J, McNay EC. Insulin modulates hippocampally-mediated spatial working memory via glucose transporter-4. *Behav Brain Res* 2018; **338:** 32–9.
159 Winkler EA, Nishida Y, Sagare AP *et al.* GLUT1 reductions exacerbate Alzheimer's disease vasculo-neuronal dysfunction and degeneration. *Nat Neurosci* 2015; **18:** 521–30.
160 Kapogiannis D, Mattson MP. Disrupted energy metabolism and neuronal circuit dysfunction in cognitive impairment and Alzheimer's disease. *Lancet Neurol* 2011; **10:** 187–98.
161 Cunnane S, Nugent S, Roy M *et al.* Brain fuel metabolism, aging, and Alzheimer's disease. *Nutrition* 2011; **27:** 3–20.
162 Calkins MJ, Manczak M, Mao P, Shirendeb U, Reddy PH. Impaired mitochondrial biogenesis, defective axonal transport of mitochondria, abnormal mitochondrial dynamics and synaptic degeneration in a mouse model of Alzheimer's disease. *Hum Mol Genet* 2011; **20:** 4515–29.
163 Bubber P, Haroutunian V, Fisch G, Blass JP, Gibson GE. Mitochondrial abnormalities in Alzheimer brain: mechanistic implications. *Ann Neurol* 2005; **57:** 695–703.
164 Melo JB, Agostinho P, Oliveira CR. Amyloid beta-peptide 25-35 reduces [3H]acetylcholine release in retinal neurons. Involvement of metabolic dysfunction. *Amyloid* 2002; **9:** 221–8.
165 Onyango IG, Dennis J, Khan SM. Mitochondrial dysfunction in Alzheimer's disease and the rationale for bioenergetics based therapies. *Aging Dis* 2016; **7:** 201–14.
166 Yao J, Zhao L, Mao Z *et al.* Potentiation of brain mitochondrial function by S-equol and R/S-equol estrogen receptor beta-selective phytoSERM treatments. *Brain Res* 2013; **1514:** 128–41.
167 Fang D, Wang Y, Zhang Z *et al.* Increased neuronal PreP activity reduces Abeta accumulation, attenuates neuroinflammation and improves mitochondrial and synaptic function in Alzheimer disease's mouse model. *Hum Mol Genet* 2015; **24:** 5198–210.
168 Yao J, Irwin R, Chen S, Hamilton R, Cadenas E, Brinton RD. Ovarian hormone loss induces bioenergetic deficits and mitochondrial beta-amyloid. *Neurobiol Aging* 2012; **33:** 1507–21.
169 Mosconi L, de Leon M, Murray J *et al.* Reduced mitochondria cytochrome oxidase activity in adult children of mothers with Alzheimer's disease. *J Alzheimers Dis* 2011; **27:** 483–90.
170 Swerdlow RH. Mitochondria and cell bioenergetics: increasingly recognized components and a possible etiologic cause of Alzheimer's disease. *Antioxid Redox Signal* 2012; **16:** 1434–55.
171 Yu Q, Du F, Douglas JT, Yu H, Yan SS, Yan SF. Mitochondrial dysfunction triggers synaptic deficits via activation of p38 MAP kinase signaling in differentiated Alzheimer's disease trans-mitochondrial cybrid cells. *J Alzheimers Dis* 2017; **59:** 223–39.
172 Amorim JA, Canas PM, Tome AR *et al.* Mitochondria in excitatory and inhibitory synapses have similar susceptibility to amyloid-beta peptides modeling Alzheimer's disease. *J Alzheimers Dis* 2017; **60:** 525–36.
173 Fang D, Yan S, Yu Q, Chen D, Yan SS. Mfn2 is required for mitochondrial development and synapse formation in human induced pluripotent stem cells/hiPSC derived cortical neurons. *Sci Rep* 2016; **6:** 31462.
174 Gan X, Huang S, Wu L *et al.* Inhibition of ERK-DLP1 signaling and mitochondrial division alleviates mitochondrial dysfunction in Alzheimer's disease cybrid cell. *Biochim Biophys Acta* 2014; **1842:** 220–31.
175 Du H, Guo L, Yan S, Sosunov AA, McKhann GM, Yan SS. Early deficits in synaptic mitochondria in an Alzheimer's disease mouse model. *Proc Natl Acad Sci USA* 2010; **107:** 18670–5.
176 Tortelli R, Lozupone M, Guerra V *et al.* Midlife metabolic profile and the risk of late-life cognitive decline. *J Alzheimers Dis* 2017; **59:** 121–30.
177 Mosconi L, Murray J, Tsui WH *et al.* Brain imaging of cognitively normal individuals with 2 parents affected by late-onset AD. *Neurology* 2014; **82:** 752–60.
178 Verdile G, Fuller SJ, Martins RN. The role of type 2 diabetes in neurodegeneration. *Neurobiol Dis* 2015; **84:** 22–38.
179 Verdile G, Laws SM, Henley D *et al.* Associations between gonadotropins, testosterone and beta amyloid in men at risk of Alzheimer's disease. *Mol Psychiatry* 2014; **19:** 69–75.
180 Duarte AI, Santos MS, Oliveira CR, Moreira PI. Brain insulin signalling, glucose metabolism and females' reproductive aging: a dangerous triad in Alzheimer's disease. *Neuropharmacology* 2018; **136:** 223–42.
181 Medina M, Avila J. New perspectives on the role of tau in Alzheimer's disease. Implications for therapy. *Biochem Pharmacol* 2014; **88:** 540–7.
182 Shaikh S, Rizvi SM, Shakil S, Riyaz S, Biswas D, Jahan R. Forxiga (dapagliflozin): plausible role in the treatment of diabetes-associated neurological disorders. *Biotechnol Appl Biochem* 2016; **63:** 145–50.

183 Corbett A, Ballard C. Is a potential Alzheimer's therapy already in use for other conditions? Can medications for hypertension, diabetes and acne help with the symptoms? *Expert Opin Investig Drugs* 2013; **22:** 941–3.

184 Sandhir R, Gupta S. Molecular and biochemical trajectories from diabetes to Alzheimer's disease: a critical appraisal. *World J Diabetes* 2015; **6:** 1223–42.

185 Sebastiao I, Candeias E, Santos MS, de Oliveira CR, Moreira PI, Duarte AI. Insulin as a bridge between type 2 diabetes and alzheimer disease - how anti-diabetics could be a solution for dementia. *Front Endocrinol (Lausanne)* 2014; **5:** 110.

186 Ahmed S, Mahmood Z, Javed A *et al.* Effect of metformin on adult hippocampal neurogenesis: comparison with donepezil and links to cognition. *J Mol Neurosci* 2017; **62:** 88–98.

187 Li J, Deng J, Sheng W, Zuo Z. Metformin attenuates Alzheimer's disease-like neuropathology in obese, leptin-resistant mice. *Pharmacol Biochem Behav* 2012; **101:** 564–74.

188 Carvalho C, Correia S, Santos MS, Seiça R, Oliveira CR, Moreira PI. Metformin promotes isolated rat liver mitochondria impairment. *Mol Cell Biochem* 2007; **308:** 75–83.

189 Hsu CC, Wahlqvist ML, Lee MS, Tsai HN. Incidence of dementia is increased in type 2 diabetes and reduced by the use of sulfonylureas and metformin. *J Alzheimers Dis* 2011; **24:** 485–93.

