Frontiers in Alzheimer's disease therapeutics

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***Abstract*:** Alzheimer's disease (AD) is a progressive neurodegenerative disease which begins with insidious deterioration of higher cognition and progresses to severe dementia. Clinical symptoms typically involve impairment of memory and at least one other cognitive domain. Owing to the exponential increase in the incidence of AD with age, the aging population across the world has seen a congruous increase in AD, emphasizing the importance of disease-altering therapy. Current therapeutics on the market, including cholinesterase inhibitors and *N*-methyl-$_D$-aspartate receptor antagonists, provide symptomatic relief but do not alter progression of the disease. Therefore, progress in the areas of prevention and disease modification may be of critical interest. In this review, we summarize novel AD therapeutics that are currently being explored, and also mechanisms of action of specific drugs within the context of current knowledge of AD pathologic pathways.

***Keywords*:** Alzheimer's disease, amyloid, antioxidants, cholinesterase inhibitors, luteinizing hormone, mitochondrial therapy, neurodegenerative drugs, NMDA antagonists, tau

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Introduction

Alzheimer's disease (AD) is a neurodegenerative disease that initially presents clinically with an insidious impairment of cognitive function, and within 5–10 years results in debilitation, often encompassing severe memory loss, confusion, behavior and personality changes, speech dysfunction, an inability to live independently, and ultimately a near-vegetative state. Death from pneumonia is typical [Smith, 1998]. As the most common cause of dementia in the elderly, AD is an increasingly problematic medical, economic, and social threat to societies with growing elderly populations. Strongly correlated with age, the incidence of AD is 1% for those 60 years of age or less, and roughly doubles every 5 years thereafter [Ziegler-Graham *et al.* 2008]. Considering prevalence, 4 million people are currently afflicted with AD in the United States and the elderly population is growing precipitously [Qiu *et al.* 2007]. Current estimates for the year 2050 place the number of people in the United States suffering from AD at approximately 16 million [Brookmeyer *et al.* 2007].

Although presentation of clinical symptoms may differ significantly between individuals, accurate diagnosis is made in 80–90% of cases and is based on clinical assessment paired with continuously improving radiologic techniques. Microscopically, AD is characterized by several hallmark pathological lesions, namely neuritic plaques, neurofibrillary tangles (NFTs), neuropil threads, and diffuse plaques [Kaminsky *et al.* 2010]. NFTs are filamentous aggregates of hyperphosphorylated tau protein which encircle or displace the neuronal nucleus; the intracellular, insoluble lesions are highly resistant to cellular clearance *in vivo*. Neuritic plaques, conversely, are found extracellularly and are composed primarily of aggregated amyloid-β (Aβ) protein. Although plaques and tangles have long been seen as the characteristic pathological structures of AD, they are no longer considered the primary progenitors of the disease pathway, but instead are viewed as downstream sequelae of prior cellular insults [Zhu *et al.* 2007; Smith *et al.* 2000]. Alternatively, oxidative stress and dysfunction of mitochondrial dynamics are now considered two primary role-players in the early pathological cascade [Smith *et al.* 2010; Wang *et al.* 2009; Petersen *et al.* 2007; Zhu *et al.* 2001]. If early oxidative stress and mitochondrial dysfunction are allowed to proceed unabated, secretion and aggregation of Aβ and NFTs, as well as microglial and astrocytic activation, neuroinflammation, and cell cycle aberration soon follow [Bonda *et al.* 2010a, 2009; Mancuso

et al. 2008; Zhu *et al.* 2004]. As such, additional components of neuritic plaques include pro-inflammatory cytokines, apolipoproteins, α1-antichymotrypsin, and various constituents of the complement cascade. While the precise role of senile plaques and NFTs in the pathologic progression of AD is contentious and the subject of ongoing research [Castellani *et al.* 2009, 2008], pathological changes ultimately result in neuronal death first evident in the entorhinal cortex, then the hippocampus and isocortex, and finally the neocortex [Whitehouse, 2006; Burns *et al.* 1997].

