A review of immunotherapies targeting the accumulation of amyloid-beta. A cure for Alzheimer’s disease?

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Alzheimer’s disease (AD) is the most prevalent form of dementia in the world. It is characterised by widespread neurodegeneration in the cerebral cortex and limbic system, which leads to the progressive deterioration of memory and cognitive functions. The accumulation of amyloid-beta (Aβ) and hyperphosphorylated tau protein produces the two primary neuropathological hallmarks of AD: amyloid plaques and neurofibrillary tangles (NFTs). Both genetic and experimental evidence supports the role of Aβ in instigating AD and being an upstream regulator of hyperphosphorylated tau, making it a desirable target for novel disease-modifying therapeutics. Active and passive immunisation targeting Aβ in murine models of AD have provided promising therapeutic results. However, both immunisation strategies have failed to reduce cognitive decline in mild to moderate AD patients in late clinical trials. Limited antibody exposure to the brain and therapeutic treatment implemented too late in AD progression could account for a lack of clinical efficacy. Therefore, preventative passive immunotherapy trials are currently investigating whether earlier treatment intervention in the preliminary stages of AD can slow or prevent disease progression. Furthermore, hybrid antibodies that capitalise on the use of endogenous transport systems to improve antibody concentration and penetration in the cerebral parenchyma could provide a much-needed solution to the problem of limited brain exposure. This review aims to explain the advances, failures and the potential future of Aβ immunotherapies, with particular emphasis on our current understanding of the pathological mechanisms of the amyloid pathway.

A4 – The Asymptomatic Alzheimer’s disease trial; ACh – Acetylcholine; AD – Alzheimer’s disease; API – Alzheimer’s Prevention Initiative; APOE – Apolipoprotein E; APP – Amyloid precursor protein; ARIA – Amyloid-related imaging abnormalities; Aβ – Amyloid-beta; BACE1 – β-secretase 1; BBB – Blood-brain barrier; CNS – Central nervous system; CSF – Cerebrospinal fluid; DIAN – The Dominantly Inherited Alzheimer’s Network; Fab – Fragment antigen-binding; FAD – Familial Alzheimer’s disease; FTDP – Frontotemporal dementia with Parkinsonism; IgG – Immunoglobulin; KO – Knock out; LDLR – Low-density lipoprotein receptors; mAb – Monoclonal antibodies; NFTs – Neurofibrillary tangles; PDAPP – APP transgenic mice; PET – Positron emission tomography; PS1 – Presenilin 1; PS2 – Presenilin 2; RMT – Receptor mediated transcytosis; sAPPα – Soluble amyloid precursor protein alpha; sAPPβ – Soluble amyloid precursor protein beta; scFV – Fc free single chain antibody; TIR – Transferrin receptor; WT – Wild type; 11C-PiB – Carbon 11-labelled Pittsburgh compound B

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**Introduction**

An undesirable result of the aging process for the growing population worldwide is the increasing incidence of neurodegenerative diseases. The brains of cognitively intact individuals display age-related changes including: brain atrophy in areas, such as the frontal cortex and the hippocampus, accompanied by enlargement of brain ventricles, which is partly the result of shrinkage of neuronal cell bodies and loss of neurons. However, genetic and environmental factors can accelerate this process causing pathological brain aging and dementia (Anderton, 2002). In 2015 the total estimated
cost of dementia worldwide was US$818 billion, a figure set to increase to $2 trillion by 2030 as it is projected that 74.7 million people will be living with dementia in 2030, in comparison with 46.8 million in 2015 (Price et al., 2015). There are many subtypes of dementia including: vascular dementia, Lewy body dementia, frontotemporal dementia and Parkinson’s disease. However, AD is the most prevalent form of dementia, accounting for 50-70% of cases (Winblad et al., 2016).

The neocortex and hippocampus are the brain regions most affected by the characteristic neuropathologies of AD: extraneuronal amyloid plaques, derived from the amyloid precursor protein (APP) and intraneuronal NFT, which contain hyperphosphorylated tau protein (Francis et al., 1999). These pathological hallmarks have been linked to synaptic loss in both neurons and pyramidal neurons (Francis et al., 1999; Martorana et al., 2010). The consequence of these changes is the progressive loss of episodic memory and cognitive functions, including impaired judgment and decision-making, language disturbances and a range of behavioral dysfunctions, such as anxiety, apathy, depression and delusions (Blennow et al., 2006; Martorana et al., 2010; Han et al., 2012; Yiannopoulou and Papageorgiou, 2013).

The currently available therapeutics for AD are based on neurotransmitter dysfunctions apparent in AD patients, including: the deterioration of cholinergic neurotransmission in the cerebral cortex and disturbances in hippocampal glutamate neurotransmission, through chronic mild activation (excitotoxicity) of NMDA receptors (Martorana et al., 2010; Danysz and Parsons, 2012). The treatments include the cholinesterase inhibitors rivastigmine, donepezil, and galantamine for mild to moderate AD, and the NMDA antagonist memantine for moderate to severe AD (Chiang and Koo, 2014). These therapies offer some symptomatic relief for AD patients however they fail to slow cognitive decline. Therefore, it is important that the development of new therapies target the underlying pathologies of AD, such as Aβ and tau (Yiannopoulou and Papageorgiou, 2013).

Aβ has been shown to have multiple binding partners at the neuronal synapse, including the NMDA, mGlu5 and α7-nicotinic receptors, which could in part account for the neurotransmitter dysfunction and loss of synaptic plasticity seen in AD patients (Spire-Jones and Hyman, 2014). Furthermore, genetic and experimental evidence supports the role of Aβ in initiating AD and being an upstream regulator of hyperphosphorylated tau, making it a desirable target for novel disease-modifying therapeutics (Schenk et al., 1999; Oakley et al., 2006; Huang and Mucke, 2012; Sanchez et al., 2012).

Immunotherapy is one method that has advanced recently, namely for its ability to reduce the accumulation of the Aβ plaques and slow disease progression in preclinical studies, by enhancing the body’s immune system to target this pathological hallmark. However, limited antibody penetration across the blood-brain barrier (BBB), and off-target side effects in humans, has led to the cessation of several immunotherapy clinical trials (Yu and Watts, 2013).

