BioscienceHorizons Volume 11 2018 10.1093/biohorizons/hzy001

Review article

Immunotherapeutic and pharmacological approaches for the treatment of Alzheimer's disease

Marcela Usmari Moraes[†*] and Timothy J. Gaudet[†]

Department of Applied Sciences, University of the West of England, Coldharbour Ln, Frenchay, Stoke Gifford, Bristol BS16 1QY, UK

***Corresponding author:** Department of Applied Sciences, University of the West of England, Coldharbour Ln, Frenchay, Stoke Gifford, Bristol BS16 1QY, UK. Email: marcelausmari@live.com

Supervisor: Dr Jonathon Hull, Professor Myra Conway, Department of Applied Sciences, University of the West of England, Coldharbour Ln, Frenchay, Stoke Gifford, Bristol BS16 1QY, UK

The development of efficacious treatments targeting Alzheimer's disease (AD) aetiology has been proved an extensive, time-consuming task. Currently prescribed therapies, primarily acetylcholinesterase inhibitors such as donepezil and the NMDA-receptor antagonist memantine, concentrate on the improvement of symptoms such as cognitive decline and memory loss, however, do not address the underlying pathology of the condition. More recently, efforts have focused on developing drugs that target the hallmarks of AD, amyloid-β (Aβ) plaques and hyperphosphorylated tau tangles. Many clinical trials focusing on the removal of these proteins are presently ongoing, primarily exploring immunotherapeutic avenues such as the antibody aducanumab (targeting Aβ) and the vaccine AADvac-1 (targeting tau). However, the incomplete understanding of AD progression presents a challenge, and further studies focusing on the development of the disease are essential to the expansion of novel, efficient therapeutic routes.

Key words: amyloid, antibody, dementia, immunotherapy, tau, therapeutics

Submitted on 3 November 2017; editorial decision on 13 February 2018

Introduction

Alzheimer's disease (AD) is one of the most common neurodegenerative diseases characterized pathologically by progressive neurodegeneration, subsequent to accumulation of extracellular amyloid plaques and intracellular neurofibrillary tangles of the microtubule-associated protein tau (Perl, 2010). This manifests clinically as progressive cognitive impairment, commonly amnestic in nature, with sporadic late-onset AD (LOAD) being the predominant cause of senile dementia (Vardarajan *et al.*, 2014). Worldwide, it is estimated 1 in 10 people over the age of 65 have some form of dementia, with AD accounting for 50–70% of all cases (Qiu, Kivipelto, Von Strauss, 2009).

Understanding of AD pathology is incomplete. However, some of the most developed hypotheses reflect the dysregulation of the cholinergic system and glutamatergic system, in addition to protein aggregation (tau and amyloid) within the brain. Currently, prescribed treatments for AD are based upon the acetylcholine and glutamate dysregulation hypotheses as monotherapy with either acetylcholinesterase inhibitors (AChEIs) or memantine, respectively (National Institute for Health and Clinical Excellence, 2011). Therapies targeting tau and amyloid, most distinctively those exploring the effects of immunotherapy, are still under development but have yet to show clear clinical efficacy over that of current treatment regimens. According to a systematic review by Ehret and Chamberlin (2015), the optimal concentrations for the available acetylcholinesterase inhibitors

[†]These authors have equally contributed towards this publication.

(galantamine, rivastigmine and dopenezil) and the NMDA-receptor antagonist memantine are unknown, albeit being the only drugs that have demonstrated consistent clinical effects throughout late-phase trials. Whilst short-term trials of 3–6 months were considered acceptable and approved by the US Food and Drug Administration (FDA), they did not consider the long-term efficacy of the drug, nor whether the positive outcomes can be sustained. Although extensive meta-analyses concluded that these drugs are efficacious in reducing major symptoms such as cognitive decline and memory loss, the improvement is modest and temporary (approximately 6 months) and does not address the underlying pathology or life expectancy (Birks, 2006; Molino *et al.*, 2013). Ultimately, a major challenge for future drug development would be a balance between economic output and evaluation of drug efficacy. Furthermore, memantine/AChEI combination therapy does not appear to provide a solution to the limits of either monotherapy, nor is it likely to be a worthwhile avenue of future development (Molino *et al.*, 2013). Therefore, a shift from the currently inadequate symptomatic treatment strategies to targeting or preventing AD aetiology is highly desirable. Drugs such as AChEI and memantine are primarily symptomatic, however, targeting not only the symptoms but also the underlying mechanism through which AD develops is fundamental. A range of disease-modifying drugs are currently under development targeting amyloid and hyperphosphorylated tau. The combination of drugs which provide symptomatic relief and disease-modifying therapies that may alter the pathological steps leading to the disease, may prove to be a worthwhile route for future treatment.