Owing to the loss of cortical neurons and cholinergic function in AD, initial therapeutic research targeted cholinergic deficiency [Farlow and Evans, 1998]. Consequently, cholinesterase inhibitors (ChEIs) were the first pharmaceutical agents approved in the treatment of AD [Moreira *et al.* 2006]. This strategy, although valuable in mild-to-moderate cases, only provides symptomatic relief and does not alter disease progression [Giacobini, 2002, 2001, 2000]. In addition to ChEIs, the *N*-methyl-$_D$-aspartate (NMDA) receptor antagonist, memantine, is the only additional drug currently approved by the US Food and Drug Administration (FDA) for treatment in AD and is indicated in moderate-to-severe cases [Kornhuber *et al.* 1989]. Overactivation of NMDA receptors by excess synaptic glutamate is thought to lead to neuronal death through excitotoxicity. Similar to ChEIs, memantine shows some symptomatic benefit [van Dyck *et al.* 2007; McShane *et al.* 2006]. Given the paucity and ineffectiveness of symptomatic treatments, novel methods of AD treatment are increasingly being pursued and span from modifying disease-specific protein targets to additions to the diet including fish oil or pharmacologically created medical food supplements (Figure 1).

In addition to symptomatic treatments, novel drug development includes an emphasis on prevention of primary cellular insults which initiate AD, and intervention measures which modulate the neurotoxic pathways once in motion. As such, this paper will review therapeutic interventions under current investigation divided among three categories: preventative, disease-modifying, and symptomatic treatment.

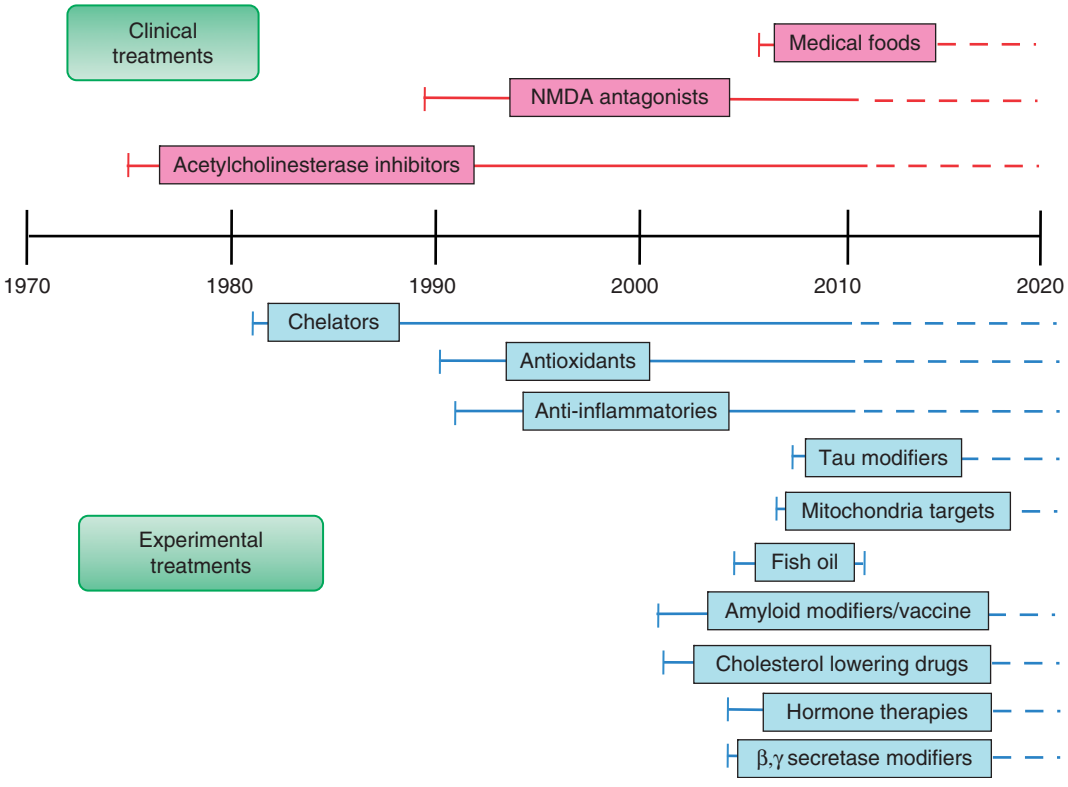

Figure 1. Timeline of the emergence of various experimental and clinical therapies for Alzheimer's disease.

Since the AD pathological pathway is multifactorial involving a host of genetic, environmental, and behavioral components, *potential* therapeutic targets are numerous [Shah *et al.* 2008], leaving some room for cautious optimism (Figure 1).