The focus of this review will be on the advances and failures of immunisation against AD, with particular emphasis on our current understanding of the pathological mechanisms of the amyloid pathway. The future of immunotherapy targeting AD will be discussed in detail, namely the development of hybrid antibody therapeutics that utilise endogenous transport mechanisms to enhance antibody transport across the BBB into the brain, increasing cerebral exposure, providing a potential breakthrough therapeutic for AD (Yu and Watts, 2013). However, firstly, it is important that the hypothesised roles of Aβ and tau in the development and progression of AD are addressed.

**Section 1: Alzheimer’s Disease Pathology**

Along with the degeneration and loss of neurons and synapses (Blennow et al., 2006), the accumulation of Aβ and hyperphosphorylated tau protein produces the two primary neuropathological hallmarks of AD: extraneuronal amyloid plaques and intraneuronal NFTs (Chiang and Koo, 2014).
The relationship between Aβ plaques and NFTs is still unclear and is the focus of ongoing research.

1.1 Amyloid-beta Pathology

The amyloid precursor protein (APP) is a protein that is involved in synaptic plasticity and synaptogenesis (Gralle and Ferreira, 2007). It is composed of a large extracellular domain, a single transmembrane spanning region and a small cytoplasmic tail (Gralle and Ferreira, 2007). APP can be processed through two separate proteolytic pathways (Figure 1) (Allinson et al., 2003; Spencer and Masliah, 2014). In the non-amyloidogenic pathway, proteases with α-secretase activity, such as metalloproteinase and disintegrin (Kojro and Fahrenholz, 2005), cleave APP within the Aβ sequence (Vassar et al., 1999; Allinson et al., 2003). This first proteolytic event leaves behind an 83-residue membrane-bound carboxyl terminal, which undergoes cleavage by the complex intramembrane γ-secretase to produce the peptide p3 (Vassar et al., 1999; Chow et al., 2010). Although the role of p3 is not fully understood, sAPPα is believed to have neurotrophic (Gralle and Ferreira, 2007) and neuroprotective (Allinson et al., 2003) properties. In contrast, in the amyloidogenic pathway, the systematic processing of APP by the proteolytic enzymes β-secretase and γ-secretase creates Aβ fragments (Patel and Jhamandas, 2012). The enzyme β-secretase (BACE1) cleaves APP at the amino terminus producing soluble amyloid precursor protein beta (sAPPβ) and a 99-residue carboxyl terminal that remains located in the membrane (Vassar et al., 1999; Patel and Jhamandas, 2012). The carboxyl terminal fragment of APP is again cleaved by γ-secretase, which forms Aβ fragments of lengths varying between 38 and 43 residues (Patel and Jhamandas, 2012).

Aβ40 is the predominant isoform of the amyloidogenic pathway, however, Aβ42 is considered to be more toxic (Kuperstein et al., 2010); as Aβ42 aggregates into amyloid oligomers and insoluble deposits more rapidly than Aβ40 (Iwatsubo et al., 1994). Strong genetic links between Aβ42 and AD have been shown to exist (Oakley et al., 2006). Missense mutations in genes coding for APP, presenilin 1 (PS1) and presenilin 2 (PS2) (multi-pass transmembrane proteins that are part of the γ-secretase complex) increase Aβ42 production, which causes dominantly inherited familial Alzheimer’s disease (FAD) (Oakley et al., 2006; Huang and Mucke, 2012). Moreover, the link between APP and AD has been cemented further on the basis that adult Down syndrome patients display Aβ pathology and commonly develop early onset AD as a result of having an extra copy of chromosome 21, where the APP gene is localised (Sanchez et al., 2012).

The most influential genetic risk factor in late-onset AD is the apolipoprotein E (APOE) gene (Genin et al., 2011; Cruchaga et al., 2012), which encodes a cholesterol carrier
protein that supports lipid transport in the brain by binding to low-density lipoprotein receptors (LDLR) (Laws et al., 2003; Liu et al., 2013). It has been suggested that APOE facilitates the uptake and redistribution of cholesterol within the central nervous system (CNS), as well as being involved in specialised forms of neuronal homeostasis including the mobilisation of cholesterol required for neural plasticity (Laws et al., 2003). APOE is polymorphic and exists as three main isoforms denoted as APOE2, APOE3 and APOE4 (Laws et al., 2003). APOE2 is thought to have a protective mechanism against late-onset AD reducing the risk of developing the disease (Kim et al., 2014). Yet individuals who carry two copies of the APOE4 allele (~2% of the Caucasian population) are 30% more likely to develop AD by age 75 and more than 50% by age 85 (Genin et al., 2011). Several hypotheses for how APOE influences AD onset and progression have been proposed, but predominantly evidence has focused on the differential effects of APOE isoforms on the aggregations and clearance Aβ (Kim et al., 2009; Cruchaga et al., 2012), as APOE is known to be an Aβ binding protein (Deane et al., 2008). APOE4 carriers have been shown to have increased Aβ plaque density in cerebral locations affected in AD in comparison to APOE4 non-carriers; detected by Carbon 11-labelled Pittsburgh compound B (11C-PiB) (Reiman et al., 2009). This is a radioactive marker that is used in Positron emission tomography (PET) to image fibrillar Aβ in cortical neurons (Rinne et al., 2010). Furthermore, evidence suggests APOE modifies the transport and metabolism of Aβ in the brain (Kim et al., 2009). Preclinically APOE4 has been shown to shift the efflux of unbound Aβ40 and Aβ42 completely from fast brain capillary transcytosis via LDLR-related protein 1 to the very low-density lipoprotein receptor, a receptor that has a considerably slower endocytotic rate (Deane et al., 2008). However, APOE2 and APOE3 only moderately inhibited Aβ clearance due to their ability to interact partially with LDLR-related protein 1 (Deane et al., 2008). Despite the aforementioned evidence, more data is needed to better understand the exact role APOE isoforms on Aβ aggregation and Aβ clearance across the BBB (Kim et al., 2009).