The amyloid hypothesis

At the centre of the amyloid hypothesis is the neuronal surface-membrane-bound protein, the amyloid precursor protein (APP) (Fig. 1). Physiologically, APP co-localizes to neuronal growth cones with the extracellular tropic molecule netrin-1 during neural development. Here, netrin-1 binds its main receptor, DCC (deleted in colorectal cancer), and co-receptor, APP, acting to navigate commissural axon outgrowth via chemoattraction (Rama *et al.*, 2012). Netrin-1 is also expressed and active in the adult brain, including the hippocampus and dentate gyrus (Lourenço *et al.*, 2009)—areas associated with memory formation, storage and major sites of AD pathology (Reilly *et al.*, 2003).

APP undergoes either α or β processing to engender functionally polar intracellular signalling fragments (Fig. 2) (Bredesen, 2009; Dong *et al.*, 2012). Products from its α-pathway proteolysis regulate neurite outgrowth, synaptic plasticity, maintenance and spatial memory—all promoting memory retention and cell survival in cognitive neural pathways via netrin-1 interactions. Conversely, the β-pathway promotes neurite retraction, synaptic reorganization and neuronal apoptosis—promoting memory loss and reorganization (Bredesen, 2009). In particular, β-processing produces an anomalous Aβ variant with a 42-amino acid sequence, rather than normally produced Aβ 1–40, which possesses marked neurotoxicity and aggregative capacity, forming hallmark amyloid plaques in AD brains (Dong *et al.*, 2012).

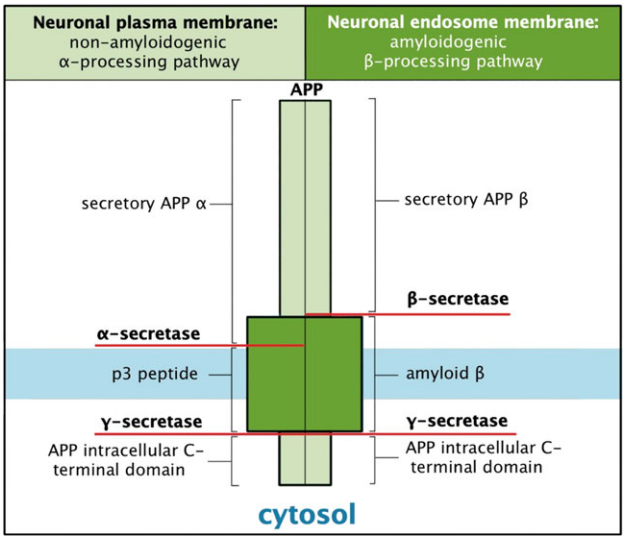

Figure 1. APP structure and differential processing. In the plasma membrane, APP processing and subsequential cleavage occur via the non-amyloidogenic α-processing pathway, whereas amyloidogenic β-processing occurs in the endosomal membrane.

Lourenço *et al.* (2009) demonstrated that production of Aβ is inhibited by APP–netrin-1 interaction, and that the netrin-1 binding-region of APP lies in its ectodomain (sAPPα region), including the 17 amino acid residues that overlap with the Aβ region. As such, Aβ also binds netrin-1, with the $A\beta_{1–42}$ variant possessing a higher binding affinity than the $A\beta_{1–17}$ fragment alone, although the authors comment that this is possibly only due to the inherently adherent properties of Aβ, and not necessarily biologically relevant.

Aβ production begins with APP proteolysis by β-site APP cleavage enzyme 1 (BACE1) into secretory APP-β and β C-terminal fragment (β-CTF), followed by further cleavage of β-CTF by γ-secretase to Aβ (Fig. 2, ❷) (Chow *et al.*, 2010). Holsinger *et al.* (2002) found that BACE1 is upregulated in AD brains 2.7-fold compared with age-matched controls, although this may not only reflect increased Aβ production, as BACE1 has multiple substrates (Chow *et al.*, 2010). The next Aβ production mediator, γ-secretase, also has multiple substrates, including Notch receptors, and is composed of several components: presenilin 1 or 2 (PS1/PS2), nicastrin, Aph-1 and Pen2 (Chow *et al.*, 2010). Genes coding for these presenilin components contain ~90% of mutations associated with early-onset familial AD (Dong *et al.*, 2012). This upregulation of BACE1 and genetic risk associated with γ-secretase in AD, coupled with their pivotal role in Aβ production, make both enzymes prime candidates for inhibitory drugs.