190 Cai Z, Ratka A. Opioid system and Alzheimer's disease. *NeuroMol Med* 2012; **14:** 91–111.

191 Moore EM, Mander AG, Ames D *et al.* Increased risk of cognitive impairment in patients with diabetes is associated with metformin. *Diabetes Care* 2013; **36:** 2981–7.

192 Tahrani AA, Barnett AH, Bailey CJ. Pharmacology and therapeutic implications of current drugs for type 2 diabetes mellitus. *Nat Rev Endocrinol* 2016; **12:** 566–92.

193 Imfeld P, Bodmer M, Jick SS, Meier CR. Metformin, other antidiabetic drugs, and risk of Alzheimer's disease: a population-based case-control study. *J Am Geriatr Soc* 2012; **60:** 916–21.

194 Gavin JR 3rd, Stolar MW, Freeman JS, Spellman CW. Improving outcomes in patients with type 2 diabetes mellitus: practical solutions for clinical challenges. *J Am Osteopath Assoc* 2010; **110:** S2–14; quiz S5–6.

195 Tureyen K, Kapadia R, Bowen KK *et al.* Peroxisome proliferator-activated receptor-gamma agonists induce neuroprotection following transient focal ischemia in normotensive, normoglycemic as well as hypertensive and type-2 diabetic rodents. *J Neurochem* 2007; **101:** 41–56.

196 Peymani M, Ghoochani A, Ghaedi K *et al.* Dual effects of peroxisome proliferator-activated receptor γ on embryonic stem cell self-renewal in presence and absence of leukemia inhibitory factor. *Eur J Cell Biol* 2013; **92:** 160–8.

197 Risner ME, Saunders AM, Altman JFB *et al.* Efficacy of rosiglitazone in a genetically defined population with mild-to-moderate Alzheimer's disease. *Pharmacogenomics J* 2006; **6:** 246–54.

198 Gold M, Alderton C, Zvartau-Hind M *et al.* Rosiglitazone monotherapy in mild-to-moderate Alzheimer's disease: results from a randomized, double-blind, placebo-controlled phase III study. *Dement Geriatr Cogn Disord* 2010; **30:** 131–46.

199 Morsink LM, Smits MM, Diamant M. Advances in pharmacologic therapies for type 2 diabetes. *Curr Atheroscler Rep* 2013; **15.**

200 Craft S. Insulin and Alzheimer disease—reply. *Arch Neurol* 2012; **69:** 670.

201 Naia L, Ferreira IL, Cunha-Oliveira T *et al.* Activation of IGF-1 and insulin signaling pathways ameliorate mitochondrial function and energy metabolism in Huntington's disease human lymphoblasts. *Mol Neurobiol* 2014; **51:** 331–48.

202 Naia L, Ribeiro M, Rodrigues J *et al.* Insulin and IGF-1 regularize energy metabolites in neural cells expressing full-length mutant huntingtin. *Neuropeptides* 2016; **58:** 73–81.

203 Lopes C, Ribeiro M, Duarte AI *et al.* IGF-1 intranasal administration rescues Huntington's disease phenotypes in YAC128 mice. *Mol Neurobiol* 2013; **49:** 1126–42.

204 Duarte AI, Petit GH, Ranganathan S *et al.* IGF-1 protects against diabetic features in an in vivo model of Huntington's disease. *Exp Neurol* 2011; **231:** 314–9.

205 Pandini G, Pace V, Copani A, Squatrito S, Milardi D, Vigneri R. Insulin has multiple antiamyloidogenic effects on human neuronal cells. *Endocrinology* 2013; **154:** 375–87.

206 De Felice FG, Lourenco MV, Ferreira ST. How does brain insulin resistance develop in Alzheimer's disease? *Alzheimers Dement* 2014; **10:** S26–32.

207 De Felice FG, Vieira MN, Bomfim TR *et al.* Protection of synapses against Alzheimer's-linked toxins: insulin signaling prevents the pathogenic binding of Abeta oligomers. *Proc Natl Acad Sci USA* 2009; **106:** 1971–6.

208 Cardoso S, Santos RX, Correia SC *et al.* Insulin-induced recurrent hypoglycemia exacerbates diabetic brain mitochondrial dysfunction and oxidative imbalance. *Neurobiol Dis* 2013; **49:** 1–12.

209 Cardoso S, Carvalho C, Santos R *et al.* Impact of STZ-induced hyperglycemia and insulin-induced hypoglycemia in plasma amino acids and cortical synaptosomal neurotransmitters. *Synapse* 2010; **65:** 457–66.

210 Maimaiti S, Frazier HN, Anderson KL *et al.* Novel calcium-related targets of insulin in hippocampal neurons. *Neuroscience* 2017; **364:** 130–42.

211 Craft S, Claxton A, Baker LD *et al.* Effects of regular and long-acting insulin on cognition and Alzheimer's disease biomarkers: a pilot clinical trial. *J Alzheimers Dis* 2017; **57:** 1325–34.

212 Reger MA, Watson GS, Green PS *et al.* Intranasal insulin improves cognition and modulates -amyloid in early AD. *Neurology* 2007; **70:** 440–8.

213 Ghasemi R, Dargahi L, Haeri A, Moosavi M, Mohamed Z, Ahmadiani A. Brain insulin dysregulation: implication for neurological and neuropsychiatric disorders. *Mol Neurobiol* 2013; **47:** 1045–65.

214 Candeias EM. Gut-brain connection: the neuroprotective effects of the anti-diabetic drug liraglutide. *World J Diabetes* 2015; **6:** 807.

215 Stolar MW, Grimm M, Chen S. Comparison of extended release GLP-1 receptor agonist therapy versus sitagliptin in the management of type 2 diabetes. *Diabetes Metab Syndr Obes* 2013; **6:** 435–44.

216 Montanya E. A comparison of currently available GLP-1 receptor agonists for the treatment of type 2 diabetes. *Expert Opin Pharmacother* 2012; **13:** 1451–67.

217 Hamilton A, Patterson S, Porter D, Gault VA, Holscher C. Novel GLP-1 mimetics developed to treat type 2 diabetes promote progenitor cell proliferation in the brain. *J Neurosci Res* 2011; **89:** 481–9.

218 D'Amico M, Di Filippo C, Marfella R *et al.* Corrigendum to "LONG-TERM inhibition of dipeptidyl peptidase-4 in Alzheimer's prone mice" [Exp. Gerontol. (2010) 202–207]. *Exp Gerontol* 2013; **48:** 1002.

219 Beeri MS, Schmeidler J, Silverman JM *et al.* Insulin in combination with other diabetes medication is associated with less Alzheimer neuropathology. *Neurology* 2008; **71:** 750–7.

220 Pintana H, Apaijai N, Chattipakorn N, Chattipakorn SC. DPP-4 inhibitors improve cognition and brain mitochondrial function of insulin-resistant rats. *J Endocrinol* 2013; **218:** 1–11.

221 Darsalia V, Klein T, Nystrom T, Patrone C. Glucagon-like receptor 1 agonists and DPP-4 inhibitors: anti-diabetic drugs with anti-stroke potential. *Neuropharmacology* 2018; **136:** 280–6.

