2016

Examining the potential of anti-A(beta) antibodies as Alzheimer's therapeutics

BOSTON UNIVERSITY

SCHOOL OF MEDICINE

Thesis

EXAMINING THE POTENTIAL OF ANTI-A(BETA) ANTIBODIES AS ALZHEIMER'S THERAPEUTICS

by

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B.S., University of California, Irvine, 2014

Submitted in partial fulfillment of the

requirements for the degree of

Master of Science

2016

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DEDICATION

I would like to dedicate this work to my family, who have been so supportive of me thus far and continue to do so in all that I strive for.

ACKNOWLEDGMENTS

I would like to thank Dr. Charles Glabe for giving me the opportunity to work as an integral member of his research lab and for teaching me along the way. I would also like to acknowledge the help from my colleagues in the Glabe lab – Dr. Jorge Mauricio Reyes-Ruiz, Ricardo Albay, Margaret Ho, and Roula Arch. Finally, I would like to thank my advisor and mentor at Boston University, Dr. Karen Symes, who worked and guided me through every step of the graduate program. Without the aid of these individuals, I would not have been able to get where I am today.

EXAMINING THE POTENTIAL OF ANTI-A(BETA) ANTIBODIES AS ALZHEIMER'S THERAPEUTICS

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ABSTRACT

Alzheimer's disease results from an accumulation of aggregated amyloid beta peptide into oligomeric forms. Soluble oligomers are neurotoxic species, which are believed to be the pathophysiological cause of Alzheimer's neurodegeneration. Amyloid β species (Aβ) are formed via normal physiological cleavage of amyloid precursor protein by β and γ secretases. Cleaved isoforms aggregate further to form oligomeric configurations of Aβ peptide. To target toxic soluble Aβ oligomers, monoclonal antibodies have been synthesized. Experimental analysis demonstrates the ability of these antibodies to recognize synthetic and endogenous oligomers. In transgenic mice designed to overexpress oligomeric isoforms of Aβ, the antibodies were able to reduce the cerebral amyloid load with proceeding improvements in cognitive abilities. However, large-scale clinical trials corroborated results indicating diminished amyloid load, but failed to produce observable improvements in clinical outcome in patients with Alzheimer's disease. Simply put, the removal of amyloidogenic species was insufficient in alleviating the associated neurodegeneration and elicited no improvement in cognitive ability, suggesting that Aβ might not be the responsible pathogen in Alzheimer's. The successes of antibodies in in vitro and transgenic mice studies suggest the potential of antibodies in the treatment of Alzheimer's, but the inability of these drugs to produce

marked improvements in clinical trials questions the role of amyloid in the pathophysiology of the disease.

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Aβ..... Amyloid β

AD..... Alzheimer's Disease

APP ..... Amyloid Precursor Protein

INTRODUCTION

In 1906, Alois Alzheimer presented his observations of a 51 year-old female patient, Auguste D., who exhibited signs of rapid memory loss and cognitive deterioration consequent to her death four and half years later (Small & Cappai, 2006). Upon post-mortem examination, the brain was shown to have atrophied with unique neurofibrillary tangles and deposits (Small & Cappai, 2006). The extracellular deposits of what we now know to be amyloid beta protein (Aβ) are characteristic of the neurodegenerative disease named for Alzheimer, and are processed via endogenously present proteolytic processing cascades (Shoji *et al*. 1992). The protein is produced from a precursor known as amyloid precursor protein (APP), which is cleaved proteolytically via endosomal lysosomes to produce C-terminal and N-terminal fragments (Shoji *et al*. 1992).

Initial cleavage of APP is carried out by either α or β-secretase, and their enzymatic products can undergo secondary cleavage by γ-secretase to produce p3 peptide and Aβ, respectively (Ling *et al*. 2003). The two major forms of Aβ, Aβ40 and Aβ42, are normal endogenous products of proteolysis via secretases – they can be detected in the cerebrospinal fluid (CSF), along with various C-terminal fragments (CTF), of both individuals with Alzheimer's and those without (Ling *et al*. 2003, Selkoe, 1994). In short, the pathophysiology of Alzheimer's originates from normal proteolytic cleavage to produce potentially pathogenic Aβ species.

Though controversial, the amyloid hypothesis of Alzheimer's has guided modern research and therapeutic development. Current results suggest that the neurotoxicity and cognitive impairment associated with Alzheimer's originates from the accumulation of

Aβ into soluble oligomers. In vitro accumulation of Aβ species into oligomeric forms implicates the Aβ42 isoforms as the predominant component of toxic neurodegenerative oligomers (El-Agnaf *et al*. 2000). The 42 amino acid long Aβ peptide aggregates more rapidly when compared to Aβ40, and its administration to cells in culture exhibits lower cell viability (El-Agnaf *et al*. 2000). The results of in vitro comparisons of Aβ isoforms point to Aβ42 as the pathogenic species of Alzheimer's.

Research has pointed to soluble oligomeric intermediates of Aβ as the toxic species responsible for the cognitive symptoms of Alzheimer's, which can be recognized using monoclonal antibodies designed (Kayed *et al*. 2003). Antibodies to Aβ are designed by vaccinating rabbits with a molecular mimic of the soluble oligomers (Kayed *et al*. 2003). The rabbit immune response produces anti-amyloid antibodies, which are then collected and isolated from polyclonal serum (Kayed *et al*. 2003). Studies with the antibodies in neuroblastoma cell lines demonstrate that the antibodies can mediate the neurotoxicity associated with soluble Aβ oligomers, results that have been corroborated by other research (Kayed *et al*. 2003). The ability of oligomer-specific antibodies to selectively recognize and neutralize the neurotoxicity of pathological species implicated in Alzheimer's makes them a promising avenue for new Alzheimer's therapeutics. This thesis will review the literature available to highlight the current uses of antibodies in research and clinical studies to demonstrate their potential as new treatment options for Alzheimer's disease.

PUBLISHED STUDIES

Source material was obtained using PubMed and Google Scholar. Articles regarding the currently available treatment options for Alzheimer's were found using the keywords "treatments" and "Alzheimer's disease." Individual therapeutics were then cross referenced with "Alzheimer's disease" to gather more information on each drug. The research uses of monoclonal antibodies were found using key terms "antibodies", "amyloid", "Aβ", and "Alzheimer's disease." Transgenic mice trials were located by adding the keyword "transgenic" to the previously mentioned terms. The key term "immunotherapy" was added to the aforementioned terms along with "clinical" to identify the application of antibodies in clinical trials.