Based on such experimental evidence, however, Bredesen (2009) proposes a convincing model for AD that sees Aβ corrupting neural growth/death signalling by shifting the APP α:β processing ratio towards the β-pathway. Aβ (particularly $A\beta_{42}$) acts as an anti-trophin, competing with APP for netrin-1

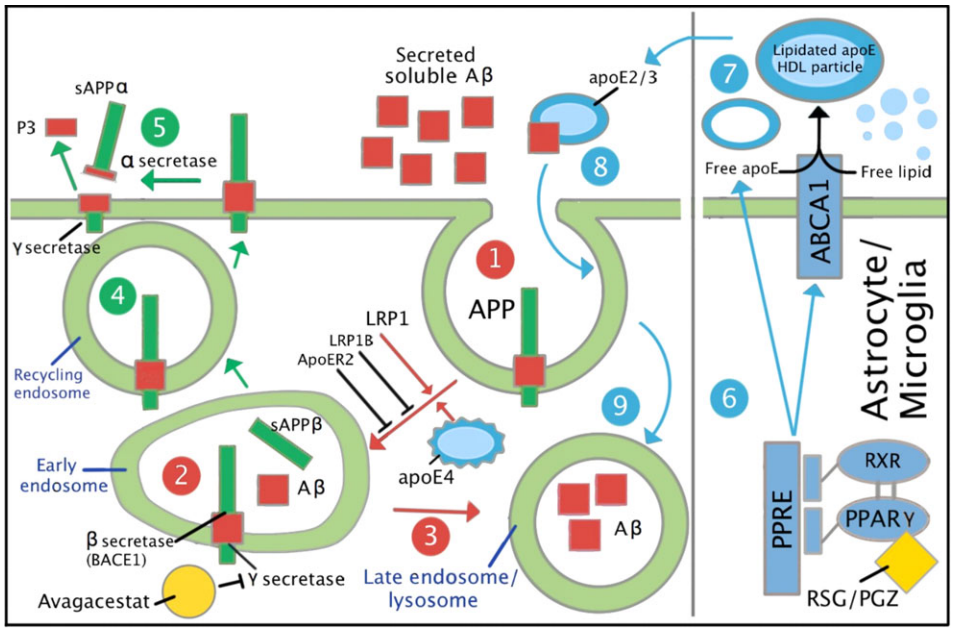

Figure 2. Aβ production via amyloidogenic and non-amyloidogenic APP processing pathways, apolipoprotein E-mediated Aβ clearance in neurons, and related drug interventions. Ab production—amyloidogenic pathway (red arrows): 1 LRP mediated endocytosis of APP into a clathirin-coated p8it. LDLR family receptors apoER2 and LRP1b act to inhibit this mechanism by retaining APP at the cell surface when bound. apoE4 promotes LRP1-mediated APP endocytosis. 2 The acidic environment of the endosome activates β-secretase, which cleaves APP, shedding secretory APPβ (aSPPβ) and leaving a membrane bound β C-terminal (β-CTF). Next, γ-secretase cleaves β-CTF, producing Aβ (38-43 amino acids long), and APP intracellular C-terminal domain (AICD—not shown). 3 Aβ is then degraded in the lysosomal/late endosome by proteolytic enzymes; Aβ production—non-amyloidogenic pathway (green arrows): 4 APP may also be restored to the plasma membrane via recycling endosomes. 5 α-secretase cleaves APP through the Aβ region, shedding secretory APPα (sAPPα). Next, γ-secretase cleaves the remaining α-CTF into non-toxic peptide P3 and AICD (not shown); Aβ clearance—apolipoprotein E-mediated degradation (blue arrows): 6 Activation of the peroxisome proliferator activated receptor γ (PPARγ) and the retinoid X receptor (RXR) cause the two to form a heterodimer, which then binds to the peroxisome proliferator response element (PPRE) DNA region, inducing transcription of APOE and abca1 genes. 7 ABCA1 lipidates apoE, forming a high-density lipoprotein (HDL) particle. 8 apoE in this particle binds secreted soluble Aβ, directing its LPR1-mediated endocytosis and autophagic degradation as in (3). Alternatively, Aβ is degraded by insulin degrading enzyme or neprilysin proteases, or removed from the brain parenchyma via de blood–brain barrier or interstitial fluid drainage pathway (not shown); Aβ-targeting drugs (yellow): Avagacestat binds and inhibits γ-secretase (2), thus preventing Aβ production. Rosiglitazone/pioglitazone (RSG/PGZ) activate PPARγ (6), inducing apoE transcription and ultimately increasing Aβ clearance. Adapted from Bu (2009) and Mandrekar-Colucci *et al.* (2012).