222 Kosaraju J, Murthy V, Khatwal RB *et al.* Vildagliptin: an anti-diabetes agent ameliorates cognitive deficits and pathology observed in streptozotocin-induced Alzheimer's disease. *J Pharm Pharmacol* 2013; **65:** 1773–84.

223 Bae C, Song J. The role of glucagon-like peptide 1 (GLP1) in type 3 diabetes: GLP-1 controls insulin resistance, neuroinflammation and neurogenesis in the brain. *Int J Mol Sci* 2017; **18:** 2493.

224 Li Z, Ni C-L, Yao Z, Chen L-M, Niu W-Y. Liraglutide enhances glucose transporter 4 translocation via regulation of AMP-activated protein kinase signaling pathways in mouse skeletal muscle cells. *Metabolism* 2014; **63:** 1022–30.

225 Li Y, Li L, Holscher C. Incretin-based therapy for type 2 diabetes mellitus is promising for treating neurodegenerative diseases. *Rev Neurosci* 2016; **27:** 689–711.

226 Muscogiuri G, DeFronzo RA, Gastaldelli A, Holst JJ. Glucagon-like peptide-1 and the central/peripheral nervous system: crosstalk in diabetes. *Trends Endocrinol Metab* 2017; **28:** 88–103.

227 Kickstein E, Krauss S, Thornhill P *et al.* Biguanide metformin acts on tau phosphorylation via mTOR/protein phosphatase 2A (PP2A) signaling. *Proc Natl Acad Sci USA* 2010; **107:** 21830–5.

228 Majewski C, Bakris GL. Blood pressure reduction: an added benefit of sodium-glucose cotransporter 2 inhibitors in patients with type 2 diabetes: table 1. *Diabetes Care* 2015; **38:** 429–30.

229 Solini A. Extra-glycaemic properties of empagliflozin. *Diabetes Metab Res Rev* 2015; **32:** 230–7.

230 Cai HY, Wang ZJ, Holscher C *et al.* Lixisenatide attenuates the detrimental effects of amyloid beta protein on spatial working memory and hippocampal neurons in rats. *Behav Brain Res* 2017; **318:** 28–35.

231 Ferrannini E, Muscelli E, Frascerra S *et al.* Metabolic response to sodium-glucose cotransporter 2 inhibition in type 2 diabetic patients. *J Clin Invest* 2014; **124:** 499–508.

232 Solini A. Extra-glycaemic properties of empagliflozin. *Diabetes Metab Res Rev* 2016; **32:** 230–7.

233 Vickers SP, Cheetham SC, Headland KR *et al.* Combination of the sodium-glucose cotransporter-2 inhibitor empagliflozin with orlistat or sibutramine further improves the body-weight reduction and glucose homeostasis of obese rats fed a cafeteria diet. *Diabetes Metab Syndr Obes* 2014; **7:** 265–75.

234 Chilton R, Tikkanen I, Crowe S, Johansen OE, Broedl UC, Woerle HJ, Hach T. Empagliflozin reduces systolic blood pressure in dipper and non-dipper patients with type 2 diabetes and hypertension. *Am Heart Assoc* 2014.

235 Tikkanen I, Chilton R, Johansen OE. Potential role of sodium glucose cotransporter 2 inhibitors in the treatment of hypertension. *Curr Opin Nephrol Hypertens* 2016; **25:** 81–6.

236 Ott C, Jumar A, Striepe K *et al.* A randomised study of the impact of the SGLT2 inhibitor dapagliflozin on microvascular and macrovascular circulation. *Cardiovasc Diabetol* 2017; **16:** 26.

237 Lin B, Koibuchi N, Hasegawa Y *et al.* Glycemic control with empagliflozin, a novel selective SGLT2 inhibitor, ameliorates cardiovascular injury and cognitive dysfunction in obese and type 2 diabetic mice. *Cardiovasc Diabetol* 2014; **13:** 148.

238 Millar P, Pathak N, Parthsarathy V *et al.* Metabolic and neuroprotective effects of dapagliflozin and liraglutide in diabetic mice. *J Endocrinol* 2017; **234:** 255–67.

239 Di Paolo G, Kim TW. Linking lipids to Alzheimer's disease: cholesterol and beyond. *Nat Rev Neurosci* 2011; **12:** 284–96.

240 Karasinska JM, Hayden MR. Cholesterol metabolism in Huntington disease. *Nat Rev Neurol* 2011; **7:** 561–72.

241 Jones L, Holmans PA, Hamshere ML *et al.* Genetic evidence implicates the immune system and cholesterol metabolism in the aetiology of Alzheimer's disease. *PLoS ONE* 2010; **5:** e13950.

242 Zissimopoulos JM, Barthold D, Brinton RD, Joyce G. Sex and race differences in the association between statin use and the incidence of Alzheimer disease. *JAMA Neurol* 2017; **74:** 225–32.

243 Petek B, Villa-Lopez M, Loera-Valencia R *et al.* Connecting the brain cholesterol and renin-angiotensin systems: potential role of statins and RAS-modifying medications in dementia. *J Intern Med* 2018; **284:** 620–42.

244 Bjorkhem I, Meaney S. Brain cholesterol: long secret life behind a barrier. *Arterioscler Thromb Vasc Biol* 2004; **24:** 806–15.

245 Lund EG, Guileyardo JM, Russell DW. cDNA cloning of cholesterol 24-hydroxylase, a mediator of cholesterol homeostasis in the brain. *Proc Natl Acad Sci USA* 1999; **96:** 7238–43.

246 Bjorkhem I, Diczfalusy U. 24(S),25-epoxycholesterol–a potential friend. *Arterioscler Thromb Vasc Biol* 2004; **24:** 2209–10.

247 Sodero AO, Trovo L, Iannilli F, Van Veldhoven P, Dotti CG, Martin MG. Regulation of tyrosine kinase B activity by the Cyp46/cholesterol loss pathway in mature hippocampal neurons: relevance for neuronal survival under stress and in aging. *J Neurochem* 2011; **116:** 747–55.

248 Lutjohann D, Breuer O. vol **931996; 9799–804.**

249 Burlot MA, Braudeau J, Michaelsen-Preusse K *et al.* Cholesterol 24-hydroxylase defect is implicated in memory impairments associated with Alzheimer-like Tau pathology. *Hum Mol Genet* 2015; **24:** 5965–76.

250 Djelti F, Braudeau J, Hudry E *et al.* CYP46A1 inhibition, brain cholesterol accumulation and neurodegeneration pave the way for Alzheimer's disease. *Brain* 2015; **138:** 2383–98.

251 Hudry E, Van Dam D, Kulik W *et al.* Adeno-associated virus gene therapy with cholesterol 24-hydroxylase reduces the amyloid pathology before or after the onset of amyloid plaques in mouse models of Alzheimer's disease. *Mol Ther* 2010; **18:** 44–53.

252 Lannfelt L, Moller C, Basun H *et al.* Perspectives on future Alzheimer therapies: amyloid-beta protofibrils - a new target for immunotherapy with BAN2401 in Alzheimer's disease. *Alzheimers Res Ther* 2014; **6:** 16.

253 Boussicault L, Alves S, Lamaziere A *et al.* CYP46A1, the rate-limiting enzyme for cholesterol degradation, is neuroprotective in Huntington's disease. *Brain* 2016; **139:** 953–70.

254 Piguet F, Alves S, Cartier N. Clinical gene therapy for neurodegenerative diseases: past, present, and future. *Hum Gene Ther* 2017; **28:** 988–1003.