POTENTIAL OF ANTI-Aβ ANTIBODIES AS ALZHEIMER'S THERAPEUTICS

Treatment of Alzheimer's disease

Current therapeutics to treat Alzheimer's attempt to delay the associated cognitive degeneration – treatment is directed toward slowing the progression of the disease rather than reversing or curing. The two lines of treatment for remedying memory loss and dementia are acetylcholinesterase inhibitors and memantine.

The cognitive decline associated with Alzheimer's is a result of the neuronal degeneration. Experimental analysis in mice with the disease demonstrated reduced labeling with tritiated acetylcholine, representing a loss of cholinergic binding sites in the pathological state (Whitehouse *et al*. 1985). Radioactively labeled nicotine discriminates the preferential loss of nicotinic receptors over muscarinic (Whitehouse *et al*. 1985). Label studies in transgenic mice also elucidated the region of mass neuronal degeneration as the nucleus basalis; thus as the nucleus degrades in the pathological state, the number cholinergic binding sites decreases (Whitehouse *et al*. 1985). Subsequent immunoreactivity analyses of diseased brain tissue from the frontal cortex confirm the loss of nicotinic acetylcholine receptors due to Alzheimer's (Schroder *et al*. 1991). Comparison of immunoprecipitate with Nissl stain demonstrates that the pathology causes a decreased expression of nicotinic cholinergic binding sites rather than simply a loss of chemoreactivity (Schroder *et al*. 1991).

In addition to the loss of nicotinic binding sites, Alzheimer's reduces neurotransmission by decreasing the activity of choline acetyltransferase. The absence of this enzyme diminishes the synthesis and availability of neurotransmitter acetylcholine

for cognitive activity (Kellar *et al*. 1985). Post-mortem examinations of individuals suffering from Alzheimer's and dementia demonstrate a deficiency of choline acetyltransferase, primarily in the temporal lobe (Wilcock *et al*. 1982). The enzyme deficit is correlated to the increase of neuritic plaques as well as the increase in severity of dementia (Wilcock *et al*. 1982). As activity of choline acetyltransferase declines, memory and cognition steadily degenerate consequently (Wilcock *et al*. 1982).

Because of the cholinergic basis of the Alzheimer's pathology, it follows that treatment of the associated cognitive symptoms would target increasing neurotransmission via acetylcholine. By administering acetylcholinesterase inhibitors in treating Alzheimer's, the aim is to raise levels of acetylcholine to sustain cognitive ability and alleviate symptoms. However, drugs of this type inhibit peripheral cholinesterase, causing intolerable gastrointestinal and hepatotoxic side effects (Rogers *et al*. 1998). To improve efficacy along with safety and tolerability, the selectivity of cholinesterase inhibitors must be addressed. Preclinical testing with donepezil demonstrated improved selectively for acetylcholinesterase over butyrylcholinesterase, and in clinical studies, exhibited positive cognition effects and good tolerability when compared with placebo (Rogers *et al*. 1998). Another acetylcholinesterase inhibitor, rivastigmine, produces synaptic effects by competitively binding with the enzyme directly. Rivastigmine is hydrolyzed similarly to acetylcholine, but its carbamoyl moiety remains bound to enzyme, preventing it from further degradation (Polinsky, 1998). Unlike donepezil, rivastigmine inhibits acetylcholinesterase and butyrylcholinesterase; the latter has been implicated in the severity of dementia (Farlow *et al*. 2000). Clinical studies demonstrate

rivastigmine's ability to delay cognitive and mental decline, displaying side effects related to peripheral inhibition, similar to those of other cholinesterase inhibitors (Farlow *et al*. 2000).

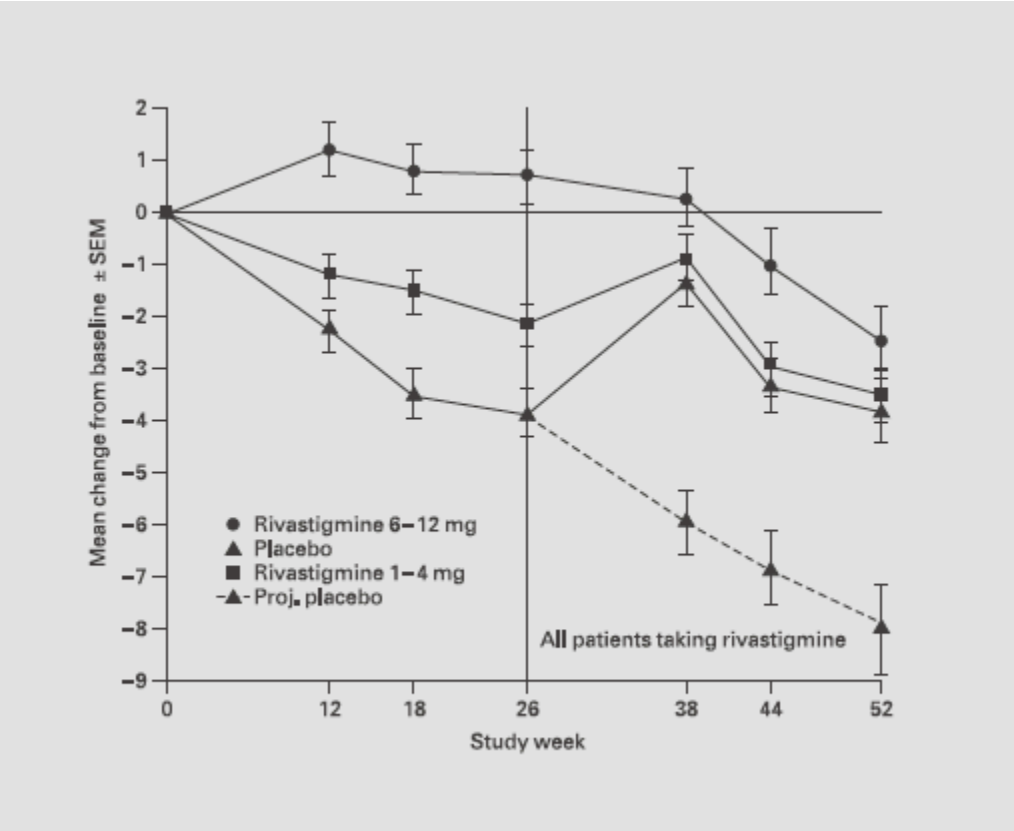

Figure 1: Compared to placebo, rivastigmine delays cognitive decline and improves mental health over an extended period. Additionally, administration of rivastigmine in the placebo group after 26 weeks produced marked improvements (Farlow *et al.* 2000).