binding, preventing neurite outgrowth and consequently impeding cognitive maintenance, learning and memory formation. Additionally, this netrin-1–APP inhibition of Aβ production is itself inhibited. This initiates a positive feedback loop of Aβ generation, as increasing volumes of Aβ surmount the regulatory function of APP–netrin-1 in the brain parenchyma. Aβ ultimately alters neuronal proteostasis to create a microenvironment that is both amyloidogenic and opposes neuronal cell growth signalling, subsequently reflected by the AD phenotype of progressive cognitive impairment as synapses are lost and new neural circuits fail to form.

Pharmacological targeting of amyloid β

Treating Aβ as the lynch-pin of AD pathogenesis offers many opportunities for therapeutic intervention which can be divided into those targeting either production or clearance. Ohno *et al.* (2004) reported that BACE1 ablation in an AD murine model rescued memory deficits, which was attributed to the 'near abolition' of Aβ production. Current phase III drug verubecestat (MK-8931), a small molecule inhibiting BACE1 and BACE2, has delivered promising results. A study by Kennedy *et al.* (2016) demonstrated a decrease in overall CSF Aβ in mice, monkeys and AD patients, following from phase I trials showing a likewise decrease within volunteers using variable dosage from 2.5 to 550 mg/day, with minimal side effects (ALZ Forum, 2012).

Conversely, two Phase III trials of the first AD candidate γ-secretase inhibitor, semagacestat, were aborted due to unforeseen toxicity, with serious adverse effects including susceptibility to skin cancer and infections due to decreased immunity being observed in patients treated with the drug (Doody *et al.*, 2013). Retrospective analysis of this failure concluded that toxicity was caused by inhibition of Notch-receptor pathways

via γ-secretase (Ghezzi, Scarpini and Galimberti, 2013). However, γ-secretase may still be a worthwhile target, with Li *et al.* (2007) suppressing γ-secretase activity by 30% via heterozygous knockout of the γ-secretase subunit Aph-1 in AD-model mice, resulting in a 35 ± 16% reduction in Aβ burden. The authors concluded that such moderate suppression avoids adverse side effects associated with impairment of Notch-pathways. The γ-secretase inhibitor avagacestat builds on this success, inhibiting the Aβ cleavage function of γ-secretase with a 193-fold selectivity over Notch-processing (Gillman *et al.*, 2010). Furthermore, Albright *et al.* (2013) demonstrated a single 200 mg dose of avagacestat caused a 47% decrease in cerebrospinal fluid (CSF) Aβ levels after 12 h in healthy human volunteers, in contrast with a steady increase in those participants in the placebo group. Moreover, the γ-secretase and inflammation modulator CHF5074 was also shown to decrease Aβ plaque burden and learning deficit in rodent models (Imbimbo *et al.*, 2009; Porrini *et al.*, 2015).

Although recent progress has been made by pharmaceutical companies in developing BACE1 inhibitors, some drugs have not made past phase I and II clinical trials. Trials involving small molecule BACE1 inhibitors such as RG7129 from Roche and LY2886721 from Lilly were either dropped or halted before reaching phase III, respectively. Whereas the development of RG7129 was terminated by Roche with no official explanation, LY2886721, an orally administered non-peptidic BACE1 inhibitor, showed promising results in animal models by reducing Aβ and successfully succeeded in phase I trials, however the continuous development was cancelled due to abnormal liver biochemistry being reported in a small number of phase II candidates (Vassar, 2014).

More recently, efforts have been placed upon the development of efficient Aβ immunotherapy. Aducanumab, a human monoclonal antibody selectively targeting aggregates and insoluble Aβ deposits, showed promising results in mice models and its latest clinical trial including AD patients, being the only drug in AD trials to suggest disease modification at a higher dose in Phase I trials (Sevigny *et al.*, 2016). In this study, 165 patients with clinical AD diagnosis and positive Aβ PET scan were given monthly doses of 1, 3, 6 or 10 mg kg^{-1} of aducanumab over the course of a year. The results showed the antibody readily transverses the blood–brain barrier and reduces Aβ aggregates in a time- and dosage-dependant manner. Moreover, in correlation with the decrease in Aβ burden, cognitive studies performed determined a steady improvement in cognition. Although the efficacy of aducanumab requires confirmation in larger studies, it provides further evidence that the development of immunotherapies as treatment for AD are a promising target route. In contrast, drugs such as solanezumab, an anti-amyloid monoclonal antibody similarly designed to target and bind to soluble Aβ peptide, showed no significant improvements to cognition or functional abilities in two Phase III trials (Doody *et al.*, 2014). Bapineuzumab, another anti-amyloid monoclonal antibody candidate, was also unsuccessful during Phase III trials (Salloway *et al.*, 2014). However, advantages and disadvantages are inevitable when exploring Aβ active and passive immunization, including variable antibody response and the necessity for repeated antibody infusions over time. Conversely, immunotherapy remains a promising target route and several clinical trials are still ongoing, having passed phase II of clinical development (Lannfelt, Relkin, Siemers, 2014).

