Alzheimer's disease immunization strategies : a review of current research

Laura Ona Sirgedas
The University of Toledo

Alzheimer's Disease Immunization Strategies: A Review of Current Research

Laura Ona Sirgedas

The University of Toledo

2009

Dedication

First and foremost, I thank God for providing me with the strength and patience necessary to guide me through PA school and this Scholarly Project.

I would like to thank my parents for supporting and encouraging me in my journey through PA school. Without their love and understanding, this goal would have not been possible to accomplish.

I would like to thank three best friends I have been lucky enough to meet in PA school. Erin, Jena, and Laurie, we have shared the most exciting and hardest moments of our lives together. Thank you for the memories and bond that cannot be broken.

Acknowledgments

I would like to thank my wonderful advisor, Karen Graham, for her guidance in finishing this Scholarly Project. She instilled in me an interest in geriatrics and neurologic diseases from the beginning of my education at University of Toledo. Her support, insight, and instruction throughout this Scholarly Project journey allowed it to be fulfilling and successful.

I would also like to thank Jolene Miller for her help in deciphering EndNote and the various difficulties encountered with it.

Table of Contents

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disease affecting over 26 million people in the world (Brookmeyer, Johnson, Ziegler-Graham, & Arrighi, 2007). As the proportion of the population over 65 years old increases, the incidence of AD is forecasted to increase exponentially. In the next 50 years AD is expected to increase between 11 million and 16 million people in the United States alone (Sano, Grossman, & Van Dyk, 2008). These numbers may further increase as developing technologies allow for earlier, more precise detection of dementia-related illnesses.

Alzheimer's disease is characterized primarily by progressive cognitive impairment. The full onset of the disease is often preceded by a syndrome known as mild cognitive impairment (MCI), which is found to commonly lead to dementia and more specifically, Alzheimer's disease. Many emerging therapies could potentially be used to prevent AD in patients exhibiting symptoms of MCI. Once the dementia manifests, the patient with AD will progressively lose cognitive function, with late stages including complete loss of activities of daily living (ADLs) and communication. The duration of disease to the terminal stage is variable among patients.

This debilitating disease carries a tremendous burden to not only those afflicted but also to their caretakers. Thus, treatment options are constantly being developed, evaluated, and tested. The progression has been tedious, however, as the pathophysiology of AD has only recently been established, and gaps in the research continue to exist. Current treatments have focused primarily on the relief of symptoms associated with AD, specifically cognitive deficits. Cholinesterase inhibitors such as donepezil, rivastigmine, and galantamine, as well as the N-methyl-D-aspartic acid (NMDA) receptor antagonist, memantine, are being used purely for their positive effect on learning and memory in patients with dementia. These medications are only

used after the onset of MCI or AD, as they have not shown to have any effect on the prevention of AD (Sano et al., 2008). Since current medications will not decrease the incidence of AD, preventative treatments have been the focus of research.

Successful preventative treatments would have an immense impact on the community of AD patients, even if the prevention is not 100%. Brookmeyer, Gray, and Kawas (1998) estimate that if the onset of AD is delayed by just one year, prevalence over the next 50 years could be reduced almost 25%. Healthcare costs would be reduced tremendously with a small delay in onset. With health care costs rising, prevention is becoming imperative to practicing medicine in an efficient manner. Unfortunately, prevention of neurodegenerative disorders proves to be a daunting task. The clinical trials for these treatments are considered primary preventative trials. In order for results to be valid, first the trials have to begin a few years before the onset of cognitive decline. A second setback is defining what constitutes cognitive decline or dementia, as strict diagnostic criteria do not exist. Finally, there are ethical concerns in prophylactically treating subjects for a condition they are not guaranteed to develop (Vellas, Coley, & Andrieu, 2008). These obstacles have contributed to the slow development of clinical trials; however, much of the research on treatment and preventative therapies for AD are on the cusp of generating significant clinical results.

Primary care physician assistants, as well as physician assistants working specifically with the geriatric population, will benefit from this clinical review. The new therapies involve complicated biochemistry and are unique approaches to the treatment of neurodegenerative disease. The PA should understand this information in order to follow advances in research and to explain these advances to patients. This review will sort through the vast research on

immunization for AD, allowing the PA to gain a basic understanding of therapies that may soon be available.

Background

Recently, there has been immense progress in the identification of the genetics, microbiology, and pathophysiology associated with Alzheimer's disease. Even with all of the new findings, Alzheimer's is still a very complex disease to interpret, as there is no single gene nor single predisposing risk factor that can accurately determine the onset of AD. Ultimately, AD is a disease involving protein misfolding. The protein misfolding is more common in patients carrying specific genes, and the misfolding itself leads to different biologic changes that explain the degenerative properties of AD.