Preventative approaches

Given the lack of current pharmacological options that target the primary disease process, and the probable advanced nature of the disease when clinically symptomatic [Su *et al.* 2010; Castellani *et al.* 2001], a preventative approach appears to have some merit. Estimates in fact suggest that if therapeutic interventions delay disease onset by a even 1 year, approximately 9.2 million fewer cases of AD would be observed in 2050 than expected [Brookmeyer *et al.* 2007]. Despite logistical difficulties in research, several large clinical studies focusing on preventative strategies such as antioxidant therapy, protection of mitochondrial dynamics, use of cholesterol-lowering drugs, and anti-inflammatory treatment have noteworthy findings.

Antioxidant therapy

Recent evidence suggests that oxidative stress plays an early and active role in the AD pathological cascade [Zhu *et al.* 2007]. Reactive oxygen species (ROS), such as the highly reactive molecules superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\bullet -}$) are naturally created in the mitochondria as the cell generates adenosine triphosphate (ATP) through cellular respiration [Petersen *et al.* 2007]. Although the cell has defensive machinery to reduce ROS such as the enzymes superoxide dismutase and catalase, some ROS inevitably leak from mitochondria and damage literally every cellular component including phospholipids, proteins, and notably, mitochondrial DNA (mtDNA) [Ansari and Scheff, 2010]. As ROS damage shows an increased incidence in AD and occurs temporally early in the initiation of the disease [Zhu *et al.* 2004, 2001], antioxidant therapy may present a valuable preventative strategy [Aliev *et al.* 2008; Liu *et al.* 2007].

Readily available through dietary supplementation, naturally occurring antioxidants, such as several vitamin moieties, present an ideal preventative measure. Evidence of protection from AD by supplements such as antioxidant vitamins (E, C, and carotenoids) and B vitamins (B6, B12, and folate) is inconsistent [Middleton and Yaffe, 2009; Gillette Guyonnet *et al.* 2007; Perrig *et al.* 1997]. However, several large longitudinal studies found folate supplementation is associated with a reduced risk of AD [Corrada *et al.* 2005], combination therapy with vitamins E and C is associated with reduced incidence and prevalence of AD [Zandi *et al.* 2004], and treatment with vitamin E may slow the progression of AD [Sano *et al.* 1997]. Additional studies that control carefully for confounding factors are certainly warranted in the case of antioxidant vitamin protection.

In addition to naturally occurring antioxidant vitamins, several other drugs are under investigation for the prevention of ROS-based damage. As mitochondria are the principal producers of ROS [Lee *et al.* 2004] and mitochondrial components undergo the initial oxidative insults temporally [Zhu *et al.* 2006, 2004; Aliev *et al.* 2003; Ogawa *et al.* 2002], mitochondrial antioxidant pharmaceuticals present a promising target for therapeutic development. One such therapy in development involves treatment with Coenzyme Q_{10} (CoQ_{10}), the protein responsible for carrying high- energy electrons from complex I and complex II in the electron transport chain (ETC) through subsequent oxidation and reduction. Studies show potential neuroprotective effects of CoQ_{10} including the suppression of toxic free-radical production, reduction of ROS injury, and stabilization of mitochondrial function [Lee *et al.* 2009; Wadsworth *et al.* 2008; Beal, 2004] Despite positive study results, CoQ_{10} treatment presents important obstacles. First, proper functioning of CoQ_{10} within the cellular respiration pathway requires an intact and fully functional ETC. Oxidatively damaged mitochondria, observed early in AD, often do not contain intact ETCs as many enzymes involved with oxidative phosphorylation become irreversibly damaged; thus, CoQ_{10} administration to cells with mitochondria exhibiting early stages of dysfunction is ineffective [Lass *et al.* 1999]. In addition, oral administration of CoQ_{10} did not significantly increase the protein levels of CoQ_{10} in the brain [Kwong *et al.* 2002]. This finding suggests that the current formulation of CoQ_{10} lacks the ability to penetrate the blood–brain barrier (BBB). Owing to the drawbacks of CoQ10 therapy, other moieties, which possess greater BBB permeability and do not require an intact ETC for proper functioning, are under investigation.

One such formulation is a triphenylphosphonium-linked ubiquinone derivative known as MitoQ [Murphy, 2001]. Like CoQ10, MitoQ is shown to inhibit ROS production, protect mitochondrial oxidative phosphorylation, preserve mitochondrial structural integrity, and ultimately prevent cell death [Murphy and Smith, 2007]. In addition, MitoQ addresses the shortcomings of CoQ10; MitoQ is shown to effectively operate in the absence of an intact ETC [Lu *et al.* 2008] and concentrate several hundred-fold in mitochondria [Smith *et al.* 2004]. At this time, MitoQ remains on the horizon for AD treatment as the therapy was found to be effective in phase II clinical trials for liver damage associated with HCV infection.