Microtubule-associated protein tau hypothesis

Deposition of neurofibrillary tangles (NFTs) formed from the hyperphosphorylated microtubule-associated protein (MAP) tau correlates closely with AD progression. Tau is produced with alternative mRNA splicing of a single gene (Stoothoff and Johnson, 2005), approximately 80 serine/threonine and 5 tyrosine phosphorylation sites (Wang *et al.*, 2013). This protein can be differentially post translationally modified (which includes, but is not limited to, aggregation, polyamination and nitration), with phosphorylation described as the most common under normal and pathological conditions. Although tau phosphorylation is present in the healthy brain, the concentrations are three to four times lower than those found in the AD brain, and the impact in biological function varies greatly depending on the phosphorylation site (Gong and Iqbal, 2008). Martin, Latypova, Terro (2011) reported that post-translational modifications are most likely responsible for excessive tau phosphorylation (hyperphosphorylation), which reduces its microtubule affinity and destabilizes neuronal cytoskeleton. However, tau hyperphosphorylation is a key mechanism for foetal neurodevelopment, where it is thought to contribute towards neuronal growth and plasticity by maintaining a less stable cytoskeleton (Lovestone and Reynolds, 1997). In the developed brain, aggregation of tau increases according to levels of hyperphosphorylated tau, also causing impairment in the functionality of other proteins (Götz, Ittner, Ittner, 2012). While tau hyperphosphorylation occurs prior to the development of NFTs, the mechanism behind its abnormal formation is not completely understood.

Current pharmacological targets of tau

Approaches including stimulation of tau disassembly and inhibition of tau aggregation are the most widely explored therapeutic treatments, and are based on the principle that an imbalance in phosphatases and kinases may lead to hyperphosphorylation of tau and to the development of NFTs (Mangialasche *et al.*, 2010). Phosphoseryl/phosphothreonyl protein phosphatase (PP-2A), responsible for tau dephosphorylation and inhibition of phosphorylation-inducing kinases such as mitogen-associated protein kinase (MAPK), has been shown to directly impact AD pathogenesis and is a strong candidate for new pharmacological therapy (Mangialasche *et al.*, 2010; Voronkov, Braithwaite, Stock, 2011; Götz, Ittner, Ittner, 2012). Downregulation of PP-2A and increased levels of PP-2A endogenous inhibitors such as inhibitor 2 (I2) have been shown in post mortem evaluation of AD brains, which resulted in increased Aβ deposition and tau hyperphosphorylation in *in vivo* models (Voronkov,

Braithwaite, Stock, 2011). Over-activation of glycogen synthase kinase 3 β (GSK-3β), involved in Aβ and tau processing, gene transcription and cell signalling, is hypothesized to be linked to tau hyperphosphorylation, Aβ deposition and memory impairment, all of which are observed in AD (Hooper *et al.*, 2008). Furthermore, a study by Wen *et al.* (2008) has shown GSK-3β activity increases with aging, and its inhibition may reverse hyperphosphorylation both *in vitro* and *in vivo* (Engel, 2006).

Therapeutic treatments targeting PP-2A stimulation and GSK-3β inhibition are therefore highly desirable. Aβ-candidate drug memantine has been linked to decreased tau hyperphosphorylation by reversing PP-2A inhibition (Mangialasche *et al.*, 2010), potentially reducing the necessity for combination therapy or simultaneous treatments. Lithium, one of the most studied pharmacological treatments for GSK-3β inhibition, has been correlated with decreased tauopathy and tau lesions *in vivo* (Nakashima *et al.*, 2005; Noble *et al.*, 2005; Pérez *et al.*, 2003). However, both GSK-3β and PP-2A are essential for normal development and play key roles in cellular regulation, requiring further pharmacological investigation and development of pathological-specific drugs rather than broad targeting of these substrates.