In addition to the effects on synaptic transmission, acetylcholinesterase inhibitors seem to have neuroprotective effects on the Alzheimer's brain. Studies with human neuroblastoma cell cultures demonstrate the protective properties of three different inhibitors commercially available for treatment of Alzheimer's: donepezil, rivastigmine,

and galantamine. Pretreatment of cell lineages with these drugs offers protection against neuronal death induced by okadaic acid (Arias *et al*. 2005). By mediating Aβ toxicity, the drugs can also confer a degree of anti-apoptotic effects to further mediate the extent of cell death (Arias *et al*. 2005). The neuroprotective benefits of cholinesterase inhibitors are impeded with the administration of antagonists of nicotinic acetylcholine receptors, suggesting that neuroprotection is imparted by the receptors (Arias *et al*. 2005). Blockades of the receptors at high concentrations of drug create U-shaped curves of efficacy, in which maximal levels of protection are achieved at intermediate concentrations (Arias *et al*. 2005).

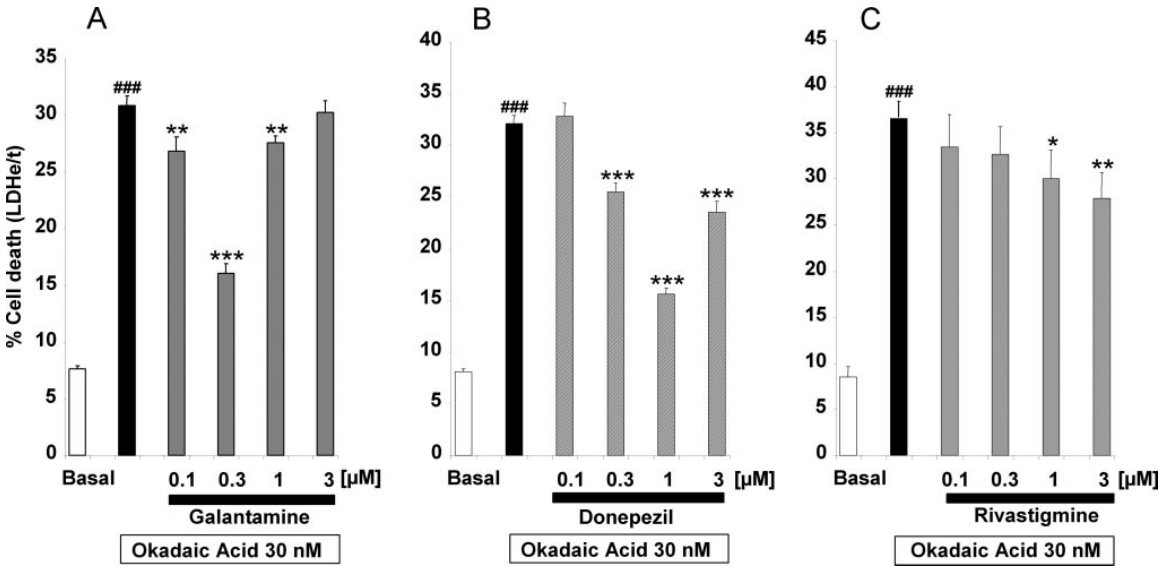

Figure 2: Comparison of the optimal concentrations of acetylcholinesterase inhibitors, galantamine, donepezil, and rivastigmine, for neutralizing neurotoxicity induced by okadaic acid administration (Arias *et al*. 2005).

A downstream effect of Aβ oligomers is the over-excitation of NMDA receptors, which are crucial components for long-term potentiation and long-term memory (De

Felice *et al*. 2007). This over stimulation effect produces reactive oxidative species that interfere in synaptic transmission, long-term potentiation, and plasticity exhibitory in Alzheimer's (De Felice *et al*. 2007). The production of neurodegenerative reactive oxidative species via amyloid derived diffusible ligands (ADDLs) can be blocked by memantine (De Felice *et al*. 2007). Specifically, memantine blocks Ca^{2+} influx caused by ADDL binding to the NMDA receptor, and by doing so, memantine inhibits mitochondrial production of superoxides and peroxides (De Felice *et al*. 2007). Because of its ability to mediate elevated levels of reactive oxidative species, memantine is an effective therapeutic in delaying the onset of cognitive decay.

In phase III clinical trials, subjects on memantine exhibited significant improvements in cognition when compared to placebo controls (Winblad *et al*. 2007). Safety and tolerability were similar amongst the two groups, with the incidence of minor adverse events marginally higher for the memantine group (Winblad *et al*. 2007). Patients with moderate to severe progressions of Alzheimer's were able to recover mechanical capabilities, such as independent bathing and dressing, as well as improve behavioral aspects (Reisberg *et al*. 2003). Treatment with memantine relieves distress on both patient and caretaker by mediating burdens associated with later stages of Alzheimer's (Reisberg *et al*. 2003).

In combination therapy with acetylcholinesterase inhibitors, memantine demonstrates significant improvement in cognitive ability when compared to treatment with cholinesterase inhibitors alone. Administration of memantine to patients with mild to moderate Alzheimer's who were already on stable treatment with the cholinesterase

inhibitor donepezil elicited observable improvements in behavior and memory without discernible differences in incidence of adverse events (Tariot *et al*. 2004). Six areas of cognitive abilities as well as functionality in everyday tasks exhibited improvement in the memantine group (Tariot *et al*. 2004). Individuals in the memantine group remained more steadily above baseline whereas the placebo group observed steady declines in cognition (Tariot *et al*. 2004). Additionally, the memantine exhibits decreased frequency of mental disturbance and psychiatric symptoms (Tariot *et al*. 2004).

However, as efficacious as current therapeutics may be, they are directed solely toward delaying the onset of inevitable cognitive degeneration. More research is required in order to discover suitable treatment options to reverse or cure the disease progression.

Monoclonal Antibodies

Monoclonal antibodies provide a hopeful avenue for the future of Alzheimer's therapeutics. Because of their unique specificity and manipulability, monoclonal antibodies can be used to precisely target pathological species and neutralize their toxicity. They have been used universally in research, and their successes show promise for drug development.