Additional antioxidants under investigation for future treatment of AD include acetyl-L-carnitine (ALCAR) and R-α-lipoic acid (LA). Differing combinations of these two antioxidants are shown to reduce cellular insults by ROS, and in particular, prevent mitochondrial abnormalities in mouse models of AD [Siedlak *et al.* 2009], and prevent cognitive decline and/or restore cognitive functioning in aged rats and dogs [Long *et al.* 2009; Milgram *et al.* 2007; Ames and Liu, 2004; Liu *et al.* 2002a, 2002b, 2002c] Notably, mitochondrial structural integrity was preserved and the number of mitochondria increased in the hippocampal region by ALCAR/LA administration [Aliev *et al.* 2009]. While much investigation remains to be completed regarding the role antioxidants may or may not play in AD treatment, the above-mentioned therapies under current investigation present exciting possibilities.

Anti-inflammatory therapy

Although the precise role of inflammatory processes in AD pathogenesis remains elusive, several *in vitro* and *in vivo* animal and human studies implicate neuroinflammation in disease progression [Moore and O'Banion, 2002; Kalaria, 1999]. In particular, activated microglia, the resident guardian macrophages of the central nervous system (CNS), and reactive astrocytes, the most abundant CNS cells responsible for a multitude of critical functions including buffering of extracellular environment, preservation of the BBB, and generation of energy substrates, are involved directly in neuroinflammation. In the presence of an inflammatory stimulus, reactive astrocytes and activated microglia express and/or secrete increased concentrations of cytokines, chemokines, ROS, growth factors, major histocompatibility complex type II, and complement proteins: all pro-inflammatory mediators. In relation to AD, human postmortem studies and transgenic (Tg) animal models of AD show neuritic plaque development associated with glial activation [Apelt and Schliebs, 2001; Wegiel *et al.* 2001], suggest microglia assist in conversion of Aβ to its fibrillar form, induce diffuse plaques to aggregate into neuritic plaques [Sasaki *et al.* 1997; Cotman *et al.* 1996; Griffin *et al.* 1995; Mackenzie *et al.* 1995], and induce glutamate release contributing to excitotoxicity [Piani *et al.* 1992]. Owing to an increasing body of research suggesting a progenitive role of neuroinflammation in AD, several anti-inflammatory therapies have been studied.

Notably, several large longitudinal studies show use of anti-inflammatory drugs are associated with a lower incidence of AD [Cornelius *et al.* 2004; Lindsay *et al.* 2002; in t' Veld *et al.* 2001]. In particular, findings show that nonaspirin nonsteroidal anti-inflammatory drugs (NSAIDs) protect against the development of AD [Etminan *et al.* 2003; McGeer *et al.* 1996], increased duration of NSAID use is associated with a lower relative risk for AD [Stewart *et al.* 1997], and naproxen in particular has a positive preventative effect [Cole and Frautschy, 2010]. Importantly, although anti-inflammatory administration shows preventative potential, several studies show treatment of patients with clinically diagnosed mild-to-moderate AD has no effect [Reines *et al.* 2004; Aisen *et al.* 2003, 2000; Van Gool *et al.* 2001]. This lends further support that once early pathological pathways, including neuroinflammation, initiate, preventative strategies quickly become futile.

Disease-modifying approaches

While preventing the onset of AD entirely is an ideal aim, the multitude of factors influencing the onset and progression of AD as well as poorly understood nature of the primary pathogenic processes and outright misconceptions inherent in leading hypotheses, make prevention unlikely; therefore, therapies that blunt the secondary effects of established disease are in high demand for both current and future AD patients.

Stabilization of mitochondrial dynamics

One such critical secondary cellular insult is disruption of mitochondrial function. Mitochondria are highly dynamic organelles which constantly

fuse and divide within cells in response to energy demands [Chan, 2006]. Given that 95% of the cell's energy is provided by the citric acid (TCA) cycle and oxidative phosphorylation of mitochondria, and neurons have extremely high metabolic demands, proper functioning of mitochondria is absolutely critical to neuronal viability. As mentioned prior, the integrity of mitochondrial structure and utility are significantly compromised in AD [Wang *et al.* 2008a, 2008b; Hirai *et al.* 2001]; mitochondria, therefore, present a valuable potential target for the modulation of AD.