255 Orth M, Bellosta S. Cholesterol: its regulation and role in central nervous system disorders. *Cholesterol* 2012; **2012:** 292598.

256 Cedazo-Minguez A, Ismail MA, Mateos L. Plasma cholesterol and risk for late-onset Alzheimer's disease. *Expert Rev Neurother* 2011; **11:** 495–8.

257 Kivipelto M, Ngandu T, Fratiglioni L *et al.* Obesity and vascular risk factors at midlife and the risk of dementia and Alzheimer disease. *Arch Neurol* 2005; **62:** 1556–60.

258 Kivipelto M, Helkala EL, Laakso MP *et al.* Apolipoprotein E epsilon4 allele, elevated midlife total cholesterol level, and high midlife systolic blood pressure are independent risk factors for late-life Alzheimer disease. *Ann Intern Med* 2002; **137:** 149–55.

259 Cedazo-Minguez A, Cowburn RF. Apolipoprotein E: a major piece in the Alzheimer's disease puzzle. *J Cell Mol Med* 2001; **5:** 254–66.

260 Bjorkhem I, Cedazo-Minguez A, Leoni V, Meaney S. Oxysterols and neurodegenerative diseases. *Mol Aspects Med* 2009; **30:** 171–9.

261 Bjorkhem I. Crossing the barrier: oxysterols as cholesterol transporters and metabolic modulators in the brain. *J Intern Med* 2006; **260:** 493–508.

262 Lutjohann D, Bjorkhem I, Locatelli S *et al.* Cholesterol dynamics in the foetal and neonatal brain as reflected by circulatory levels of 24S-hydroxycholesterol. *Acta Paediatr* 2001; **90:** 652–7.

263 Meaney S, Hassan M, Sakinis A *et al.* Evidence that the major oxysterols in human circulation originate from distinct pools of cholesterol: a stable isotope study. *J Lipid Res* 2001; **42:** 70–8.

264 Meaney S, Lutjohann D, Diczfalusy U, Bjorkhem I. Formation of oxysterols from different pools of cholesterol as studied by stable isotope technique: cerebral origin of most circulating 24S-hydroxycholesterol in rats, but not in mice. *Biochem Biophys Acta* 2000; **1486:** 293–8.

265 Vaya J, Schipper HM. Oxysterols, cholesterol homeostasis, and Alzheimer disease. *J Neurochem* 2007; **102:** 1727–37.

266 Lathe R, Sapronova A, Kotelevtsev Y. Atherosclerosis and Alzheimer–diseases with a common cause? Inflammation, oxysterols, vasculature. *BMC Geriatr* 2014; **14:** 36.

267 Marwarha G, Rhen T, Schommer T, Ghribi O. The oxysterol 27-hydroxycholesterol regulates alpha-synuclein and tyrosine hydroxylase expression levels in human neuroblastoma cells through modulation of liver X receptors and estrogen receptors–relevance to Parkinson's disease. *J Neurochem* 2011; **119:** 1119–36.

268 Bjorkhem I, Lovgren-Sandblom A, Leoni V *et al.* Oxysterols and Parkinson's disease: evidence that levels of 24S-hydroxycholesterol in cerebrospinal fluid correlates with the duration of the disease. *Neurosci Lett* 2013; **555:** 102–5.

269 Teunissen CE, Dijkstra C, Polman C. Biological markers in CSF and blood for axonal degeneration in multiple sclerosis. *Lancet Neurol* 2005; **4:** 32–41.

270 Baek AE, Yu YA, He S *et al.* The cholesterol metabolite 27 hydroxycholesterol facilitates breast cancer metastasis through its actions on immune cells. *Nat Commun* 2017; **8:** 864.

271 Siam A, Brancale A, Simons C. Comparative modeling of 25-hydroxycholesterol-7alpha-hydroxylase (CYP7B1): ligand binding and analysis of hereditary spastic paraplegia type 5 CYP7B1 mutations. *J Mol Model* 2012; **18:** 441–53.

272 Schols L, Rattay TW, Martus P *et al.* Hereditary spastic paraplegia type 5: natural history, biomarkers and a randomized controlled trial. *Brain* 2017; **140:** 3112–27.

273 Ismail MA, Mateos L, Maioli S *et al.* 27-Hydroxycholesterol impairs neuronal glucose uptake through an IRAP/GLUT4 system dysregulation. *J Exp Med* 2017; **214:** 699–717.

274 Mateos L, Akterin S, Gil-Bea FJ *et al.* Activity-regulated cytoskeleton-associated protein in rodent brain is down-regulated by high fat diet in vivo and by 27-hydroxycholesterol in vitro. *Brain Pathol* 2009; **19:** 69–80.

275 Heverin M, Maioli S, Pham T *et al.* 27-hydroxycholesterol mediates negative effects of dietary cholesterol on cognition in mice. *Behav Brain Res* 2015; **278:** 356–9.

276 Mateos L, Ismail M-A-M, Gil-Bea F-J *et al.* Side chain-oxidized oxysterols regulate the brain renin-angiotensin system through a liver X receptor-dependent mechanism. *J Biol Chem* 2011; **286:** 25574–85.

277 Mateos L, Ismail MA, Gil-Bea FJ *et al.* Upregulation of brain renin angiotensin system by 27-hydroxycholesterol in Alzheimer's disease. *J Alzheimers Dis* 2011; **24:** 669–79.

278 Meir K, Kitsberg D, Alkalay I *et al.* Human sterol 27-hydroxylase (CYP27) overexpressor transgenic mouse model. Evidence against 27-hydroxycholesterol as a critical regulator of cholesterol homeostasis. *J Biol Chem* 2002; **277:** 34036–41.

279 Albiston AL, McDowall SG, Matsacos D *et al.* Evidence that the angiotensin IV (AT(4)) receptor is the enzyme insulin-regulated aminopeptidase. *J Biol Chem* 2001; **276:** 48623–6.

280 Mast N, Lin JB, Pikuleva IA. Marketed drugs can inhibit cytochrome P450 27A1, a potential new target for breast cancer adjuvant therapy. *Mol Pharmacol* 2015; **88:** 428–36.

281 Marciniak E, Leboucher A, Caron E *et al.* Tau deletion promotes brain insulin resistance. *J Exp Med* 2017; **214:** 2257–69.

282 Spolcova A, Mikulaskova B, Krskova K *et al.* Deficient hippocampal insulin signaling and augmented Tau phosphorylation is related to obesity- and age-induced peripheral insulin resistance: a study in Zucker rats. *BMC Neurosci* 2014; **15:** 111.

283 Marwarha G, Dasari B, Prasanthi JR, Schommer J, Ghribi O. Leptin reduces the accumulation of Abeta and phosphorylated tau induced by 27-hydroxycholesterol in rabbit organotypic slices. *J Alzheimers Dis* 2010; **19:** 1007–19.

284 Rahman A, Akterin S, Flores-Morales A *et al.* High cholesterol diet induces tau hyperphosphorylation in apolipoprotein E deficient mice. *FEBS Lett* 2005; **579:** 6411–6.

285 LaFerla FM, Troncoso JC, Strickland DK, Kawas CH, Jay G. Neuronal cell death in Alzheimer's disease correlates with apoE uptake and intracellular Abeta stabilization. *J Clin Invest* 1997; **100:** 310–20.