The toxic species associated with Alzheimer's, Aβ peptide, can be recognized using monoclonal antibodies. By exposing rabbits to synthetic forms of Aβ peptide, antibodies

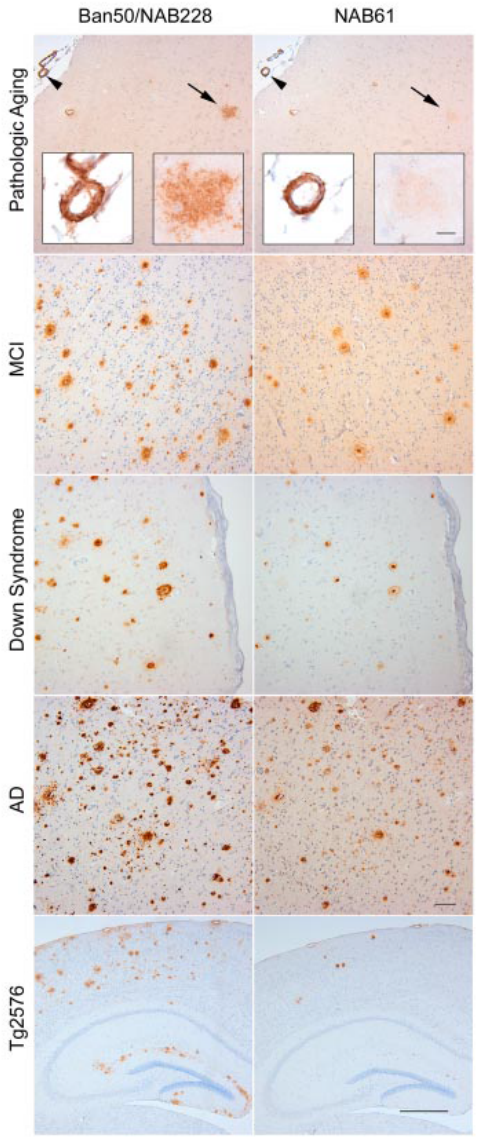

Figure 3: NAB61 specifically stains oligomeric forms of amyloid as seen in immunohistochemical studies (Lee *et al*. 2006).

against amyloid can be produced endogenously (Solomon *et al*. 1996). When anti-amyloid antibody is introduced to fibrillar conformations of Aβ, the aggregated forms of

revert back to an amorphous state (Solomon *et al*. 1996). The ability to prevent the in vitro aggregation of amyloid, implicated in the pathogenesis of Alzheimer's, present potential for the development of such antibodies as therapeutics (Solomon *et al*. 1996).

Monoclonal antibody, NAB61, was designed to recognize oligomeric forms of the Aβ peptide (Lee *et al*. 2006). Immunoreactivity and specificity of the antibody was assessed using ELISA and synthetic Aβ aggregates, revealing its selectivity for amyloid oligomers over non-oligomer forms as well as its recognition of a conformation dependent N-terminus epitope (Lee *at el*. 2006). Immunohistochemical studies also confirm that NAB61 recognizes amyloid plaques in diseased brain samples (Lee *et al*. 2006). In studies of different brain regions, NAB61 demonstrates regional selectivity for plaques in the mid-frontal cortex (Lee *et al*. 2006). The pathology of Alzheimer's originates in association cortices with increasing densities and aggregations of amyloid as the disease progresses; the localization of NAB61 onto oligomeric forms of Aβ in the

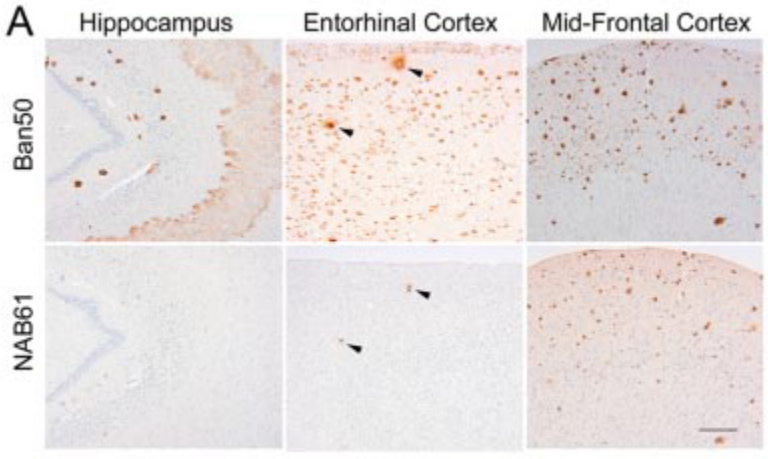

Figure 4: NAB61 displays regional selectivity for amyloid in the mid-frontal cortex (Lee *et al*. 2006).

mid-frontal cortex suggests that the antibody recognizes mature forms of amyloid peptide (Lee *et al*. 2006). When administered to transgenic mice, NAB61 elicited improvements in spatial reference memory as tested using underwater mazes – the performance of antibody-treated transgenic mice was comparable to that of non-transgenic mice and significantly better than IgG-treated transgenic control mice (Lee *et al*. 2006). However, assessment of levels of amyloid plaques and amyloid precursor protein in transgenic mice immunized with NAB61 demonstrated no significant difference in steady state levels between the antibody-treated transgenic mice and control groups (Lee *et al*. 2006). The ability of NAB61 to improve cognition and memory does not affect amyloid pathogenesis, but relies on neutralizing the toxicity of existing amyloid oligomers (Lee *et al*. 2006).

The application of antibodies in transgenic mice has elucidated that amyloid can be cleared using monoclonal antibodies *in vivo* via a Fc-independent mechanism (Bacskai *et al*. 2002). To determine whether removing Aβ species by administration of antibodies can remediate amyloid associated neuritic dystrophy, transgenic mice where passively immunized with anti-amyloid antibodies (Brendza *et al*. 2005). Brains were imaged *in vivo* using yellow fluorescent protein to monitor the number and area of dystrophic neurites over 72 hour and weeklong periods (Brendza *et al*. 2005). Without treatment, there was no improvement in neuritic dystrophy; however, in the presence of antibody 10D5, recovery of neuritic dystrophy following the clearance of amyloid deposits occurred as rapidly as 3 days (Brendza *et al*. 2005). Comparison of 18 plaque deposits

from 7 different transgenic mice demonstrates consistent reduction in number and area dystrophic neurites as a result of the 10D5 treatment (Brendza *et al*. 2005).