Recently, in vitro (Wei et al., 2010), post-mortem (Näslund et al., 2000) and transgenic mice (Jacobsen et al., 2006) studies have provided evidence supporting the hypothesis that neuronal dysfunction occurs before the accumulation of insoluble Aβ plaques and NFTs (Jacobsen et al., 2006). Transgenic mice that express the APP mutation are shown to have increased levels of soluble Aβ40 and Aβ42 compared to wild type (WT) mice, which is detectable in its early stages of life (Jacobsen et al., 2006). These murine models display behavioural deficits, impairments in long-term potentiation and decreased dendritic spine density months before Aβ plaques are detected (Jacobsen et al., 2006). Näslund et al. (2000) also observed increased soluble Aβ40 and Aβ42 in patients who were in the early stages of AD. The increased levels of these soluble Aβ fragments correlated strongly with cognitive decline and also preceded tau pathology (Näslund et al., 2000). These results, along with the overwhelming genetic evidence described above, supports the idea that Aβ initiates the pathogenic events in AD, which makes it a viable drug target.

1.2 Tau Pathology

Tau protein is involved in promoting the assembly and stability of microtubules through tubulin-binding motifs (Köpke et al., 1993; Mazanetz and Fischer, 2007). This is important, as microtubules are a component essential for axonal transport, the intracellular process that transports organelles along the axon that is necessary for the maintenance and optimal function of a neuron (Köpke et al., 1993; Millecamps and Julien, 2013). Neighboring the tubulin-binding motifs on tau are a number of serine and threonine residues that can be phosphorylated by a range of kinases including GSK3β, CDK5, and ERK2 (Mazanetz and Fischer, 2007). Normally tau contains 2-3 mol of phosphate/mol of tau, whereas in AD preparations this is increased to between 5-15 mol of phosphate/mol of tau (Köpke et al., 1993). This pathological hyperphosphorylation of tau weakens its microtubule assembly activity by preventing tubulin from binding to the tubulin-binding
motifs and causes the formation of paired helical filaments and straight filaments, which accumulate into NFT in the neuronal cell body and dendritic spines of a neuron (Köpke et al., 1993; Mazanetz and Fischer, 2007).

During the development of AD, NFTs are first identified in the transentorhinal cortex and hippocampus, particularly in the CA1 region (Braak and Braak, 1991; Nath et al., 2012). As the disease progresses NFTs broadly extend into the neocortex spreading to the sensory and motor areas of the brain in the end-stages of AD (Braak and Braak, 1991; Braak et al., 2006). The buildup of NFTs causes neuronal degeneration, leaving behind ghost tangles, which are NFTs in the extracellular space (Köpke et al., 1993). Loss of neurons in these areas causes functional deficits that contribute to the clinical manifestations of AD (Braak and Braak, 1991; Braak et al., 2006). This strict spatiotemporal pattern of lesion formation is not observed with Aβ plaques (Braak and Braak, 1991).

It is hypothesised that tau is more strongly correlated with AD pathogenesis than Aβ pathology, due to the following reasons: Increasing levels of the cerebrospinal fluid (CSF) biomarker, tau protein, have been associated with a worsening degree of neuronal damage and rapid cognitive decline in AD patients (Kester et al., 2009). Furthermore, many studies have identified high levels of Aβ plaques in the cortex of cognitively intact patients who are of a comparable age to AD patients (Braak and Braak, 1991; Savva et al., 2009). There is a strong association between cognitive decline and NFT levels in the hippocampus and neocortical areas at all ages (Braak and Braak, 1991; Savva et al., 2009). Moreover, transgenic Aβ mice models have also failed to recapitulate progressive NFT formation and neuronal loss, suggesting the amyloid and tau pathogenic pathways are independent of one another (Lewis et al., 2001).

However, if abnormal tau proteins are the sole driving force behind AD pathology, and Aβ plaques are absent, AD patients would display clinical symptoms similar to inherited frontotemporal dementia, with Parkinsonism (FTDP) linked to chromosome 17 (Hardy and Selkoe, 2002). Indeed, this notion is supported by the JNPL3 transgenic mouse, which develops NFTs and progressive motor dysfunctions comparable to FTDP, as a result of having a mutant (P301L) tau protein (Lewis et al., 2001).

Section 2: Amyloid Based Therapeutics

Numerous approaches that reduce production, block aggregation, and promote clearance of Aβ have been investigated (Yu and Watts, 2013). The subsequent section will touch on therapies that interfere with secretase processing, followed by a more detailed evaluation of active and passive Aβ immunotherapy.

2.1 Secretase Inhibitors

The production on Aβ requires the sequential cleavage of APP by BACE1 at the amino terminal of Aβ followed by γ-secretase at the carboxyl terminal (Vassar et al., 1999; Patel and Jhamandas, 2012). Therefore, interfering with secretase processing to decrease the production of Aβ has been a prime therapeutic target for AD.

γ-secretase Inhibitors

Promising in vivo results showed that inhibiting γ-secretase with semagacestat reduced Aβ levels by 25% in the brains and CSF of APP transgenic mice over a period of 24 hours (Doody et al., 2013). However, in a phase III multinational placebo-controlled, double-blind clinical trial, the γ-secretase inhibitor semagacestat caused accelerated cognitive and functional decline along with other severe side effects including: adverse gastrointestinal symptoms, skin irritations, altered immune function, and an increased incidence of non-melanoma skin cancer (Doody et al., 2013). Similar side effects were also recorded in a phase II clinical trial that assessed the safety and tolerability of another γ-secretase inhibitor named avagacestat (Coric et al., 2012). The adverse events observed in the semagacestat and avagacestat clinical trials can be explained by the fact that γ-secretase does not exclusively cleave APP; it acts on other transmembrane
substances including the Notch family of receptors (Coric et al., 2012; Wolfe, 2012; Doody et al., 2013). Notch signaling is essential in the neural and non-neural cell differentiation and cell-fate processes during development and in the adult (Selkoe and Kopan, 2003). Ligand-activated proteolysis cleavage of Notch receptors releases the Notch intracellular domain, which enters the nucleus and associates with transcription factors that control gene expression involved in cell fate (Selkoe and Kopan, 2003; Wolfe, 2012). The enzyme γ-secretase is essential in the cleavage of the Notch intracellular domain thus, disrupting this process with γ-secretase inhibitors leads to functional consequences for many tissues and cell types within each tissue (Selkoe and Kopan, 2003; Wolfe, 2012). The problematic off-target effects of γ-secretase and worsening of cognition have effectively negated γ-secretase inhibitors as a disease transforming therapeutic for AD (Doody et al., 2013). Consequently, recent efforts have been focused on identifying a γ-secretase modulator that has minimal or no effects on normal Notch processing (Wolfe, 2012).