286 Cummings BJ, Cotman CW. Image analysis of beta-amyloid load in Alzheimer's disease and relation to dementia severity. *Lancet* 1995; **346:** 1524–8.

287 Kalaria RN, Cohen DL, Greenberg BD *et al.* Abundance of the longer A beta 42 in neocortical and cerebrovascular amyloid beta deposits in Swedish familial Alzheimer's disease and Down's syndrome. *NeuroReport* 1996; **7:** 1377–81.

288 Mattsson N, Zetterberg H, Hansson O *et al.* CSF biomarkers and incipient Alzheimer disease in patients with mild cognitive impairment. *JAMA* 2009; **302:** 385–93.

289 Eckman CB, Mehta ND, Crook R *et al.* A new pathogenic mutation in the APP gene (I716V) increases the relative proportion of A beta 42(43). *Hum Mol Genet* 1997; **6:** 2087–9.

290 Sandebring A, Welander H, Winblad B, Graff C, Tjernberg LO. The pathogenic abeta43 is enriched in familial and sporadic Alzheimer disease. *PLoS ONE* 2013; **8:** e55847.

291 Sturchler-Pierrat C, Abramowski D, Duke M *et al.* Two amyloid precursor protein transgenic mouse models with Alzheimer disease-like pathology. *Proc Natl Acad Sci USA* 1997; **94:** 13287–92.

292 Holcomb L, Gordon MN, McGowan E *et al.* Accelerated Alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes. *Nat Med* 1998; **4:** 97–100.

293 Games D, Adams D, Alessandrini R *et al.* Alzheimer-type neuropathology in transgenic mice overexpressing V717F beta-amyloid precursor protein. *Nature* 1995; **373:** 523–7.

294 Oddo S, Caccamo A, Shepherd JD *et al.* Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Abeta and synaptic dysfunction. *Neuron* 2003; **39:** 409–21.

295 Golde TE, Dickson D, Hutton M. Filling the gaps in the abeta cascade hypothesis of Alzheimer's disease. *Curr Alzheimer Res* 2006; **3:** 421–30.

296 Saito T, Matsuba Y, Yamazaki N, Hashimoto S, Saido TC. Calpain activation in Alzheimer's model mice is an artifact of APP and presenilin overexpression. *J Neurosci* 2016; **36:** 9933–6.

297 Saito T, Matsuba Y, Mihira N *et al.* Single App knock-in mouse models of Alzheimer's disease. *Nat Neurosci* 2014; **17:** 661–3.

298 Geula C, Nagykery N, Wu CK. Amyloid-beta deposits in the cerebral cortex of the aged common marmoset (*Callithrix jacchus*): incidence and chemical composition. *Acta Neuropathol* 2002; **103:** 48–58.

299 Sasaguri H, Nilsson P, Hashimoto S *et al.* APP mouse models for Alzheimer's disease preclinical studies. *EMBO J* 2017; **36:** 2473–87.

300 Castellano JM, Kim J, Stewart FR *et al.* Human apoE isoforms differentially regulate brain amyloid-beta peptide clearance. *Sci Transl Med* 2011; **3:** 89ra57.

301 Hardy J, Bogdanovic N, Winblad B *et al.* Pathways to Alzheimer's disease. *J Intern Med* 2014; **275:** 296–303.

302 Hardy J. Alzheimer's disease: the amyloid cascade hypothesis: an update and reappraisal. *J Alzheimers Dis* 2006; **9:** 151–3.

303 Harold D, Abraham R, Hollingworth P *et al.* Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. *Nat Genet* 2009; **41:** 1088–93.

304 Jones B. Alzheimer disease: TREM2 linked to late-onset AD. *Nat Rev Neurol* 2013; **9:** 5.

305 Forsberg A, Almkvist O, Engler H, Wall A, Langstrom B, Nordberg A. High PIB retention in Alzheimer's disease is an early event with complex relationship with CSF biomarkers and functional parameters. *Curr Alzheimer Res* 2010; **7:** 56–66.

306 Klunk WE, Engler H, Nordberg A *et al.* Imaging brain amyloid in Alzheimer's disease with Pittsburgh Compound-B. *Ann Neurol* 2004; **55:** 306–19.

307 Rodriguez-Vieitez E, Saint-Aubert L, Carter SF *et al.* Diverging longitudinal changes in astrocytosis and amyloid PET in autosomal dominant Alzheimer's disease. *Brain* 2016; **139:** 922–36.

308 Aisen PS, Petersen RC, Donohue M, Weiner MW. Alzheimer's disease neuroimaging I. Alzheimer's disease neuroimaging initiative 2 clinical core: progress and plans. *Alzheimers Dement* 2015; **11:** 734–9.

309 Aisen PS, Petersen RC, Donohue MC *et al.* Clinical core of the Alzheimer's disease neuroimaging initiative: progress and plans. *Alzheimers Dement* 2010; **6:** 239–46.

310 Jack CR Jr, Bennett DA, Blennow K *et al.* NIA-AA research framework: toward a biological definition of Alzheimer's disease. *Alzheimers Dement* 2018; **14:** 535–62.

311 Forsberg A, Engler H, Almkvist O *et al.* PET imaging of amyloid deposition in patients with mild cognitive impairment. *Neurobiol Aging* 2008; **29:** 1456–65.

312 Budd Haeberlein S, O'Gorman J, Chiao P *et al.* Clinical development of aducanumab, an anti-abeta human monoclonal antibody being investigated for the treatment of early Alzheimer's disease. *J Prev Alzheimers Dis* 2017; **4:** 255–63.

313 Honig LS, Vellas B, Woodward M *et al.* Trial of solanezumab for mild dementia due to Alzheimer's disease. *N Engl J Med* 2018; **378:** 321–30.

314 Egan MF, Kost J, Tariot PN *et al.* Randomized trial of verubecestat for mild-to-moderate Alzheimer's disease. *N Engl J Med* 2018; **378:** 1691–703.

315 Gulisano W, Maugeri D, Baltrons MA *et al.* Role of amyloid-beta and tau proteins in Alzheimer's disease: confuting the amyloid cascade. *J Alzheimers Dis* 2018; **64:** S611–31.

316 Gold M. Phase II clinical trials of anti-amyloid beta antibodies: when is enough, enough? *Alzheimers Dement (N Y)* 2017; **3:** 402–9.

317 Wisniewski T. Active immunotherapy for Alzheimer's disease. *Lancet Neurol* 2012; **11:** 571–2.

318 Dodel RC, Hampel H, Du Y. Immunotherapy for Alzheimer's disease. *Lancet Neurol* 2003; **2:** 215–20.

319 Sigurdsson EM, Scholtzova H, Mehta PD, Frangione B, Wisniewski T. Immunization with a nontoxic/nonfibrillar amyloid-beta homologous peptide reduces Alzheimer's disease-associated pathology in transgenic mice. *Am J Pathol* 2001; **159:** 439–47.

320 Morgan D. Immunotherapy for Alzheimer's disease. *J Intern Med* 2011; **269:** 54–63.

321 Novak P, Schmidt R, Kontsekova E *et al.* Safety and immunogenicity of the tau vaccine AADvac1 in patients with Alzheimer's disease: a randomised, double-blind, placebo-controlled, phase 1 trial. *Lancet Neurol* 2017; **16:** 123–34.