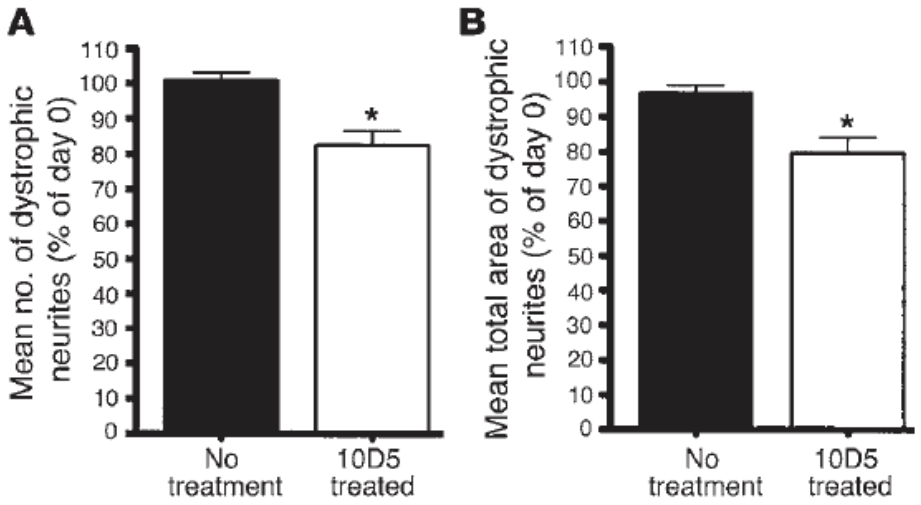

Figure 5: Treatment with anti-amyloid 10D5 reduces neuritic dystrophy within 3 days (Brendza *et al*. 2005).

Soluble forms of amyloid have been implicated as the neurotoxic species responsible for the pathogenesis of Alzheimer's (Kayed *et al*. 2003). The effect of anti-Aβ-protofibril antibodies in mediating the toxicity of soluble protofibrils was assessed in transgenic mice (Lord *et al*. 2009). Transgenic mice were passively immunized with one of two antibodies, mAb1C3 and mAb158, which recognize linear and conformational epitopes of Aβ, respectively (Lord *et al*. 2009). Because of its conformation dependent nature, mAb158 displays better selectivity to Aβ protofibrils (Lord *et al*. 2009). However, administration of either antibody elicited a neuro-protective effect, but the conformation dependent antibody, mAb158, does so at a lower concentration as a result of its selective binding to toxic soluble protofibrils (Lord *et al*. 2009). Additionally, both antibodies prevent fibrillation of amyloid peptide *in vitro* (Lord *et al*. 2009). The

antibodies both exhibited preventative properties, diminishing the aggregation of protofibrils and plaque formation in transgenic mice when compared to controls (Lord *et al*. 2009).

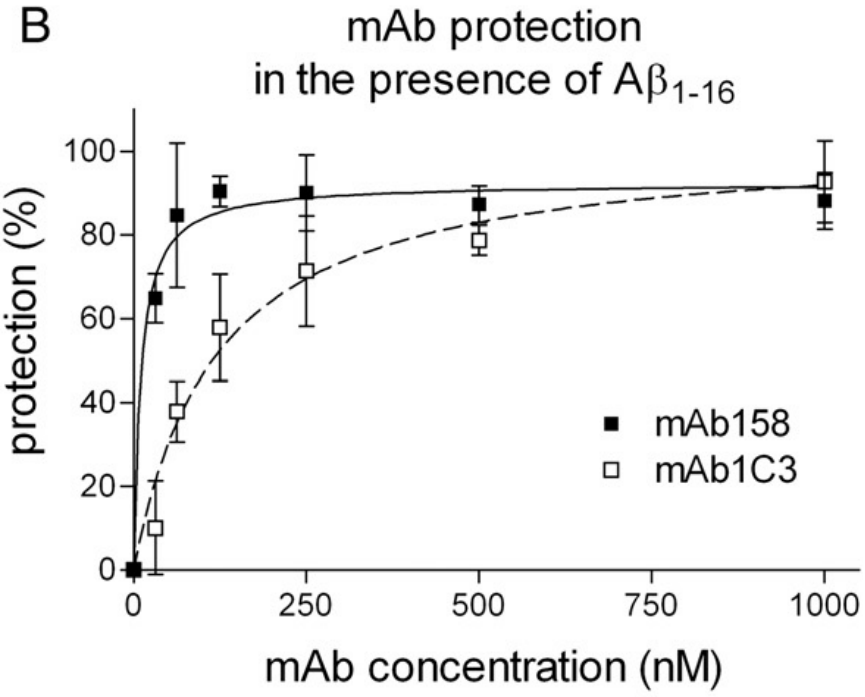

Figure 6: Both antibodies impart equal levels of protection to the mice, but mAb158 reaches maximum protection at lower concentrations, demonstrating its selectivity (Lord *et al*. 2009).

In clinical trials, an increased level of serum anti-Aβ antibodies seems to be causal to decreased disease progression (Geylis *et al*. 2005). To determine if commercial production of human antibodies would be viable for vaccines, cell lines were established to determine if human anti-Aβ antibodies could be synthesized *in vitro* (Geylis *et al*. 2005). The cell lines were produced from lymphocytes obtained from healthy blood samples, which were immortalized with Epstein-Barr virus and fused with mouse plasmacytoma cells to retain antibody specificity (Geylis *et al*. 2005). Anti-Aβ

antibodies were detected in the supernatant after centrifugation, with specificity to synthetic Aβ as determined by ELISA, specifically an N-terminus epitope of amyloid peptide (Geylis *et al*. 2005). Immunohistochemcial analysis of synthesized antibody determined that the antibody does bind to endogenous amyloid in the Alzheimer's brain (Geylis *et al*. 2005). The success of human antibody synthesis via hybridoma manufacture promotes the development of anti-Aβ vaccination without the immunogenicity associated with murine or chimeric antibodies.

Active and passive immunization has proved successful in eliminating toxic amyloid species in transgenic mice as well as in clinical trials; however, it has also elicited adverse events in human trials (Dodel *et al*. 2004). In order to mediate the cognitive decay of Alzheimer's while keeping side effects minimal, human immunoglobins containing anti-Aβ antibodies were administered to patients in clinical trial (Dodel *et al*. 2004). Five individuals suffering from Alzheimer's disease were recruited for the study, and levels of Aβ cerebrospinal fluid and serum were measured over a 6-month period (Dodel *et al*. 2004). Additional neuropsychological testing was conducted to track cognitive and behavioral progress (Dodel *et al*. 2004). Over the test period, mean values of Aβ in CSF decreased 30.1% on average and increased from an average of 240.4 pg/mL to 558.2 pg/mL in serum (Dodel *et al*. 2004). Slight improvements in mental state were observed, but no cognitive decline was evident (Dodel *et al*. 2004). There were no adverse effects associated with treatment (Dodel *et al*. 2004). This early study of immunization with anti-Aβ antibody containing immunoglobins suggests that the treatment is well tolerated and could be promising in the existing line of therapy (Dodel