In addition to the prior-mentioned mitochondrial antioxidants, dimebon is believed to target mitochondrial dysfunction in AD. Specifically, evidence indicates dimebon binds to and blocks the mitochondrial permeability pore (MPP) [Bachurin *et al.* 2003]. The MPP is a multiprotein complex involving both the inner and outer mitochondrial membranes which serves to regulate the transfer of ions and proteins in and out of mitochondria with particular focus on maintaining intracellular Ca^{2+} homeostasis. Early in the pathological pathway of AD, oxidative stress and other neurotoxic insults can irreversibly open the MPP eliciting significant cellular stress [Parks *et al.* 2001]. Through closure of faulty MPPs, dimebon may serve as a potent neuroprotector. Importantly, although dimebon showed unprecedented success in an initial relatively small phase II trial in patients with mild-to-moderate AD [Doody *et al.* 2008], a recently completed double-blind large-scale phase III clinical trial indicated a discouraging similarity in cognitive outcomes between dimebon and control groups. While the fate of dimebon is yet to be determined, the strategy of attenuating mitochondrial dysfunction in AD will certainly continue to be the subject of much future inquiry.

Cessation of luteinizing hormone expression

Dyshomeostasis of hormones, luteinizing hormone (LH) in particular, appears to play a significant role in AD. Substantiating this assertion, the incidence of AD in women who experience severe hypothalamic–pituitary–gonadal hormone changes with menopause, particularly an elevation of circulating LH, is approximately two times higher than men [Jorm and Jolley, 1998]. Evaluation of patients with Down's syndrome (DS) lend further evidence supporting the role of LH in AD. Men with DS are twice as likely as women with DS to develop AD, showing the reverse trend of the normal population [Bonda *et al.* 2010b]. Demonstrating the same phenomenon as postmenopausal women, men with DS have elevated levels of circulating LH suggesting a direct link between risk for AD and LH level [Webber *et al.* 2007a; Casadesus *et al.* 2006]. In fact, elevated LH is the only hormonal similarity discovered to date which connects the predisposition to AD observed in normal women and men with DS. Many recent investigations have, therefore, been launched into the role of LH in AD and the evaluation of LH-based treatments.

In short, patients afflicted with AD show serum concentrations of LH approximately twice as high as age-matched controls [Short *et al.* 2001; Bowen *et al.* 2000]. Elevated LH is observed in the AD vulnerable hippocampus [Bowen *et al.* 2002], and LH appears to encourage Aβ deposition into senile plaques [Webber *et al.* 2007b; Bowen *et al.* 2004]. A breakthrough on the treatment front, the novel therapeutic leuprolide acetate is shown to reduce LH levels by downregulating the expression of gonadotropin releasing hormone (GnRH) receptor levels in the anterior pituitary [Marlatt *et al.* 2005]. This appears to have a significant positive effect in AD as leuprolide acetate reduces Aβ deposition in rats [Okada *et al.* 1996], and modulates cognition in amyloid-β protein precursor (AβPP) overexpressing Tg mice and humans [Casadesus *et al.* 2006] (see ClinicalTrials.gov, NCT00076440). In addition, high doses of leuprolide acetate attenuated cognitive decline (ADCS-CGIC, ADAS-Cog) and stabilized activities of daily living (ADCS-ADL) in phase II clinical trials of patients with mild-to-moderate AD (see http://www.secinfo.com/d14D5a.z6483.htm, pp. 56–64). Although much study is still required, LH-ablating therapies are quite promising as a novel treatment avenue.

Tau-focused therapies

As tau hyperphosphorylation and aggregation into pathological NFTs is a significant aspect in the development of AD, tau-focused therapeutic advancement is of much current interest. Notably, the abundance of NFTs in AD vulnerable brain regions is correlated with cognitive decline [Castellani *et al.* 2006; Nunomura *et al.* 2006], and NFT accumulation is therefore used to definitively diagnose AD postmortem [Perry *et al.* 1985]. Consequently, targeting tau to inhibit NFT build up or encourage NFT degradation may prove to be an important therapeutic strategy.