322 Novak P, Schmidt R, Kontsekova E *et al.* FUNDAMANT: an interventional 72-week phase 1 follow-up study of AADvac1,

an active immunotherapy against tau protein pathology in Alzheimer's disease. *Alzheimers Res Ther* 2018; **10:** 108.
323 Logovinsky V, Satlin A, Lai R *et al.* Safety and tolerability of BAN2401–a clinical study in Alzheimer's disease with a protofibril selective Abeta antibody. *Alzheimers Res Ther* 2016; **8:** 14.
324 Bard F, Barbour R, Cannon C *et al.* Epitope and isotype specificities of antibodies to beta -amyloid peptide for protection against Alzheimer's disease-like neuropathology. *Proc Natl Acad Sci USA* 2003; **100:** 2023–8.
325 Lemere CA. Immunotherapy for Alzheimer's disease: hoops and hurdles. *Mol Neurodegener* 2013; **8:** 36.
326 Panza F, Frisardi V, Imbimbo BP, Seripa D, Solfrizzi V, Pilotto A. Monoclonal antibodies against beta-amyloid (Abeta) for the treatment of Alzheimer's disease: the Abeta target at a crossroads. *Expert Opin Biol Ther* 2011; **11:** 679–86.
327 Pfeifer M, Boncristiano S, Bondolfi L *et al.* Cerebral hemorrhage after passive anti-Abeta immunotherapy. *Science* 2002; **298:** 1379.
328 Crane A, Brubaker WD, Johansson JU *et al.* Peripheral complement interactions with amyloid beta peptide in Alzheimer's disease: 2. Relationship to amyloid beta immunotherapy. *Alzheimers Dement* 2018; **14:** 243–52.
329 Cehlar O, Skrabana R, Revajova V, Novak M. Structural aspects of Alzheimer's disease immunotherapy targeted against amyloid-beta peptide. *Bratisl Lek Listy* 2018; **119:** 201–4.
330 Bittar A, Sengupta U, Kayed R. Prospects for strain-specific immunotherapy in Alzheimer's disease and tauopathies. *NPJ Vaccines* 2018; **3:** 9.
331 Wilcock DM, Rojiani A, Rosenthal A *et al.* Passive immunotherapy against Abeta in aged APP-transgenic mice reverses cognitive deficits and depletes parenchymal amyloid deposits in spite of increased vascular amyloid and microhemorrhage. *J Neuroinflamm* 2004; **1:** 24.
332 Vandenberghe R, Riviere ME, Caputo A *et al.* Active Abeta immunotherapy CAD106 in Alzheimer's disease: a phase 2b study. *Alzheimers Dement (N Y)* 2017; **3:** 10–22.
333 Orgogozo JM, Gilman S, Dartigues JF *et al.* Subacute meningoencephalitis in a subset of patients with AD after Abeta42 immunization. *Neurology* 2003; **61:** 46–54.
334 Morgan D. Modulation of microglial activation state following passive immunization in amyloid depositing transgenic mice. *Neurochem Int* 2006; **49:** 190–4.
335 Lemere CA, Masliah E. Can Alzheimer disease be prevented by amyloid-beta immunotherapy? *Nat Rev Neurol* 2010; **6:** 108–19.
336 Farlow MR, Andreasen N, Riviere ME *et al.* Long-term treatment with active Abeta immunotherapy with CAD106 in mild Alzheimer's disease. *Alzheimers Res Ther* 2015; **7:** 23.
337 Winblad B, Andreasen N, Minthon L *et al.* Safety, tolerability, and antibody response of active Abeta immunotherapy with CAD106 in patients with Alzheimer's disease: randomised, double-blind, placebo-controlled, first-in-human study. *Lancet Neurol* 2012; **11:** 597–604.
338 Winblad B, Graf A, Riviere ME, Andreasen N, Ryan JM. Active immunotherapy options for Alzheimer's disease. *Alzheimers Res Ther* 2014; **6:** 7.
339 Menendez-Gonzalez M, Perez-Pinera P, Martinez-Rivera M, Muniz AL, Vega JA. Immunotherapy for Alzheimer's disease: rational basis in ongoing clinical trials. *Curr Pharm Des* 2011; **17:** 508–20.
340 Wiessner C, Wiederhold KH, Tissot AC *et al.* The second-generation active Abeta immunotherapy CAD106 reduces amyloid accumulation in APP transgenic mice while minimizing potential side effects. *J Neurosci* 2011; **31:** 9323–31.
341 Sevigny J, Chiao P, Bussiere T *et al.* The antibody aducanumab reduces Abeta plaques in Alzheimer's disease. *Nature* 2016; **537:** 50–6.
342 Sevigny J, Chiao P, Bussiere T *et al.* Addendum: The antibody aducanumab reduces Abeta plaques in Alzheimer's disease. *Nature* 2017; **546:** 564.
343 Piazza F. Reader response: a phase 3 trial of IV immunoglobulin for Alzheimer disease. *Neurology* 2018; **90:** 144–5.
344 Relkin NR, Thomas RG, Rissman RA *et al.* A phase 3 trial of IV immunoglobulin for Alzheimer disease. *Neurology* 2017; **88:** 1768–75.
345 Eriksson CA. *BioArctic Announces Positive Topline Results of BAN2401 Phase 2b at 18 months in Early Alzheimer's Disease*. BioArctic.se: BioArctic, 2018.
346 Nilsson P, Loganathan K, Sekiguchi M *et al.* Abeta secretion and plaque formation depend on autophagy. *Cell Rep* 2013; **5:** 61–9.
347 Caspersen C, Wang N, Yao J *et al.* Mitochondrial Abeta: a potential focal point for neuronal metabolic dysfunction in Alzheimer's disease. *FASEB J* 2005; **19:** 2040–1.
348 Sanchez-Pulido L, Devos D, Valencia A. BRICHOS: a conserved domain in proteins associated with dementia, respiratory distress and cancer. *Trends Biochem Sci* 2002; **27:** 329–32.
349 Hermansson E, Schultz S, Crowther D *et al.* The chaperone domain BRICHOS prevents CNS toxicity of amyloid-beta peptide in Drosophila melanogaster. *Dis Model Mech* 2014; **7:** 659–65.
350 Kim J, Chakrabarty P, Hanna A *et al.* Normal cognition in transgenic BRI2-Abeta mice. *Mol Neurodegener* 2013; **8:** 15.
351 Willander H, Askarieh G, Landreh M *et al.* High-resolution structure of a BRICHOS domain and its implications for anti-amyloid chaperone activity on lung surfactant protein C. *Proc Natl Acad Sci USA* 2012; **109:** 2325–9.
352 Walther FJ, Hernandez-Juviel JM, Waring AJ. Aerosol delivery of synthetic lung surfactant. *PeerJ* 2014; **2:** e403.
353 Leinenga G, Gotz J. Scanning ultrasound removes amyloid-beta and restores memory in an Alzheimer's disease mouse model. *Sci Transl Med* 2015; **7:** 278ra33.
354 Bray N. Biologics: transferrin' bispecific antibodies across the blood-brain barrier. *Nat Rev Drug Discov* 2015; **14:** 14–5.
355 Friden PM, Walus LR, Musso GF, Taylor MA, Malfroy B, Starzyk RM. Anti-transferrin receptor antibody and antibody-drug conjugates cross the blood-brain barrier. *Proc Natl Acad Sci USA* 1991; **88:** 4771–5.
356 Okun I, Tkachenko SE, Khvat A, Mitkin O, Kazey V, Ivachtchenko AV. From anti-allergic to anti-Alzheimer's: molecular pharmacology of dimebon. *Curr Alzheimer Res* 2010; **7:** 97–112.
357 Doody RS, Gavrilova SI, Sano M *et al.* Effect of dimebon on cognition, activities of daily living, behaviour, and global function in patients with mild-to-moderate Alzheimer's disease: a randomised, double-blind, placebo-controlled study. *Lancet* 2008; **372:** 207–15.