et al. 2004). Bapineuzumab is a humanized monoclonal antibody targeted to the N-terminus of Aβ peptide, specifically species deposited in plaques (Panza *et al*., 2010). A phase 2 clinical trial of the proposed drug investigated its effect on cortical Aβ load as measured by 11-labeled Pittsburgh compound B (^{11}C-PiB) radiotracer (Rinne *et al*., 2010). Over 78 weeks, 19 patients with mild to moderate Alzheimer's were treated with bapineuzumab and compared against 7 patients given placebo (Rinne *et al*., 2010). Assessments of ^{11}C-PiB levels indicated a mean decrease amongst the bapineuzumab group and mean increase within the placebo group, correlating to a reduction in cortical Aβ load due to administration of bapineuzumab (Rinne *et al*., 2010). Adverse effects were reported to be mild to moderate in severity and short-lived, with the exception of incidence of vasogenic edema in 2 subjects, leading to their subsequent discontinuation (Rinne *et al*., 2010).

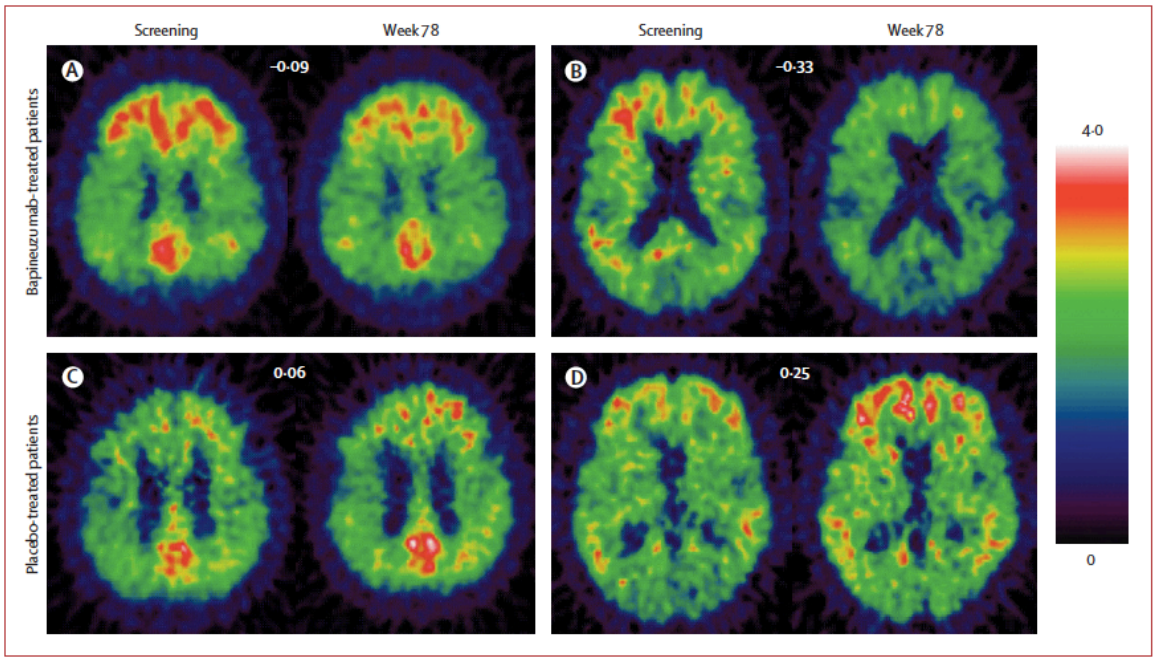

Figure 7: PET imaging of ^{11}C-PiB demonstrates reduced cortical amyloid load following administration of bapineuzumab (A, B) compared to placebo (C, D) (Rinne *et al*. 2010).

A phase 3 clinical trial of bapineuzumab assessed the ability of the antibody to change the clinical outcome of patients with mild to moderate Alzheimer's, as well as discriminate between carriers and non-carriers of ApoE ε4 allele in regards to the incidence of vasogenic edema (Salloway *et al*., 2014). The research team established groups based on presence of the ApoE ε4 allele, drug administered, bapineuzumab or placebo, and dosage (Salloway *et al*., 2014). Cognitive ability and amyloid load were measured to determine disease progression and outcomes (Salloway *et al*., 2014). Increased incidences of vasogenic edema at increasing concentrations led to the discontinuation of the group receiving 2.0 mg/mL (Salloway *et al*., 2014). Regardless of group classification, no significant differences in cognitive change from baseline were

observed (Salloway *et al*., 2014). The PET assessment of ^{11}C-PiB marker for cortical Aβ peptide determined that amongst carriers, there was a mean increase in standardized uptake value (SUVR) in the placebo group and no significant change over 71 weeks for the bapineuzumab group (Salloway *et al*., 2014). Similarly, amongst non-carriers, there was no significant change in SUVR in the bapineuzumab, but no significant increase was observed in the placebo group (Salloway *et al*., 2014). Measurements of phosphorylated tau in cerebrospinal fluid indicated significant reductions in the bapineuzumab group and significant increases in the placebo group for carriers (Salloway *et al*., 2014). Amongst non-carriers, no group differences were observed in levels of phosphorylated tau (Salloway *et al*., 2014). The incidence of adverse events were similar between carriers and non-carriers in both drug and placebo groups (Salloway *et al*., 2014). Effusion or edema was the most prominent adverse event that arose as a result of bapineuzumab administration, consistent with increasing dosage and number of ApoE ε4 alleles (Salloway *et al*., 2014). Incidence of neoplasm amongst 20 carriers and 23 non-carriers as a result of drug administration led to death (Salloway *et al*., 2014).