Immunotherapy focusing on pathological tau removal is also a strong candidate for drug development. AADvac-1, active immunization therapy targeting tau peptides, has shown to decrease 95% of tau hyperphosphorylation in AD rodent models. The study also indicated levels of tau oligomers and NFTs were reduced, with clinical phenotype of models improving considerably (Kontsekova *et al.*, 2014). Moreover, a double blind, phase I clinical trial conducted from June 2013 to March 2015 demonstrated positive results for AADvac-1, with 29 out of 30 patients developing the IgG immune response expected from the drug (Novak *et al.*, 2016). In conjunction, these results offer promising routes for the development of novel tau immunotherapy.

Conclusion

Clinical trials in AD remain challenging due to the struggle to recruit participants, participant compliance and technological limitations related to sensitive markers/measures of cognitive or biochemical improvements as a result of treatment. Moreover, the development of therapeutics targeting multiple pathways responsible for the pathogenesis of AD is undoubtedly complex, and is yet to produce a disease-modifying agent. Acetylcholinesterase inhibitors such as rivastigmine, dopenezil and galantamine, and the NMDA-receptor antagonist memantine are thus far the only therapeutics approved by the FDA and available for AD treatment. These treatments, however, offer no more than symptomatic relief over a short period of time, and have variable efficacy from patient to patient. Although any improvement must be considered a triumph, without efficacious treatments concentrating on the underlying pathology there is very little possibility of long-term recovery. Moreover, while passive and active immunization targeting either Aβ or hyperphosphorylated tau are still under development, most studies were halted after unsuccessful Phase III trials. There is, however, a growing expectation of those clinical trials persisting for longer periods and focusing on specific populations, therefore gathering more relevant data that could lead to the development of an effective treatment.

Author's biography

[Timothy J Gaudet] Having attained a First in BSc (Hons) Biomedical Science, Timothy has directed the data handling skills he has learned towards finance. He is now a Director of a sportswear clothing company. They developed the study, provided essential materials, analysed data, wrote the article and had primary responsibility for final content. [Marcela Usmari Moraes] Having recently obtained a degree in BSc (Hons) Biomedical Science, Marcela currently works as a Research Assistant in a biochemistry laboratory. She has worked with Professor Myra Conway from her first academic year, completing two summer placements and a year placement in brain biochemistry, which have contributed towards the preparation of two manuscripts. Marcela has plans to pursue a PhD and continue her career in academia in the following years. They provided essential materials, analysed data, wrote the paper and had primary responsibility for final content.

Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

Albright, C. F., Dockens, R. C., Meredith, J. E., Jr. et al. (2013) Pharmacodynamics of selective inhibition of gamma-secretase by avagacestat, *Journal of Pharmacology and Experimental Therapeutics*, 344 (3), 686–695. [online].

ALZ Forum. (2012) *Wave of New BACE Inhibitors Heading to Phase 2*, accessed at: http://www.alzforum.org/news/conference-coverage/wave-new-bace-inhibitors-heading-phase-2. (29 February 2016).

Birks, J. (2006) Cholinesterase inhibitors for Alzheimer's disease, *The Cochrane Database of Systematic Reviews*, 1 (5), 1–102.

Bredesen, D. E. (2009) Neurodegeneration in Alzheimer's disease: caspases and synaptic element interdependence, *Molecular Neurodegeneration*, 4, 27.

Bu, G. (2009) Apolipoprotein E and its receptors in Alzheimer's disease: pathways, pathogenesis and therapy, *Nature Reviews Neuroscience*, 10 (5), 333–344. [online].

Chow, V. W., Mattson, M. P., Wong, P. C. et al. (2010) An overview of APP processing enzymes and products, *NeuroMolecular Medicine*, 12 (1), 1–12. [online].

Dong, S., Duan, Y., Gu, F. et al. (2012) Advances in the pathogenesis of Alzheimer's disease: a re-evaluation of amyloid cascade hypothesis, *Translational Neurodegeneration*, 1 (1), 18. [online].

Doody, R. S., Raman, R., Farlow, M. et al. (2013) A phase 3 trial of semagacestat for treatment of Alzheimer's disease, *The New England Journal of Medicine*, 369 (4), 341–350.

Doody, R. S., Thomas, R. G., Farlow, M. et al. (2014) Phase 3 trials of solanezumab for mild-to-moderate Alzheimer's disease, *The New England Journal of Medicine*, 370 (4), 311–321. [online].

Ehret, M. J. and Chamberlin, K. W. (2015) Current practices in the treatment of Alzheimer disease: where is the evidence after the phase III trials? *Clinical Therapeutics*, 37 (8), 1604–1616. [online].