**β-secretase Inhibitors**

Since the β-secretase, BACE1, was discovered over a decade ago (Vassar et al., 1999), the search for a therapeutic agent that targets this enzyme that initiates the generation of Aβ has been extensive. However, the pursuit of a BACE1 inhibitor has been challenging for a number of reasons. BACE1 has a large hydrophobic catalytic pocket, which has been difficult to target with small nonpeptidic compounds. Furthermore, these compounds need to be sufficiently hydrophobic to translocate across plasma and intracellular membranes to reach β-secretase. Lastly, developing an efficacious BACE1 inhibitor that reaches high enough concentrations in the brain has been an ongoing obstacle not only for BACE1 inhibitors, but also other drugs targeting AD, due to limited BBB penetration (Vassar and Kandalepas, 2011).

As with γ-secretase, β-secretase also has other substrates. This suggests that BACE1 has a myriad of physiological roles, which can be exhibited in the phenotypes of knockout (KO) murine models (BACE1-/-). Neuregulin-1, a factor expressed in neuronal axons, is necessary for glial myelination and development; in BACE1-/- mice the lack of β-secretase results in build-up of unprocessed neuregulin-1 (Willem et al., 2006). This causes hypomyelination of the central, peripheral (Hu et al., 2006; Willem et al., 2006), and optic neurons (Hu et al., 2006), as well as affecting sciatic neuronal cell remyelination (Hu et al., 2008). Hypomyelination of the central and peripheral neurons undesirably modifies neurological behaviours, causing hyperalgesia and reduced grip strength (Hu et al., 2006). Electroencephalographic recordings revealed approximately 30% of BACE1-/- mice (Hitt et al., 2010) had epileptic abnormalities, including generalised tonic-clonic and absence seizures, as well as more frequent pharmacologically induced seizures (Hitt et al., 2010; Hu et al., 2010). However, it is currently unknown whether these behavioural changes exhibited are related to embryonic and postnatal development (Vassar and Kandalepas, 2011).

Despite these challenges, BACE1-/- murine models did eradicate Aβ accumulation (Kobayashi et al., 2008); thus two novel BACE1 inhibitors, MK-8931 and E2609, are currently in clinical development. MK-8931 was well tolerated in a phase I trial, with mild to moderate side effects and reduced CSF Aβ by up to 94% (Forman et al., 2013). MK-8931 is now in two phase II/III clinical trials to assess efficacy and safety compared with placebo treatment (Merck, 2016; Merck and Dohme, 2016). E2609 inhibited the production of Aβ40 and Aβ42 in the brain and CSF preclinical models and was well tolerated in a phase I clinical trial, which had a prolonged reduction in plasma Aβ after a single dose (Lai et al., 2012).

An alternative to small-molecule BACE1 inhibitors is monoclonal antibodies (mAb). These can either bind competitively to the proteolytic cleavage site (Rabinovich-Nikitin et al., 2012) or noncompetitively to an exosite on BACE1 (Atwal et al., 2011). An in vivo study in triple transgenic mice showed reduced intraneuronal and extracellular Aβ concentrations and decreased total phosphorylated tau levels after a month’s treatment of competitive mAb (Rabinovich-
Nikitin et al., 2012). Furthermore, noncompetitive anti-BACE1 mAb reduced peripheral and central Aβ concentrations in murine and nonhuman primate models (Atwal et al., 2011). Although the results from these early stage clinical trials and in vivo mAb studies are promising, given the knowledge gained from KO mice studies careful anti-BACE1 drug administration and extreme safety monitoring in humans is vital.

As targeting Aβ production with secretase inhibitors has not yet yielded effective treatments due to off target effects and limited brain exposure, alternative approaches are required. Active and passive immunisation aimed at increasing Aβ peptide clearance or inhibiting Aβ aggregation into insoluble Aβ plaques in the cerebral parenchyma offers a very promising therapeutic method.

2.2 Active Immunisation

Active immunisation involves the administration of an antigen, typically with an adjuvant, which initiates endogenous antibody production against the foreign body (Chiang and Koo, 2014; Spencer and Masliah, 2014). Since Edward Jenner pioneered the first vaccine against smallpox in 1798 (Brody and Holtzman, 2008), active immunisations have been commonly developed against a plethora of bacterial and viral agents. However, recent attentions have focused on utilising the human immune system to remove endogenously produced toxic and harmful proteins such as Aβ (Spencer and Masliah, 2014).

Preclinical Studies

In 1999, an in vivo study involving APP transgenic mice (PDAPP) demonstrated the potential of immunotherapy for reducing the neuropathological progression of AD (Schenk et al., 1999). PDAPP transgenic mice express elevated levels of human APP, develop Aβ plaques, and synaptic loss similar to that seen in AD patients (Games et al., 1995). Immunisation with synthetic human Aβ42 resulted in almost complete prevention of Aβ deposition in young PDAPP mice, and profoundly slowed the progression of neuropathologies associated with AD in older mice including: Aβ plaques, degeneration of neurons and astrogliosis (Schenk et al., 1999). Soon after, other groups replicated these results independently, showing immunisation with Aβ42 caused a marked reduction of Aβ accumulation and reduced cognitive dysfunction in murine models of AD (Janus et al., 2000; Das et al., 2001). One plausible mechanism of anti-Aβ42 immunisation is that the antibody induces phagocytosis of Aβ deposits in vaccinated PDAPP mice (Figure 2, after Brody and Holtzman (2008)) (Schenk et al., 1999), as there is a significant correlation between microglial activation and Aβ clearance (Wilcock et al., 2001).

Clinical Studies

The encouraging preclinical results rapidly translated to human clinical trials. A phase I study with 80 mild to moderate AD patients evaluated the safety and tolerability of AN1792, a human aggregated Aβ42 combined with an immunogenic adjuvant QS-21 (Bayer et al., 2005). Patients were randomly assigned in a double-blind manner to one of four AN1792 conditions or to the control QS-21 group (Bayer et al., 2005). Ten patients withdrew prematurely due to adverse effects, including hallucinations, depression, and hostility. However, AN1792 combined with QS-21 did elicit a positive response to Aβ42 in 58.8% of treated patients (Bayer et al., 2005).