Inhibition of tau hyperphosphorylation. Evidence suggests the hyperphosphorylation of tau precedes NFT formation. Therefore, the strategy of inhibiting tau phosphorylation is under investigation as a potential AD modulating therapy. In particular, tau phosphorylation at threonine 231, and serines 235 and 262 appears to be responsible for the fibrillization and aggregation that produces NFTs. Glycogen synthase kinase 3 (GSK3) is believed to be the primary kinase which induces the hyperphosphorylation of tau [Shiurba *et al.* 1996] as it is shown to colocalize to NFTs in AD vulnerable neurons [Imahori and Uchida, 1997], and Tg mice engineered to overexpress GSK3 show increased tau hyperphosphorylation and AD symptomology [Hernandez *et al.* 2002; Lucas *et al.* 2001]. Hence, several therapeutic agents aimed at inhibiting GSK3 are in early stages of development and show promising initial results. In this regard, administration of the GSK3 inhibitor, LiCl, to tau-overexpressing Tg mice prevented the development of NFTs [Engel *et al.* 2006], reduced tau phosphorylation [Noble *et al.* 2005], and reduced the concentration of insoluble tau [Perez *et al.* 2003]. In addition, other GSK3 inhibitors, SB216763 and CHIR-98014 [Selenica *et al.* 2007], as well as a nonspecific kinase inhibitor, SRN-003-556 [Hampel *et al.* 2009], are under early investigation for efficacy in AD treatment.

In addition to preventing tau phosphorylation by direct inhibition of tau kinases, another strategy under inquiry is the inhibition of the β-N-acetylglucosamine (O-GlcNAc) cleaving enzyme O-GlcNAcase. Tau is shown to be postranslationally glycosylated with O-GlcNAc at the same threonine and serine residues that become pathologically phosphorylated. Consequently, O-GlcNAc glycosylation acts as a competitive inhibitor to tau hyperphosphorylation and subsequent aggregation into NFTs [Liu *et al.* 2004; Lefebvre *et al.* 2003], so preventing the cleavage of O-GlcNAc may therefore be a valuable strategy. In this regard, thiamet-G, a potent O-GlcNAcase inhibitor, induced a reduction of tau phosphorylation at threonine 231, and serines 396 and 404 in rats [Liu *et al.* 2004]. Although the molecules under current investigation which seek to inhibit tau hyperphosphorylation are in early stages and many obstacles still must be addressed, tau phosphorylation as a target in AD treatment remains valid nonetheless.

Inhibition of tau oligomerization and fibrillization. Following tau hyperphosphorylation, monomeric tau begins to aggregate into oligomers which proceed to further accumulate into fibrils. Therefore, in lieu of hyperphosphorylation inhibition, another potential strategy seeks to prevent formation of toxic oligomers and fibrils [Brunden *et al.* 2010, 2009]. Closest to the therapeutic market in this category, methylene blue, a histologic dye, recently completed a phase II clinical trial with positive results and will begin phase III trials soon [Neugroschl and Sano, 2009]. Several additional tau aggregation inhibitors are under preclinical research as well and show promise for the future [Crowe *et al.* 2009; Larbig *et al.* 2007; Pickhardt *et al.* 2005].

Degradation of hyperphosphorylated tau. In addition to the prevention of hyperphosphorylation and aggregation of tau, an additional strategy aimed at ameliorating presumed toxic deposition of phosphorylated tau is to increase its intracellular degradation through the ubiquitin proteosome system [Brunden *et al.* 2009]. Evidence suggests this may be accomplished through the inhibition of heat shock protein 90 (HSP90). HSP90 serves to refold denatured proteins and its inhibition is believed to attenuate the preservation of phosphorylated tau, therefore enhancing its degradation [Dickey *et al.* 2007; Zhang and Burrows, 2004]. Notably, the HSP90 inhibitor, EC102, reduced the amount of hyperphosphorylated tau in the brains of Tg mice engineered to overexpress tau [Luo *et al.* 2007] and efficiently inhibited HSP90 in human cortical AD homogenates at 1000-fold lower concentrations than control homogenates [Dickey *et al.* 2007] indicating a clinically safe dosing range is available. It is important to note that the ubiquitin proteosome degradation system involves the precise threading of a denatured peptide through the small opening of the cylindrical proteosome. Therefore, once hyperphosphorylated tau becomes fibrillized, it no longer is an available target for this system being too wide to enter the proteosome. Nevertheless, tau degradation remains an exciting and promising strategy in the modification and attenuation of destructive AD pathology.