Engel, T. (2006) Full reversal of Alzheimer's disease-like phenotype in a mouse model with conditional overexpression of glycogen synthase kinase-3, *Journal of Neuroscience*, 26 (19), 5083–5090. [online].

Ghezzi, L., Scarpini, E. and Galimberti, D. (2013) Disease-modifying drugs in Alzheimer's disease, *Drug Design, Development and Therapy*, 7, 1471–1479.

Gillman, K. W., Starrett, J. E., Parker, M. F. et al. (2010) Discovery and evaluation of BMS-708163, a potent, selective and orally bioavailable γ-secretase inhibitor, *ACS Medicinal Chemistry Letters*, 1 (3), 120–124.

Gong, C.-X. and Iqbal, K. (2008) Hyperphosphorylation of microtubule-associated protein tau: a promising therapeutic target for Alzheimer disease, *Current Medicinal Chemistry*, 15 (23), 2321–2328. [online].

Götz, J., Ittner, A. and Ittner, L. M. (2012) Tau-targeted treatment strategies in Alzheimer's disease, *British Journal of Pharmacology*, 165 (5), 1246–1259.

Holsinger, R., McLean, C., Beyreuther, K. et al. (2002) Increased expression of the amyloid precursor β-secretase in Alzheimer's disease, *Annals of Neurology*, 51 (6), 783–786.

Hooper, C., Killick, R. and Lovestone, S. (2008) The GSK3 hypothesis of Alzheimer's disease, *Journal of Neurochemistry*, 104 (6), 1433–1439.

Imbimbo, B. P., Hutter-Paier, B., Villetti, G. et al. (2009) CHF5074, a novel γ-secretase modulator, attenuates brain??—amyloid pathology and learning deficit in a mouse model of Alzheimer's disease, *British Journal of Pharmacology*, 156 (6), 982–993.

Kennedy, M. E., Stamford, A. W., Chen, X. et al. (2016) The BACE1 inhibitor verubecestat (MK-8931) reduces CNS β-amyloid in animal models and in Alzheimer's disease patients, *Science Translational Medicine*, 8 (363), 363ra150. [online].

Kontsekova, E., Zilka, N., Kovacech, B. et al. (2014) First-in-man tau vaccine targeting structural determinants essential for pathological tau–tau interaction reduces tau oligomerisation and neurofibrillary degeneration in an Alzheimer's disease model, *Alzheimer's Research & Therapy [Online]*, 6 (4), 44.

Lannfelt, L., Relkin, N. R. and Siemers, E. R. (2014) Amyloid-ß-directed immunotherapy for Alzheimer's disease, *Journal of Internal Medicine*, 275 (3), 284–295.

Li, T., Wen, H., Brayton, C. et al. (2007) Moderate reduction of -secretase attenuates amyloid burden and limits mechanism-based liabilities, *Journal of Neuroscience*, 27 (40), 10849–10859. [online].

Lourenço, F. C., Galvan, V., Fombonne, J. et al. (2009) NIH public access, *October*, 16 (5), 655–663.

Lovestone, S. and Reynolds, C. H. (1997) The phosphorylation of tau: a critical stage in neurodevelopment and neurodegenerative process, *Neuroscience*, 78 (2), 309–324.

Mandrekar-Colucci, S., Karlo, J. C. and Landreth, G. E. (2012) Mechanisms underlying the rapid peroxisome proliferator-activated receptor – Mediated amyloid clearance and reversal of cognitive deficits in a murine model of Alzheimer's disease, *Journal of Neuroscience*, 32 (30), 10117–10128. [online].

Mangialasche, F., Solomon, A., Winblad, B. et al. (2010) Alzheimer's disease: clinical trials and drug development, *The Lancet Neurology [Online]*, 9 (7), 702–716.

Martin, L., Latypova, X. and Terro, F. (2011) Post-translational modifications of tau protein: implications for Alzheimer's disease, *Neurochemistry International*, 58, 458–471.

Molino, I., Colucci, L., Fasanaro, A. M. et al. (2013) Efficacy of memantine, donepezil, or their association in moderate-severe Alzheimer's disease: a review of clinical trials, *The Scientific World Journal*, 2013, 925702.

Nakashima, H., Ishihara, T., Suguimoto, P. et al. (2005) Chronic lithium treatment decreases tau lesions by promoting ubiquitination in a mouse model of tauopathies, *Acta Neuropathologica*, 110 (6), 547–556.