AN1792 was advanced to a phase II multicentre randomised double blind placebo-controlled study with 372 mild-to-moderate AD patients (Orgogozo et al., 2003). Patients were to receive a series of AN1792 or placebo intramuscular injections (Orgogozo et al., 2003). However, the trial was promptly terminated as 18 patients developed post-immunisation aseptic meningoencephalitis (Orgogozo et al., 2003). Out of these 18 patients, 12 recovered in a few weeks, but 6 patients had worsening cognitive decline and neurological sequelae (Orgogozo et al., 2003). The severity of adverse effects was not correlated with anti-Aβ titres (Orgogozo et al., 2003). Around a similar time an autopsy report stated a patient from the initial phase I trial
developed meningoencephalitis 6-8 weeks following her last injection and died soon after (Nicoll et al., 2003). Subsequent follow-up studies found AN1792-vaccinated patients produced antibodies that had a high affinity for the pathological Aβ plaques (Hock et al., 2002) and displayed slower cognitive decline, measured by the Mini Mental State Examination (Hock et al., 2003). Furthermore, four years after the phase II AN1792 study, patients had sustained anti-AN1792 levels and exhibited significantly reduced cognitive decline in comparison with placebo treated patients (Vellas et al., 2009). However, further analysis of the AN1792 trial identified many T-cell epitopes in the middle and C-terminal regions of the full length Aβ42 (Monsonego et al., 2003; Orgogozo et al., 2003), which are responsible for mediating T-lymphocyte meningoencephalitis (Schneeberger et al., 2009).

**Ongoing and Future Active Immunotherapy Trials**

Efforts are underway to develop second-generation active immunotherapies. AFFITOPE and CAD106 are two examples of novel immunogens; they exploit the use of short antigenic peptides located at the N-terminal of Aβ acting as B-cell epitopes (Winblad et al., 2012), whilst precluding any type of T-cell immune response that is thought to underlie the adverse effects mentioned above (Schneeberger et al., 2009).

AFFITOPEs are small six-amino acid long synthetic peptides that mimic parts of the Aβ N-terminus (Schneeberger et al., 2009). It is advantageous that these peptides are non-endogenous so as to avoid self-tolerance (Schneeberger et al., 2009). This allows the synthetic antigen to be administered with aluminium hydroxide, an immunological adjuvant that has a longstanding safety profile (Schneeberger et al., 2009). Two different AFFITOPEs, ADO1 and ADO2, improved cognitive function, reduced cerebral Aβ load and AD-like neuropathology in PDAPP mice (Schneeberger et al., 2009). Furthermore, these AFFITOPEs were well tolerated with little or no side effects in preclinical toxicology studies (Schneeberger et al., 2009). ADO2 is now in a phase II trial with patients in the early stages of AD (Winblad et al., 2014).

CAD106, another novel active immunotherapeutic comprising multiple copies of Aβ1-6 covalently conjugated to the bacteriophage Qβ, effectively induced the production of efficacious Aβ antibodies in murine and non-human primate models (Wiessner et al., 2011). Furthermore, CAD106 successfully disrupted amyloid accumulation and protected the brain from Aβ toxicity in PDAPP mice (Wiessner et al., 2011), especially when administered before/in the early stages of neuropathological transformations (Wiessner et al., 2011). A 52-week double blind placebo-controlled phase I trial with mild to moderate AD patients confirmed CAD106 was well tolerated and produced an acceptable antibody response without stimulating Aβ-specific T cells (Winblad et al., 2012), thus avoiding the adverse inflammatory effects observed in the AN1792 trials. However, there was no significant difference between the CAD106 and placebo group’s total CSF Aβ42/40, phosphorylated tau and phospho-tau biomarkers (Winblad et al., 2012). Limited exposure to low concentrations of antibodies, premature sampling, and small sampling groups could account for the lack of clinical significance between treatment groups (Winblad et al., 2012), all of which should be taken into consideration for further clinical studies. There are a number of on-going phase II clinical trials investigating CAD106, which aim to establish the presence of cognitive benefit to this disease-modifying drug.

### 2.3 Passive Immunisation

Unlike active immunotherapy that primes the human immune system to generate an immune response against an antigen (Spencer and Masliah, 2014), passive immunisation involves the direct injection of mAb that target specific epitopes (Lemere, 2013). The mAb are immunoglobulins (Igs) that have been produced in vitro by fusing B-lymphocytes with myeloma cells to from hybrid cells called hybridomas. These cells propagate infinitely in vitro and secrete a single class of antibodies (Wang et al., 2008). These mAb recognise a specific antigen at predefined sites
on certain protein or peptide molecules (Solomon et al., 1996). Clinically, passive immunisation has been successful in treating some forms of cancer, including advanced breast cancer and haematological malignancies (Schuster et al., 2006), as well as autoimmune diseases (Olsen and Stein, 2004) and transplant rejection (Todd and Brogden, 1989; Beniaminovitz et al., 2000). The natural extension, consequently, was development of therapeutic mAb for neurodegenerative diseases such as AD (Spencer and Masliah, 2014).

**Preclinical Studies**

Following the publication of the first preclinical active vaccination experiment (Schenk et al., 1999), two individual groups reported that peripheral administration of anti-Aβ mAb could also sufficiently reduce amyloid burden in PDAPP mice (Bard et al., 2000; DeMattos et al., 2001). The central mechanism of plaque clearance, however, is unclear. There are two conflicting views on how this may occur, namely, the “direct action” and “peripheral sink” hypotheses (Figure 2, after Brody and Holtzman, 2008) (Yu and Watts, 2013). The peripheral sink hypothesis suggests that mAb bind and completely sequester Aβ in the plasma, preventing its reuptake into the brain and consequently disrupting the Aβ equilibrium, facilitating the movement of Aβ from the brain to the plasma (DeMattos et al., 2001; Karran et al., 2011). Whereas, the direct action hypothesis proposes that mAb bind to Aβ plaques, which stimulates microglia cells to phagocytose and remove the Aβ plaques from the brain (Karran et al., 2011). The results of a study by Bard et al. (2000) supports the more popular “direct action hypothesis” (Schenk et al., 1999; Wilcock et al., 2001; Nicoll et al., 2003), as in vivo brain preparations from PDAPP and AD patients showed anti-Aβ mAb triggered microglial cells to clear, and subsequently degrade, Aβ plaques through Fc receptor-mediated phagocytosis (Bard et al., 2000). Other evidence indicates peripherally administered mAb, such as m266 do not enter or bind to Aβ deposits in the brain, instead, they completely sequester soluble plasma Aβ and act