Amyloid-focused therapy

Along with hyperphosphorylated tau tangles, Aβ plaques compose the second hallmark pathological marker of AD. Notably, increasing evidence indicates that Aβ may actually be a protective

measure used by the cell to attenuate damage by oxidative stress [Zhu *et al.* 2007, 2001], as Aβ has antioxidant properties [Hayashi *et al.* 2007; Nakamura *et al.* 2007; Kontush, 2001; Kontush *et al.* 2001; Rottkamp *et al.* 2001; Cuajungco *et al.* 2000], and the appearance of mitochondrial abnormalities and ROS precedes that of Aβ deposition [Petersen *et al.* 2007]. However, the possibility that oversecretion of Aβ (perhaps caused by oxidative stress), and eventual Aβ aggregation into senile plaques, is a toxic event that is implicit in the literature even though it is somewhat dated in recent studies focusing on soluble oligomeric species. Hence, current amyloid-focused treatment strategies in development aim to prevent the accumulation and aggregation of insoluble Aβ and/or clear Aβ plaques postformation. Still, soluble Aβ peptides may similarly be protective *in vivo* as an ameliorative response to free-radical toxicity [Nunomura *et al.* 2010; Masters *et al.* 1985].

Inhibition of Aβ accumulation and aggregation. One therapy method currently under investigation seeks to prevent the toxicity associated with Aβ by inhibiting its accumulation *a priori*. In this vein, the modulation of enzymes responsible for the cleavage of AβPP to potentially detrimental Aβ peptides is one strategy of interest. The aspartyl proteases β- and γ-secretase work in sequence to cleave AβPP to Aβ; consequently, drug treatment aims to inhibit the enzymes and thereby limit concentrations of extracellular Aβ [Lundkvist and Naslund, 2007; Marlatt *et al.* 2005]. Numerous pharmacological agents, including NCT00594568, NCT00762411, GS1-136, and MK0752 [Neugroschl and Sano, 2009], are under current study; most notably, the β-secretase inhibitor, posiphen, is in phase I clinical trials [Sabbagh, 2009], and the γ-secretase inhibitor, LY450139, is in phase III clinical trials [Lundkvist and Naslund, 2007; Barten *et al.* 2005; Wong *et al.* 2004]. Results of these trials are much anticipated and will further elucidate the future role of β- and γ-secretase inhibitors in AD treatment.

In addition to prevention of Aβ accumulation, the inhibition of Aβ aggregation is of much current interest. In this regard, nanoparticle metal chelators present an exciting future avenue to accomplish anti-aggregation of Aβ. Redox metals iron and copper are shown to be elevated in AD brain [Smith *et al.* 2010b; Honda *et al.* 2004] and appear to induce Aβ oxidation and self-assembly [Exley, 2006; Jobling *et al.* 2001; Atwood *et al.* 2000; Bush *et al.* 1994]. Consequently, metal chelators attempt to obstruct the interaction of Aβ and redox metals to prevent aggregation in AD vulnerable neurons. Metal chelators such as ethylenediaminetetracetic acid (EDTA), desferrioxamine, and iodochlorhydroxyquin (clioquinol) [Ritchie *et al.* 2003; Regland *et al.* 2001; Crapper McLachlan *et al.* 1991] can prevent the detrimental interaction between redox metals and Aβ [Liu *et al.* 2006; Opazo *et al.* 2002; Schubert and Chevion, 1995]. Unfortunately however, they cannot cross the BBB. To address this, current studies suggest utilizing nanoparticle conjugation to efficiently deliver metal chelators across the BBB [Liu *et al.* 2009]. Specifically, the Nano-N2PY conjugate has been shown to thwart Aβ-induced toxicity in neuronal cell lines without impacting cell proliferation and growth [Liu *et al.* 2009]. Nanoparticle-conjugated metal chelators remain in early-stage preparation as the pharmacological agents have yet to be administered in an animal model, but present a promising future treatment nonetheless.

Further along in drug development, the neurohormone melatonin also shows promise as an inhibitor of Aβ aggregation. In detail, long-term administration of melatonin to Aβ-overexpressing Tg mice significantly attenuated senile plaque deposition in the entorhinal cortex and hippocampus [Olcese *et al.* 2009]. This effect was most likely due to melatonin's ability to prevent fibrillization of Aβ [Poeggeler *et al.* 2001; Pappolla *et al.* 1998; Fraser *et al.* 1991], although melatonin also had the beneficial effects of reducing oxidative stress and pro-inflammatory cytokines [Olcese *et al.* 2009]. The clinical safety of melatonin administration in humans is confirmed, so all that remains is the completion of a large-scale phase III clinical trial of melatonin in AD patients which will most likely take place in the near future.

Finally, in addition to nanoparticle-conjugated metal chelators and melatonin, the cyclohexanehexol stereoisomer, scyllo-inositol (ELND005), is under current study for Aβ aggregation prevention [Sabbagh, 2009]. Specifically, the molecule showed a reduction of insoluble Aβ and reversed cognitive decline in Aβ-overexpressing Tg mice [Rogers *et al.* 1993]. With phase I trials complete, ELND005 will produce much anticipated results

from a phase II clinical trial in mid-2010 [Scharf *et al.* 1999].