National Institute for Health and Clinical Excellence. (2011) *NICE Technology Appraisal Guidance 127—Donezepil, Galantamine, Rivastigmine and Memantine for the Treatment of Alzheimer's Disease*, National Institute for Health and Clinical Excellence, London.

Noble, W., Planel, E., Zehr, C. et al. (2005) Inhibition of glycogen synthase kinase-3 by lithium correlates with reduced tauopathy and degeneration in vivo, *Proceedings of the National Academy of Sciences of the United States of America*, 102 (19), 6990–6995. [online].

Novak, P., Schmidt, R., Kontsekova, E. et al. (2016) Safety and immunogenicity of the tau vaccine AADvac1 in patients with Alzheimer's disease: a randomised, double-blind, placebo-controlled, phase 1 trial, *The Lancet Neurology*, 4422 (16), 123–134. [online].

Ohno, M., Sametsky, E. A., Younkin, L. H. et al. (2004) BACE1 deficiency rescues memory deficits and cholinergic dysfunction in a mouse model of Alzheimer's disease, *Neuron*, 41 (1), 27–33.

Perl, D. P. (2010) Neuropathology of Alzheimer's disease, *The Mountt Sinai Journal of Medicine, New York*, 77 (1), 32–42.

Porrini, V., Lanzillotta, A., Branca, C. et al. (2015) CHF5074 (CSP-1103) induces microglia alternative activation in plaque-free Tg2576 mice and primary glial cultures exposed to beta-amyloid, *Neuroscience*, 302, 112–120.

Pérez, M., Hérnandez, F., Lim, F. et al. (2003) Chronic lithium treatment decreases mutant tau protein aggregation in a transgenic mouse model, *Journal of Alzheimer's Disease*, 5 (4), 301–308.

Qiu, C., Kivipelto, M. and Von Strauss, E. (2009) Epidemiology of Alzheimer's disease: occurrence, determinants, and strategies toward intervention, *Dialogues in Clinical Neuroscience*, 11 (2), 111–128.

Rama, N., Goldschneider, D., Corset, V. et al. (2012) Amyloid precursor protein regulates netrin-1-mediated commissural axon outgrowth, *The Journal of Biological Chemistry*, 287 (35), 30014–30023.

Reilly, J. F., Games, D., Rydel, R. E. et al. (2003) Amyloid deposition in the hippocampus and entorhinal cortex: quantitative analysis of a transgenic mouse model, *Proceedings of the National Academy of Sciences of the United States of America*, 100 (8), 4837–4842. [online].

Salloway, S., Sperling, R., Fox, N. C. et al. (2014) Two phase 3 trials of bapineuzumab in mild-to-moderate Alzheimer's disease, *The New England Journal of Medicine*, 370 (4), 322–333. [online].

Sevigny, J., Chiao, P., Bussiere, T. et al. (2016) The antibody aducanumab reduces AB plaques in Alzheimer's disease, *Nature*, 537, 50–56.

Stoothoff, W. H. and Johnson, G. V. W. (2005) Tau phosphorylation: physiological and pathological consequences, *Biochimica et Biophysica Acta—Molecular Basis of Disease*, 1739 (2), 280–297.

Vardarajan, B. N., Faber, K. M., Bird, T. D. et al. (2014) Age-specific incidence rates for dementia and Alzheimer disease in NIA-LOAD/NCRAD and EFIGA families: National Institute on Aging Genetics Initiative for Late-Onset Alzheimer Disease/National Cell Repository for Alzheimer Disease (NIA-LOAD/NCRAD) and Estu, *JAMA Neurology*, 71 (3), 315–323. [online].

Vassar, R. (2014) BACE1 inhibitor drugs in clinical trials for Alzheimer's disease, *Alzheimer's Research & Therapy*, 6 (9), 89. [online].

Voronkov, M., Braithwaite, S. P. and Stock, J. B. (2011) Phosphoprotein phosphatase 2A: a novel druggable target for Alzheimer's disease, *Future Medicinal Chemistry*, 3 (7), 821–833. [online].

Wang, J., Xia, Y., Grundke-Iqbal, I. et al. (2013) Abnormal hyperphosphorylation of tau: sites, regulation, and molecular mechanism of neurofibrillary degeneration, *Journal of Alzheimer's Disease*, 33 (1), 123–139.

Wen, Y., Planel, E., Herman, M. et al. (2008) Interplay between cyclin-dependent kinase 5 and glycogen synthase kinase 3beta mediated by neuregulin signaling leads to differential effects on tau phosphorylation and amyloid precursor protein processing, *Journal of Neuroscience*, 28 (10), 2624–2632. [online].