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**Figure 2. The central mechanisms of Aβ plaque clearance by immunotherapeutics targeting Aβ in AD; the peripheral sink and direct action hypotheses.** Adapted from Brody and Holtzman (2008).
as a “peripheral sink” (DeMattos et al., 2001). Disrupting CNS/ plasma Aβ equilibrium promotes the efflux or clearance of Aβ from the cerebral parenchyma (Dodart et al., 2002).

One safety concern accompanying passive immunisation that targets the amino terminal of Aβ is increased incidence and severity of cerebral microhaemorrhage related to cerebral amyloid angiopathy in PDAPP mice (Pfeifer et al., 2002; Racke et al., 2005). Cerebral amyloid angiopathy is caused by the deposition of amyloid plaques in the cerebral blood vessels (Wilcock et al., 2004), which damages smooth muscle cells and weakens the vessel walls (Pfeifer et al., 2002). A conceivable mechanism that increases the risk of haemorrhage is Aβ mAb prompting a local inflammatory response that destabilises the already compromised vessel wall (Pfeifer et al., 2002). This is an important finding and should be scrupulously monitored in human clinical trials, as the majority of AD patients have varying levels of Aβ deposition in their cerebral vasculature (Racke et al., 2005).

Irrespective of the mechanism of CNS Aβ reduction and the risk of microhaemorrhages, passive immunisation in PDAPP mice can protect against synaptotoxicity (Buttini et al., 2005) and reverse cognitive and memory impairments in object recognition (Dodart et al., 2002) and Morris water maze tasks (Kotilinek et al., 2002).

Clinical Studies

Bapineuzumab, an antibody that binds to the N-terminal of Aβ plaques with higher affinity than soluble Aβ (Panza et al., 2010), was the first passive immunisation to be trialled in AD patients (Spencer and Masliah, 2014). In phase II clinical trials with mild to moderate AD patients, the bapineuzumab-treated patients displayed reduced cortical 11C-PiB-PET levels (Rinne et al., 2010) and significantly reduced CSF biomarkers, tau, and phosphorylated tau (Blennow et al., 2012) in comparison to the placebo groups. These results were encouraging, as they suggest bapineuzumab mitigates neurodegeneration (Salloway et al., 2014).

However, amyloid-related imaging abnormalities (ARIA), such as vasogenic cerebral oedema, the accumulation of fluid in the brain parenchyma originating from blood vessels, and microhaemorrhages were recorded in some patients treated with bapineuzumab (Sperling et al., 2012). APOE4 carriers and higher bapineuzumab doses were related to the increased incidence of ARIAs (Sperling et al., 2012), findings supported by a subsequent clinical trial (Salloway et al., 2014). Two separate randomised placebo-controlled phase III trials for mild to moderate AD patients aimed to determine the safety and efficacy of bapineuzumab in APOE4 carriers and non-carriers (Salloway et al., 2014). Unfortunately, bapineuzumab failed to improve cognitive or functional outcomes, despite reduced 11C-PiB-PET and CSF biomarkers in AD patients with the APOE4 genotype (Salloway et al., 2014). This led to the termination of late stage and extension studies of bapineuzumab (Tayeb et al., 2013).

Another humanised antibody, solanezumab that preferentially binds to soluble Aβ (Doody et al., 2014) was well tolerated, with no signs of microhaemorrhage, vasogenic oedema, or meningioccephalitis (Siemers et al., 2010; Farlow et al., 2012). These early clinical trials also showed increased CSF and plasma Aβ levels following dose-dependent administration of solanezumab (Siemers et al., 2010; Farlow et al., 2012). This suggests solanezumab shifts the Aβ equilibrium, promoting Aβ efflux from the brain into the peripheral circulatory system (Farlow et al., 2012; Doody et al., 2014). A separate study proposed that solanezumab sequesters and neutralises soluble Aβ monomers intracerebrally not peripherally, thereby preventing the build-up of neurotoxic multimeric Aβ in the cerebral parenchyma (Yamada et al., 2009). Disappointingly, phase II (Siemers et al., 2010) and III (Doody et al., 2014) solanezumab trials failed to show any cognitive improvements from baseline.

Gantenerumab is the only full-human antibody in clinical trials that targets Aβ1-40 in plaques, but not soluble Aβ (Bohrmann et al., 2012). Ex vivo assays showed gantenerumab induces cellular phagocytosis of Aβ deposits in AD brain slices in the presence of human macrophages (Bohrmann et al., 2012). A multicentre, randomised double-blind placebo
control PET study with mild to moderate AD patients found gantenerumab decreased cortical brain amyloid by 15.6% and 35.7% for 60 mg and 200 mg treatment groups, respectively, in comparison to the placebo group (Ostrowitzki et al., 2012). Phase III gantenerumab trials investigating whether cerebral Aβ reductions translate into clinical improvements are on-going (Panza et al., 2014).

MABT, also known as crenezumab, is a humanised anti-Aβ mAb that can bind to numerous forms of pathogenic amyloid including monomeric, oligomeric, and fibrillar Aβ (Adolfsson et al., 2012). Crenezumab differs from other passive immunotherapies, as it is an IgG4 isotype (Adolfsson et al., 2012). This subclass of IgG reduces the risk of an Fc receptor-mediated microglial inflammatory response and therefore may provide a safer disease-modifying therapeutic (Adolfsson et al., 2012). Preliminary clinical trials support this hypothesis, as there have been no reports or indications of vasogenic oedema even in at risk AD populations (APOE4 carriers) (Adolfsson et al., 2012).