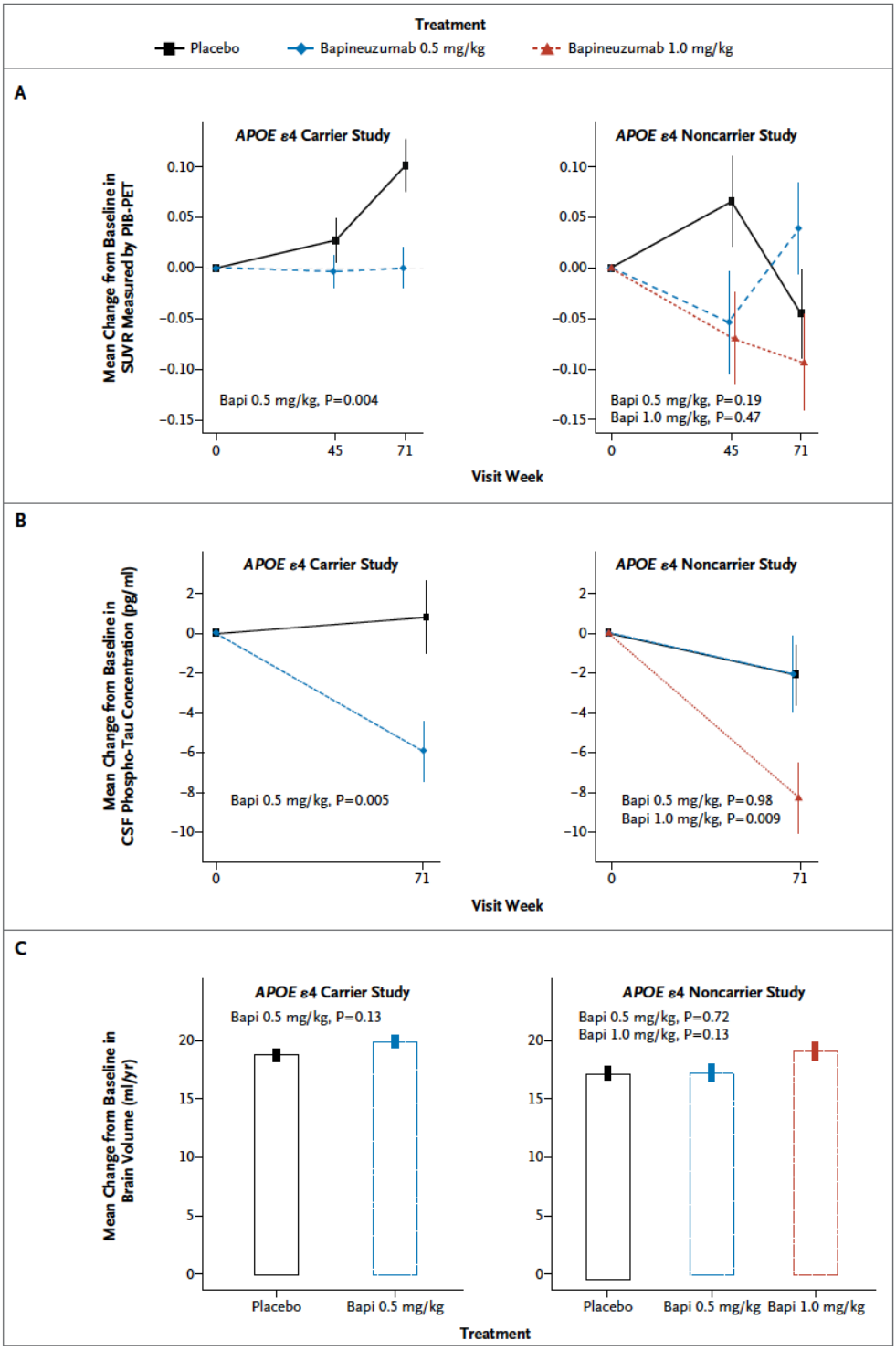

Figure 8: These graphs summarize the results for the phase 3 clinical trial, separating the results of carriers and non-carriers (Salloway *et al*., 2014).

Clinical trials were conducted to assess the safety and pharmacological properties of anti-Aβ antibody, solanezumab, in patients with mild to moderate Alzheimer's disease. The phase 2 trial involved 52 subjects taking either drug or placebo over a 12-week period (Farlow *et al*., 2012). Magnetic resonance imaging (MRI) and samples of CSF were employed to track patient progress (Farlow *et al*., 2012). Eight patients receiving solanezumab experienced 10 treatment emergent adverse events, two of which, subdural hematoma and lumbar puncture headaches, were attributed to the lumbar puncture required for CSF sampling (Farlow *et al*., 2012). Imaging via MRI revealed no instances of vasogenic edema (Farlow *et al*., 2012). Five patients experienced anti-solanezumab immunogenicity (Farlow *et al*., 2012). Total concentrations of Aβ species increased in plasma for each of the dosing regiments, but increases were largest for the groups given 100 mg weekly, 400 mg weekly, and 400 mg every 4 weeks (Farlow *et al*., 2012). No significant differences in cognitive ability from baseline were observed between antibody and placebo groups (Farlow *et al*., 2012). Solaneuzumab was deemed generally well tolerated with no incidence of adverse events consistent with other antibody trials, T-cell mediated inflammation and vasogenic edema (Farlow *et al*., 2012). However, pharmacokinetic and pharmacodynamics assessment determined that the antibodies did not seem to neutralize amyloid toxicity, as no cognitive improvement was observed in clinical trial (Farlow *et al*., 2012).

Further clinical testing was conducted for solanezumab by means of phase 3 trials. Patients over the age of 55 with a diagnosis of mild to moderate Alzheimer's disease were recruited to participate in the study for 18 months (Doody *et al*., 2014).

Efficacy was determined using cognitive measurements from baseline levels established prior to drug administration (Doody *et al*., 2014). Levels of Aβ in plasma and CSF were also monitored alongside MRI and PET imaging to observe amyloidogenic burden (Doody *et al*., 2014). At 52 and 64 weeks, there were significant improvements in cognition observed for the solanezumab group compared to placebo; however at the endpoint, at 80 weeks, there were no significant group differences (Doody *et al*., 2014). Total concentrations of Aβ peptide in plasma increased from baseline over the 80 weeks (Doody *et al*., 2014). No adverse events that affected more than 2% of the solanezumab group were evident, but there appeared to be a greater incidence of cardiac disease amongst the antibody group than placebo (Doody *et al*., 2014). Imaging studies revealed that only 0.9% of solanezumab and 0.4% of placebo group exhibited amyloid related edema (Doody *et al*., 2014). Though the incidence of adverse effects and amyloid abnormalities, edema and hemorrhage, were low for solanezumab, the phase 3 clinical trials did not demonstrate significant improvement in clinical outcome for the patients who participated (Doody *et al*., 2014).

SUMMARY

The experimental applications of monoclonal antibodies as well as their successes in targeting amyloid *in vitro* point to their potential in treating and possibly curing Alzheimer's. Results collectively corroborate that monoclonal antibodies to Aβ peptide recognize and clear amyloid species, correlating to a decline in cognitive decay in experiments with transgenic mice.