358 Grigorev VV, Dranyi OA, Bachurin SO. Comparative study of action mechanisms of dimebon and memantine on AMPA- and NMDA-subtypes glutamate receptors in rat cerebral neurons. *Bull Exp Biol Med* 2003; **136:** 474–7.

359 DeMattos RB, Bales KR, Cummins DJ, Dodart JC, Paul SM, Holtzman DM. Peripheral anti-A beta antibody alters CNS and plasma A beta clearance and decreases brain A beta burden in a mouse model of Alzheimer's disease. *Proc Natl Acad Sci USA* 2001; **98:** 8850–5.

360 He Z, Guo JL, McBride JD *et al.* Amyloid-beta plaques enhance Alzheimer's brain tau-seeded pathologies by facilitating neuritic plaque tau aggregation. *Nat Med* 2018; **24:** 29–38.

361 Ando K, Brion JP, Stygelbout V *et al.* Clathrin adaptor CALM/PICALM is associated with neurofibrillary tangles and is cleaved in Alzheimer's brains. *Acta Neuropathol* 2013; **125:** 861–78.

362 Chapuis J, Hansmannel F, Gistelinck M *et al.* Increased expression of BIN1 mediates Alzheimer genetic risk by modulating tau pathology. *Mol Psychiatry* 2013; **18:** 1225–34.

363 Dourlen P, Fernandez-Gomez FJ, Dupont C *et al.* Functional screening of Alzheimer risk loci identifies PTK2B as an in vivo modulator and early marker of Tau pathology. *Mol Psychiatry* 2017; **22:** 874–83.

364 Moreau K, Fleming A, Imarisio S *et al.* PICALM modulates autophagy activity and tau accumulation. *Nat Commun* 2014; **5:** 4998.

365 Goedert M, Spillantini MG, Cairns NJ, Crowther RA. Tau proteins of alzheimer paired helical filaments: abnormal phosphorylation of all six brain isoforms. *Neuron* 1992; **8:** 159–68.

366 Tolnay M, Sergeant N, Ghestem A *et al.* Argyrophilic grain disease and Alzheimer's disease are distinguished by their different distribution of tau protein isoforms. *Acta Neuropathol* 2002; **104:** 425–34.

367 Hof PR, Delacourte A, Bouras C. Distribution of cortical neurofibrillary tangles in progressive supranuclear palsy: a quantitative analysis of six cases. *Acta Neuropathol* 1992; **84:** 45–51.

368 Hof PR, Knabe R, Bovier P, Bouras C. Neuropathological observations in a case of autism presenting with self-injury behavior. *Acta Neuropathol* 1991; **82:** 321–6.

369 Schmidt ML, Zhukareva V, Newell KL, Lee VM, Trojanowski JQ. Tau isoform profile and phosphorylation state in dementia pugilistica recapitulate Alzheimer's disease. *Acta Neuropathol* 2001; **101:** 518–24.

370 Buée-Scherrer V, Buée L, Leveugle B *et al.* Pathological τ proteins in postencephalitic parkinsonism: comparison with Alzheimer's disease and other neurodegenerative disorders. *Ann Neurol* 1997; **42:** 356–9.

371 Ikeda K, Akiyama H, Kondo H *et al.* Thorn-shaped astrocytes: possibly secondarily induced tau-positive glial fibrillary tangles. *Acta Neuropathol* 1995; **90:** 620–5.

372 Caparros E. Avant-propos. *Revue générale de droit* 2002; **32:** 205.

373 Caparros-Lefebvre D, Golbe LI, Deramecourt V *et al.* A geographical cluster of progressive supranuclear palsy in northern France. *Neurology* 2015; **85:** 1293–300.

374 Buée-Scherrer V, Buée L, Hof PR *et al.* Tau variants in aging and neurodegenerative disorders. K.A. Jellinger *Alzheimer's Disease: Lessons from Cell Biology*. Berlin and Heidelberg: Springer, 1995; 132–49.

375 Buée-Scherrer V, Hof PR, Buée L *et al.* Hyperphosphorylated tau proteins differentiate corticobasal degeneration and Pick's disease. *Acta Neuropathol* 1996; **91:** 351–9.

376 Mailliot C, Sergeant N, Bussière T, Caillet-Boudin M-L, Delacourte A, Buée L. Phosphorylation of specific sets of tau isoforms reflects different neurofibrillary degeneration processes. *FEBS Lett* 1998; **433:** 201–4.

377 Delacourte A, Robitaille Y, Sergeant N *et al.* Specific pathological tau protein variants characterize pick's disease. *J Neuropathol Exp Neurol* 1996; **55:** 159–68.

378 Delacourte A, Sergeant N, Wattez A, Gauvreau D, Robitaille Y. Vulnerable neuronal subsets in Alzheimer's and Pick's disease are distinguished by their? Isoform distribution and phosphorylation. *Ann Neurol* 1998; **43:** 193–204.

379 Love S, Bridges LR, Case CP. Neurofibrillary tangles in Niemann—Pick disease type C. *Brain* 1995; **118:** 119–29.

380 Spillantini MG, Tolnay M, Love S, Goedert M. Microtubule-associated protein tau, heparan sulphate and alpha-synuclein in several neurodegenerative diseases with dementia. *Acta Neuropathol* 1999; **97:** 585–94.

381 Sergeant N, Sablonniere B, Schraen-Maschke S *et al.* Dysregulation of human brain microtubule-associated tau mRNA maturation in myotonic dystrophy type 1. *Hum Mol Genet* 2001; **10:** 2143–55.

382 Vermersch P, Sergeant N, Ruchoux MM *et al.* Specific tau variants in the brains of patients with myotonic dystrophy. *Neurology* 1996; **47:** 711–7.

383 Goedert M, Ghetti B, Spillantini MG. Frontotemporal dementia: implications for understanding Alzheimer disease. *Cold Spring Harb Perspect Med* 2012; **2:** a006254.

384 Smith PY, Delay C, Girard J *et al.* MicroRNA-132 loss is associated with tau exon 10 inclusion in progressive supranuclear palsy. *Hum Mol Genet* 2011; **20:** 4016–24.

385 Baker M, Litvan I, Houlden H *et al.* Association of an extended haplotype in the tau gene with progressive supranuclear palsy. *Hum Mol Genet* 1999; **8:** 711–5.

386 Jucker M, Walker LC. Pathogenic protein seeding in Alzheimer disease and other neurodegenerative disorders. *Ann Neurol* 2011; **70:** 532–40.

387 Kuchibhotla KV, Wegmann S, Kopeikina KJ *et al.* Neurofibrillary tangle-bearing neurons are functionally integrated in cortical circuits in vivo. *Proc Natl Acad Sci USA* 2014; **111:** 510–4.