Lastly, ponezumab is a humanised mAb that avoids labelling the full length APP and instead targets the C-terminal of Aβ40 (La Porte et al., 2012). In PDAPP mice systemic administration of ponezumab decreased hippocampal amyloid load and increased plasma Aβ40 levels in a dose dependent manner (La Porte et al., 2012). The latter finding was validated in early clinical trials (Burstein et al., 2013). Furthermore, ponezumab was well tolerated with no evidence of drug associated inflammatory changes, microhaemorrhages, or vasogenic oedemas (Burstein et al., 2013; Landen et al., 2013). However, as with the other passive immunotherapies described in this section; no cognitive improvement was identified (Burstein et al., 2013).

**Ongoing and Future Passive Immunotherapy Clinical Trials**

The current passive immunotherapeutic approaches have failed to show any clinical benefit in mild to moderate AD patients in late clinical trials (Siemers et al., 2010; Burstein et al., 2013; Doody et al., 2014; Salloway et al., 2014). Limited mAb delivery across the BBB and intervention too late in disease state progression, are the two principal but not exclusive theories that attempt to rationalise the lack of clinical efficacy (Spencer and Masliah, 2014). Novel technologies that improve delivery of antibodies across the BBB will be addressed in greater detail in the subsequent section.

AD is thought to begin decades before clinical symptoms arise (Bateman et al., 2012). This is important to note, as these disease-modifying therapeutics aim to reduce and eliminate Aβ accumulation, but negate the fact that irreversible synapse loss and degeneration of neurons has already occurred (Garber, 2012). Therefore, current preventative passive immunotherapy trials are investigating whether earlier treatment intervention in the preliminary stages of AD can stop or slow the progression of neurodegeneration. Other ongoing trials are examining both early and late-onset AD populations (Carrillo et al., 2013).

The Asymptomatic Alzheimer’s disease trial (A4) is a study of cognitively intact older patients who are not known to have any genetic AD predispositions, but display early signs of Aβ deposits (Carrillo et al., 2013) identified by 11C-PiB-PET scans (Garber, 2012). Patients will be treated with solanezumab over a three-year period to establish if earlier therapeutic intervention can prevent or delay the onset of AD (Carrillo et al., 2013). The Dominantly Inherited Alzheimer’s Network (DIAN), on the other hand, will treat a group of individuals who are carriers of the dominantly inherited familial FAD genes APP, PS1, or PS2 with gantenerumab and solanezumab (Garber, 2012). There is increasing evidence that suggests FAD has overlapping pathophysiological features with late-onset AD and, therefore, it may be possible to generalise some of the DIAN findings to this population of AD patients (Bateman et al., 2012).

Finally, the Alzheimer’s Prevention Initiative (API) aims to run a trial of crenezumab over a period of five years, including an interim analysis at two years (Garber, 2012; Carrillo et al., 2013), in healthy individuals who are at an increased risk of developing AD based on their genotype and age...

(Reiman et al., 2011; Bateman et al., 2012). The first API trial will be a study of 300 cognitively normal individuals from the world’s largest early onset AD population in Columbia, who carry the pathological PS1 genotype and are at least 35 years of age (Reiman et al., 2011). Whereas, the second API trial will enrol APOE4 homozygotes, and possibly heterozygotes, who are not cognitively impaired, but are near to their predicted average age of disease onset (Reiman et al., 2011).

Evaluating the efficacy of presymptomatic immunotherapy treatment may provide a revolutionary disease-modifying treatment for AD; alternatively, negative findings would call into question the validity of the amyloid hypothesis.

Section 3: Increasing Antibody Uptake Across the BBB

An inherent challenge of immunotherapy strategies targeting Aβ is limited brain exposure; only 0.1-0.2% of peripherally-administered antibodies cross the BBB (Poduslo et al., 1994; Banks et al., 2002). Exploiting endogenous transport systems to circumvent the BBB is a pioneering technique that could enhance antibody uptake (Moos and Morgan, 2001) and thus improve the clinical efficacy of anti-Aβ therapeutics. The three main BBB transport systems are receptor-mediated transcytosis (RMT), substrate-selective, carrier-mediated transport and nonspecific absorptive-mediated endocytosis (Yu and Watts, 2013). As the latter two mechanisms are incapable of transporting larger molecules, RMT provides the most promise for Aβ immunotherapy. RMT facilitates the movement of essential macromolecules from the systemic circulation to the brain via endogenous receptors located on the luminal surface of the capillary endothelium (Yu and Watts, 2013; Spencer and Masliah, 2014).

One example of RMT is the transportation of iron from the circulation to the CNS via transferrin receptor (TfR) mediated endocytosis (Jeffries et al., 1984). TfRs can independently endocytose in the absence of transferrin binding (Ajioka and Kaplan, 1986) and recycle in a matter of minutes (Maxfield and McGraw, 2004), making TfRs a desirable dynamic drug target. Preclinical studies selectively targeting cerebral TfRs with the antibody OX-26 (Jeffries et al., 1984; Pardridge et al., 1991) were able to transport conjugated drugs across the BBB without disrupting iron transport (Friden et al., 1991; Pardridge et al., 1991; Shin et al., 1995; Lee et al., 2000). However, more recent investigations found that these high-affinity radiolabelled antibodies targeting TfR remain associated with the brain capillary endothelial cells and do not cross into the CNS to reach significant levels (Moos and Morgan, 2001; Paris-Robidas et al., 2011; Yu et al., 2011). An innovative solution to this issue was the development of low-affinity TfR antibodies that have enhanced RMT across the BBB (Figure 3, after Yu et al., 2011) (Yu et al., 2011). In mice, these novel anti-TfR antibodies were able to reach therapeutic concentrations and a broad cerebral distribution 24 hours after administration (Yu et al., 2011).

Figure 3. The relationship between high-affinity and low-affinity antibody BBB uptake; the dissociation of antibody after TfR RMT is more probable with lower-affinity
antibodies, resulting in higher brain exposure. Figure adapted from Yu et al., 2011.