Experimentation with anti-Aβ antibodies elucidated multiple possible mechanisms for effectively neutralizing the toxicity of amyloid species. Transgenic study by Bacskai *et al*. determined that administration of antibodies reduced the Aβ load via F_c independent phagocytosis (Bacskai *et al*. 2002). The inhibition of fibrillation of Aβ peptide was observed by Lord *et al*., presenting an alternative mechanism for relieving Aβ burden on the brain (Lord *et al*. 2009). These studies demonstrate the ability of antibodies to reduce, and thereby neutralize, amyloid load and toxicity. This property of antibodies makes them viable options for development into Alzheimer's therapeutics.

Clinical trials involving the active immunization with aggregated Aβ species to promote endogenous production of anti-Aβ antibodies proved inconclusive, as subjects exhibited adverse effects associated with T-cell activation (Geylis & Steinitz, 2005). In subjects that succeeded in producing anti-Aβ antibodies, there was dramatic improvement in cognition as serum levels of antibody increased; however, not all subjects were able to elicit an antibody response to the vaccination of amyloid (Geylis & Steinitz, 2005). To combat side effects resulting from activation of T-lymphocytes,

passive immunization strategies, involving vaccination with the antibodies themselves, were employed with early success (Geylis & Steinitz, 2005). The various strategies available to administer the efficacious anti-amyloid antibodies highlight their developmental potential as therapeutics.

When compared to current treatment options for Alzheimer's disease, monoclonal antibodies specifically target the pathological moieties rather than on other neurological aspects that are implicated by the diseases progression. Cholinesterase inhibitors attempt to raise endogenous levels of acetylcholine to mediate the reduced neurotransmission resultant of Alzheimer's, and memantine reduces over-activity of NMDA receptors to mitigate the load of reactive oxidative species. Anti-amyloid antibodies, however, neutralize the toxicity of Aβ species, the precursor to the proceeding cognitive decline. In essence, the current treatment options address the symptoms, whereas the goal of antibodies is to target the source of Alzheimer's.

The results of clinical trials of solanezumab and bapineuzumab demonstrate not only progress of antibody therapeutics, but also the obstacles that hinder such treatments from becoming clinically viable. Both are humanized monoclonal antibodies to Aβ, developed with the hope to reverse the progression of Alzheimer's. Bapineuzumab demonstrated ability to reduce the amyloid load in the brain, but these decreases in amyloid levels did not exhibit a subsequent improvement cognitive ability (Rinne *et al*., 2010, Salloway *et al*., 2014). Additionally, bapineuzumab was associated with concerning adverse effects, namely amyloid-related effusion and vasogenic edema specific to carriers of the ApoE allele (Rinne *et al*., 2010). Solaneuzumab was also

effective at reducing amyloid load and unlike bapineuzumab, seemingly without amyloid-related adverse events (Farlow *et al*., 2012). However, clinical trials of solanezumab were unable to produce results that demonstrated improvements in cognition (Doody *et al*., 2014). The current clinical trials with monoclonal antibodies have been fruitless in demonstrating their clinical efficacy in remedying cognitive symptoms of Alzheimer's disease. Though the two antibodies, solanezumab and bapineuzumab, have produced promising results in transgenic mice, these results have not been replicated in humans. To develop antibodies into clinically useful therapeutics, it would be necessary to overcome the adverse effects associated with antibody, effusion and vasogenic edema, as well as demonstrate an ability to mediate or reverse cognitive decay.

Monoclonal antibodies demonstrate novel potential as therapeutics for the treatment of Alzheimer's. However, current strategies and antibodies have not yet demonstrated the same degree of success in humans as they have in transgenic mice. To improve the tolerability and efficacy of monoclonal antibodies, new methods need to be investigated to directly deliver anti-Aβ antibodies to the central nervous system (Vasilevko & Cribbs, 2006). Because the whole antibody molecule is not required for the clearance of amyloid species, smaller fragments of antibody (scFv) can be utilized for easier and more direct delivery (Vasilevko & Cribbs, 2006). Fragments can then be delivered via adenovirus vectors or mesenchymal stem cells to evenly distribute antibody and promote uniform clearance of Aβ to optimistically produce cognitive improvement in Alzheimer's patients (Vasilevko & Cribbs, 2006). By direct delivery to the CNS, the

hope is that antibodies will promote activation of microglia to clear amyloid species and reduce neuroinflammation and subsequent cognitive loss (Vasilevko & Cribbs, 2006).

Differing results from clinical and transgenic mice studies question the potential of anti-Aβ antibodies in treating Alzheimer's disease. Though the successes in recognition and neutralization of toxic oligomeric species in transgenic mice demonstrate promise, the inability to replicate such results in clinical trials questions their usefulness as a therapeutic. Perhaps the issue with current antibody treatments lies with the administration of drug. Research demonstrates that promoting endogenous production of anti-amyloid antibodies to neutralize oligomers elicits positive cognitive improvements. The next goal of developing antibody treatments should aim to complement the human immune system to clear amyloid load.

However, there is a school of thought that the failures of antibodies to elicit positive clinical outcomes in the treatment of Alzheimer's disease are evidence that the amyloid hypothesis itself may be flawed. Clinical trials demonstrate that antibodies are effective in reducing the amyloid load in patients with no improvement in mental state or cognition, suggesting that amyloid itself might not be the cause of Alzheimer's-associated neurodegeneration (Galimberti *et al*. 2013). It is suggested that research focus on understanding the mechanism of Alzheimer's more thoroughly ahead of developing antibody therapeutics that may be ineffective (Galimberti *et al*. 2013). There are some that consider the clinical data obtained from anti-Aβ antibody trials to be sufficient evidence to invalidate the entire amyloid hypothesis, so it may be necessary to ensure the validity of the hypothesis before moving ahead in development of treatments that address

amyloid burden (Mullane & Williams, 2013).

Despite the ability of antibodies to reduce cerebral amyloid load, and even cause improved cognition in transgenic mice, the implications that its inability to produce similar effects in clinical trials prevent antibodies from becoming viable options to develop into Alzheimer's therapeutics. The clinical outcomes that clinical trials have reported question the validity of the underlying amyloid hypothesis, which needs to be corroborated by more research before antibodies can be pursued further. With transgenic mice, the antibodies show great potential, but they stumbled when moved into the clinical phase of testing. However, further research needs to be conducted to determine if using antibodies to target amyloid load is even relevant to the treatment of the neurodegeneration of Alzheimer's disease.

LIST OF JOURNAL ABBREVIATIONS

Annu Rev Cell Biol	Annual Review of Cell Biology
Eur Neurol	European Neurology
J Clin Invest	Journal of Clinical Investigation
J Neurochem	Journal of Neurochemistry
N Engl J Med	New England Journal of Medicine

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