388 Ahmed T, Van der Jeugd A, Blum D *et al.* Cognition and hippocampal synaptic plasticity in mice with a homozygous tau deletion. *Neurobiol Aging* 2014; **35:** 2474–8.

389 Santacruz K, Lewis J, Spires T *et al.* Tau suppression in a neurodegenerative mouse model improves memory function. *Science* 2005; **309:** 476–81.

390 Sydow A, Van der Jeugd A, Zheng F *et al.* Tau-induced defects in synaptic plasticity, learning, and memory are reversible in transgenic mice after switching off the toxic Tau mutant. *J Neurosci* 2011; **31:** 2511–25.

391 Pooler AM, Usardi A, Evans CJ, Philpott KL, Noble W, Hanger DP. Dynamic association of tau with neuronal membranes is regulated by phosphorylation. *Neurobiol Aging* 2012; **33:** 431 e27–38.

392 Frost B, Hemberg M, Lewis J, Feany MB. Tau promotes neurodegeneration through global chromatin relaxation. *Nat Neurosci* 2014; **17:** 357–66.

393 Reynolds CH, Garwood CJ, Wray S *et al.* Phosphorylation regulates tau interactions with Src homology 3 domains of phosphatidylinositol 3-kinase, phospholipase Cgamma1, Grb2, and Src family kinases. *J Biol Chem* 2008; **283:** 18177–86.
394 Ittner LM, Ke YD, Delerue F *et al.* Dendritic function of tau mediates amyloid-beta toxicity in Alzheimer's disease mouse models. *Cell* 2010; **142:** 387–97.
395 Sperling RA, Aisen PS, Beckett LA *et al.* Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* 2011; **7:** 280–92.
396 Marengoni A, Rizzuto D, Fratiglioni L *et al.* The effect of a 2-year intervention consisting of diet, physical exercise, cognitive training, and monitoring of vascular risk on chronic morbidity-the FINGER randomized controlled trial. *J Am Med Dir Assoc* 2018; **19:** 355–60 e1.
397 Medina M, Khachaturian ZS, Rossor M, Avila J, Cedazo-Minguez A. Toward common mechanisms for risk factors in Alzheimer's syndrome. *Alzheimers Dement (N Y)* 2017; **3:** 571–8.
398 Vanitallie TB. Preclinical sporadic Alzheimer's disease: target for personalized diagnosis and preventive intervention. *Metabolism* 2013; **62(Suppl. 1):** S30–3.
399 Silvestre FJ, Lauritano D, Carinci F, Silvestre-Rangil J, Martinez-Herrera M, Del Olmo A. Neuroinflammation, Alzheimers disease and periodontal disease: is there an association between the two processes? *J Biol Regul Homeost Agents* 2017; **31:** 189–96.
400 Rafii MS, Tuszynski MH, Thomas RG *et al.* Adeno-associated viral vector (serotype 2)-nerve growth factor for patients with Alzheimer disease: a randomized clinical trial. *JAMA Neurol* 2018; **75:** 834–41.
401 Khachaturian AS, Hayden KM, Mielke MM *et al.* Future prospects and challenges for Alzheimer's disease drug development in the era of the NIA-AA research framework. *Alzheimers Dement* 2018; **14:** 532–4.
402 Kodamullil AT, Zekri F, Sood M *et al.* Trial watch: tracing investment in drug development for Alzheimer disease. *Nat Rev Drug Discov* 2017; **16:** 819.
403 Khachaturian ZS, Mesulam MM, Khachaturian AS, Mohs RC. The special topics section of Alzheimer's & Dementia. *Alzheimers Dement* 2015; **11:** 1261–4.
404 Haapaniemi E, Botla S, Persson J, Schmierer B, Taipale J. CRISPR-Cas9 genome editing induces a p53-mediated DNA damage response. *Nat Med* 2018; **24:** 927–30.
405 Yamamoto N, Fujii Y, Kasahara R *et al.* Simvastatin and atorvastatin facilitates amyloid beta-protein degradation in extracellular spaces by increasing neprilysin secretion from astrocytes through activation of MAPK/Erk1/2 pathways. *Glia* 2016; **64:** 952–62.
406 Janson CG. Diverse effects of statins in Alzheimer's disease. *Sci Transl Med* 2016; **8:** 330ec44-ec44.
407 Salta E, De Strooper B. microRNA-132: a key noncoding RNA operating in the cellular phase of Alzheimer's disease. *FASEB J* 2017; **31:** 424–33.
408 Chaudhuri AD, Dastgheyb RM, Yoo SW *et al.* TNFalpha and IL-1beta modify the miRNA cargo of astrocyte shed extracellular vesicles to regulate neurotrophic signaling in neurons. *Cell Death Dis* 2018; **9:** 363.
409 Martin R, Bajo-Graneras R, Moratalla R, Perea G, Araque A. Circuit-specific signaling in astrocyte-neuron networks in basal ganglia pathways. *Science* 2015; **349:** 730–4.
410 Parikshak NN, Gandal MJ, Geschwind DH. Systems biology and gene networks in neurodevelopmental and neurodegenerative disorders. *Nat Rev Genet* 2015; **16:** 441–58.
411 Colonna M, Wang Y. TREM2 variants: new keys to decipher Alzheimer disease pathogenesis. *Nat Rev Neurosci* 2016; **17:** 201–7.
412 Donath MY, Dalmas E, Sauter NS, Boni-Schnetzler M. Inflammation in obesity and diabetes: islet dysfunction and therapeutic opportunity. *Cell Metab* 2013; **17:** 860–72.
413 Iaccarino HF, Singer AC, Martorell AJ *et al.* Gamma frequency entrainment attenuates amyloid load and modifies microglia. *Nature* 2016; **540:** 230–5.
414 Deeks SG, Lewin SR, Ross AL *et al.* International AIDS Society global scientific strategy: towards an HIV cure 2016. *Nat Med* 2016; **22:** 839–50.
415 Hodge JW, Ardiani A, Farsaci B, Kwilas AR, Gameiro SR. The tipping point for combination therapy: cancer vaccines with radiation, chemotherapy, or targeted small molecule inhibitors. *Semin Oncol* 2012; **39:** 323–39.
416 LoRusso PM, Canetta R, Wagner JA *et al.* Accelerating cancer therapy development: the importance of combination strategies and collaboration. Summary of an Institute of Medicine workshop. *Clin Cancer Res* 2012; **18:** 6101–9.
417 Tumbarello M, Viale P, Viscoli C *et al.* Predictors of mortality in bloodstream infections caused by Klebsiella pneumoniae carbapenemase-producing K. pneumoniae: importance of combination therapy. *Clin Infect Dis* 2012; **55:** 943–50.
418 Barnes DE, Yaffe K. The projected effect of risk factor reduction on Alzheimer's disease prevalence. *Lancet Neurol* 2011; **10:** 819–28.
419 Sink KM, Leng X, Williamson J *et al.* Angiotensin-converting enzyme inhibitors and cognitive decline in older adults with hypertension: results from the Cardiovascular Health Study. *Arch Intern Med* 2009; **169:** 1195–202.

Correspondence: B. Winblad and R. Loera-Valencia, Division of Neurogeriatrics, Centre for Alzheimer Research, Department of Neurobiology, Care Sciences and Society, Karolinska Institutet, Solna, Sweden.
(e-mails: Bengt.winblad@ki.se and raul.loera@ki.se).