A bi-specific antibody that binds to both BACE1 and TFR was developed to test the clinical potential of anti-TFR antibodies (Yu et al., 2011). BACE1 was chosen as a therapeutic target, as it enabled investigators to measure BBB penetration and accompanying activity through analysis of Aβ concentrations (Yu et al., 2011; Couch et al., 2013). Bi-specific antibodies accumulated in the cerebral parenchyma of WT mice and led to a greater reduction in Aβ load relative to monospecific BACE1 antibodies (Yu et al., 2011). A later safety study identified that after TFR/BACE1, bi-specific dosing, mice displayed acute clinical reactions (profound lethargy, lack of locomotion and limb or whole body spastic movements), and decreased circulating immature red blood cells (reticulocytes) (Couch et al., 2013). Modification of TFR/BACE1 bi-specific antibodies removed Fc interactions with the complement system, eliminating adverse effects aforementioned (Couch et al., 2013). Although multiple doses of TFR/BACE1 bi-specific antibodies were notably effective at reducing Aβ, the findings of Couch et al. (2013) highlight important safety concerns.

Soon after the bi-specific antibody was devised a separate group developed a bi-functional antibody comprised of a single-chain fragment antigen-binding (Fab) anti-TFR mAb attached to the C-terminal of the heavy chain of the anti-Aβ mAb (Niewoehner et al., 2014). In PDAPP mice this bi-functional antibody significantly increased Aβ target engagement in comparison to anti-Aβ mAb alone (Niewoehner et al., 2014). However, extended exposure to bi-functional anti-body in vivo lead to the downregulation of TFRs, which consequently may have a negative impact on neuronal iron uptake (Niewoehner et al., 2014).

LDLRs are another well-characterised family of BBB receptors that offers potential as an antibody brain shuttle (Spencer and Verma, 2007). LDLRs predominantly bind to apolipoprotein complexes, namely APOB and APOE (Spencer and Verma, 2007). Recently, an anti-Aβ Fc free single-chain antibody (scFV) attached to the binding domain of APOB demonstrated significant BBB penetration (Spencer and Masliah, 2014). Furthermore, the APOB binding domain enables glial and neuronal cell uptake via LDLRs for degradation, evading the Fc receptor signalling cascade and undesirable inflammatory response (Spencer and Masliah, 2014). Therefore, this method of RMT with scFV antibodies targeting soluble and fibrillar Aβ (Fukuchi et al., 2006) is a promising approach to treat AD.

Although these hybrid antibodies provide encouraging results in vivo, the success of targeting RMT routes for Aβ immunotherapeutics will rely on the safety of exploiting these pathways, particularly if the receptor is expressed ubiquitously (Yu and Watts, 2013).

**Conclusion**

AD is the most prevalent form of dementia worldwide affecting 50-70% of cases; in 2015, 46.8 million people had dementia, yet this figure is set to increase to 74.7 million by 2030 as the aging population continues to increase (Price et al., 2015). The currently available drugs for AD only provide symptomatic relief, therefore, there is a desperate need for disease-modifying therapeutics that can prevent or delay the onset of AD. In *vitro* (Wei et al., 2010), post-mortem (Näslund et al., 2000), and transgenic mice (Jacobsen et al., 2006) studies, along with overwhelming genetic evidence (Oakley et al., 2006; Huang and Mucke, 2012), support the amyloid hypothesis, making Aβ a very rational drug target. Targeting Aβ production with γ- and β-secretase inhibitors has not shown clinical utility due to severe off-target side effects (Hitt et al., 2010; Hu et al., 2010). Active and passive immunisation aimed at increasing Aβ peptide clearance or inhibiting Aβ aggregation into insoluble plaques, on the other hand, continue to show more promise (Schenk et al., 1999).

Despite the early setback in AN1792 active immunisation trials using full-length human aggregated Aβ1-42 (Nicoll et al., 2003; Orgogozo et al., 2003), second-generation active immunotherapies implementing short antigenic N-terminal Aβ (Winblad et al., 2012),
and passive immunisation involving the direct injection of Aβ targeted mAb, display hopeful results with a greater therapeutic index.

Several phase III clinical trials have failed to demonstrate any cognitive improvement in mild-to-moderate AD patients (Siemers et al., 2010; Burstein et al., 2013; Doody et al., 2014; Salloway et al., 2014). Intervention too late in disease progression and inadequate antibody delivery to the brain are thought to account for lack of clinical efficacy. Current, longitudinal presymptomatic immunotherapy trials (A4, DIAN and API) aim to address the former issue by treating populations who are at high risk of developing AD and asymptomatic patients prior to the onset of symptoms (Carrillo et al., 2013). Positive clinical results will have a profound impact on the AD society, especially for individuals who are known FAD and APOE4 carriers. Furthermore, pioneering hybrid antibodies (Yu et al., 2011; Niewoehner et al., 2014) that implement the use of RMT to improve antibody concentration and penetration in the cerebral parenchyma could provide a much-needed solution to the problem of limited brain exposure. If successful, novel immunotherapeutic methods of delivering antibodies to the brain may provide groundbreaking therapies not only for AD but also other neurodegenerative diseases.

Although this review has revealed the great potential of targeting Aβ as a treatment for AD, other targets need addressing, namely, hyperphosphorylated tau and APOE4. As AD is a heterogeneous disorder, the greatest efficacy in disease modification may be achieved through combination therapy and earlier diagnoses.

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References


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Hitt BD, Jaramillo TC, Chetkovich DM, Vassar R (2010) BACE1--/ mice exhibit seizure activity that does not correlate with sodium channel level or axonal localization, Molecular Neurodegeneration 5:1.


Iwatsubo T, Odaka A, Suzuki N, Mizusawa H, Nukina N, Ihara Y (1994) Visualization of A β42 (43) and A β40 in senile plaques with end-specific Aβ monoclonals - evidence that an initially deposited species is Aβ42 (43), Neuron 13:45-53.

National Academy of Sciences of the United States of America 103:5161-5166.


Merck S (2016) An efficacy and safety trial of Verubecestat (MK-8931) in mild to moderate Alzheimer’s disease (P07738) (EPOCH). In: Merck Sharp & Dohme Corp: In: Clinical Trials.gov [Internet].
Merck S, Dohme C (2016) Efficacy and safety trial of Verubecestat (MK-8931) in participants with prodromal Alzheimer’s disease (MK-8931-019) (APECs). In: Clinical Trials.gov [Internet].
Ostrowitzki S, Depta L, Thurfjell L, Barkhof F, Bohrmann B, Brooks DJ, Klunk WE,


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2016

pharmacodynamics, Clinical Pharmacology & Therapeutics 5:548-58.