Degradation of Aβ. Once Aβ begins to accumulate and aggregate, a third treatment strategy may prove to be efficacious: degradation of insoluble extracellular Aβ. Immunotherapy directed against Aβ oligomers is highly touted as the solution to Aβ deposition despite a high risk of adverse effects and questionable patient outcomes [Smith *et al.* 2002; Perry *et al.* 2000]. Active immunotherapy involves introducing an Aβ antigen into the host system to induce the production of antibodies which bind Aβ and target the insoluble peptide oligomers for degradation and clearance. The initial trial of active immunotherapy with compound AN1792, an aggregated amyloid peptide, was prematurely terminated due to the development of meningoencephalitis in a significant percentage of enrolled patients (6%). This phenomenon was attributed to aberrant T-cell activation [Marlatt *et al.* 2005]. Hence, several new Aβ antigens are in development which produce lowered T-cell activation, including ACC-001 and CAD106, both of which are in current phase II trials.

As an alternative to active immunization, passive immunotherapy involves the injection of a preformulated anti-Aβ antibody, thus necessitating much less involvement of the patients own immune system. In this category, the humanized anti-Aβ monoclonal antibody, bapineuzumab (AAB-001), is in phase III clinical trials (see ClinicalTrials.gov, NCT00667810) and unfortunately shows a paucity of positive results. Specifically, patients treated with bapineuzumab show no temporal improvements in cognition (ADAS-cog), excluding *post hoc* analyses (see ClinicalTrials.gov, NCT00676143, NCT00575055, and NCT00574132). In addition to bapineuzumab, several other passive immunotherapeutics are in various phases of clinical development including phase II immunoglobulin and PF-04360365, and phase III solanezumad (LY2062430) [Relkin *et al.* 2009] (see ClinicalTrials.gov, NCT00329082, NCT00749216, NCT00905372, NCT00904683, and NCT00722046). As noted previously, evidence suggests Aβ is actually an antioxidant elaborated by neurons as a neuroprotective agent against primary cellular insults involved in AD pathogenesis; therefore, as we have long contended, amyloid degradation strategies will likely continue to be disappointing because of a fundamentally flawed paradigm [Smith *et al.* 2002; Perry *et al.* 2000].

Symptomatic approaches

Since preventative and disease-modifying treatments are presently unproven, additional symptomatic treatments in addition to the ChEIs mentioned are worth considering as an attempt to decrease the burden on those already afflicted with AD and improve functional outcome with symptomatic AD. Such approaches are scarce, although one potential avenue is antidepressant therapy.

Approximately 35% of AD patients present with the crippling symptom of depression at some point in the disease course [Arbus *et al.* 2010]. Depression is believed to appear in AD due to an insufficiency of the neurotransmitters serotonin, norepinephrine, and dopamine resulting from cortical atrophy. Consequently, the use of selective serotonin reuptake inhibitors (SSRIs) in AD has been widely studied. Although there are mixed results with particular drugs within the class [Rosenberg *et al.* 2010], as a whole, SSRIs seem to improve symptoms of depression in AD patients and significantly enhance quality of life [Siddique *et al.* 2009]. In addition, in patients treated with ChEIs, the addition of SSRIs to the treatment regimen may actually protect against cognitive decline [Tan and Kutlu, 1991]. Although antidepressants do not appear to target the disease process of AD [Hampel *et al.* 2009], their use is still extremely valuable in symptomatic relief. Moreover, antidepressant therapy is, to date, the only therapeutic intervention that has shown significant improvement in subjects with mild cognitive impairment [Doody *et al.* 2009].

Conclusions

Although AD pharmaceuticals on the market today, including ChEIs and NMDA antagonists, fail to alter disease progression, the development of new agents utilizing novel treatment strategies is certain. Numerous preventative treatments, specifically those aiming to avert oxidative stress (i.e. antioxidants) and neuroinflammation, are in the clinical testing phases and offer some hope for upstream therapy that may blunt the neurodegenerative process. Similarly, pharmaceuticals that stabilize mitochondrial function, ablate LH production, and obstruct the detrimental effects of hyperphosphorylated tau and Aβ, may play a role either alone or, more likely, in combination

with other approaches. Since, to date, the neurodegenerative process has been unaffected by the spectrum of approaches, a broadening of that spectrum, rather than a narrowing of the focus on failed constructs, is the logical approach.

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Conflict of interest statement

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