The key feature of AD is the increased amount of β-amyloid (Aβ) deposited as senile plaques and neurofibrillary tangles in the affected brain (Bertram & Tanzi, 2004). The β-amyloid is produced as a result of the cleavage of the Amyloid Precursor Protein (APP) by γ-secretase and β-secretase. This resulting β-amyloid begins to aggregate together forming what is known as senile plaques. Although beta-amyloid fibrils are found in normal aging, those with Alzheimer's disease have an increased amount of the plaques and fibrils. The accumulation of these plaques has many detrimental consequences in the central nervous system such as synaptic degradation, oxidation, excitotoxicity, inflammation, demyelination, and apoptosis (Salloway, Mintzer, Weiner, & Cummings, 2008).

Tau hyperphosphorylation has also been found in the pathology of Alzheimer's disease. Tau is a normal protein involved in microtubule formation and stabilization. However, when hyperphosphorylated, tau accumulates in nerve cells as neurofibrillary tangles, affecting the transmission of impulses across neurons (Goedert & Spillantini, 2006). Cholinergic receptors and synapses have been found to be dysfunctional in AD patients as well. Evidence shows that the use of cholinesterase inhibitors improves symptoms of AD such as memory, cognition,

mood, and behavior (Grutzendler & Morris, 2001), but does not treat the disease pathology itself. Finally, genetics play a significant role in Alzheimer's disease. The most well known gene is apolipopretein E (APOE) which functions to help break down beta amyloid. The ϵ4-allele of APOE is less efficient at breaking down this protein, causing those carrying this allele to be at increased risk of developing AD. The presenelins are genes that code for proteins that allow γ-secretase to cleave the APP into beta amyloid. Mutations in these genes cause γ-secretase to cleave the APP at sites that produce more of the beta-amyloid that tends to aggregate, increasing risk of AD. The β-amyloid precursor protein, presenilin 1 (PSEN1), presenilin 2 (PSEN2), and APOE, the ϵ4-allele, have all been implicated to play a role in AD pathogenesis, particularly in the increase of Aβ (Bertram & Tanzi, 2004). All of these processes contribute to the neurodegeneration found in the brains of AD patients. Anti-amyloid treatments and preventative strategies are aiming to interfere with the production of Aβ to prevent the plaques and their secondary consequences. Other neuroprotective medications directly target those secondary processes to prevent the neuronal cell damage and death that they induce.

The most recent investigation into the treatment of AD has involved the use of immunization for treatment and prevention of AD. The very first study was begun by Elan Pharmaceuticals in 1999, and research has escalated since then. There are two broad categories of immunization strategies for the treatment and prevention of Alzheimer's disease. Antibodies can be delivered either by active or passive immunization. Immunization describes the process by which immunity is induced to provide protection from a disease through artificial means. Active immunization is when the body is stimulated, usually by a vaccine, to produce antibodies and activate other immune responses. Passive immunization is the administration of exogenous antibodies previously created in humans or animals for temporary immunity (Long, 2008).

Researchers are creating therapies for AD on the principles of passive or active immunization, both of which have clinical advantages and disadvantages.

Immunization

Many theories have been developed to determine the mechanism of action of vaccines in the treatment and prevention of Alzheimer's disease. Passive immunization utilizes immunoglobulins containing a range of different antibodies whose function involves the targeting of the beta amyloid peptide, known to be a prominent protein in the development of AD. Active immunization administers the beta amyloid peptide, specifically Aβ-42, so the body's innate immune system can develop antibodies against the foreign substance. Both methods of immunization focus on the removal and degradation of β-amyloid, only one aspect in the pathophysiology of AD. The question remains as to how the antibodies remove and attack Aβ, whether the antibodies are created via passive or active immunization.

Three current theories exist as to how these antibodies work. One theory postulates that the antibodies against the beta amyloid plaques of an AD brain induce a response from microglial cells. Microglial cells remove Aβ through an Fc receptor-mediated phagocytosis mechanism (Okura & Matsumoto, 2007). Wilcock et al. (2004) tested this theory in their study using intracranial anti-Aβ antibody administration. Their results showed that after administration of the antibodies into mice with amyloid precursor protein, the sites of administration of the anti-Aβ antibodies showed a significant number of activated microglial cells. This result was enhanced by the finding that the extent of plaque removal was correlated to the number of microglial cells activated. The second theory is that anti-Aβ antibodies directly bind and attack the beta amyloid plaques and their associated fibrils and oligomers. The results of one in-vitro study demonstrated that the antibodies against beta amyloid peptides bind directly to the N-terminal regions of the assemblies and lead to their disaggregation and solubilization, thus

deactivating the neurotoxic effects of the plaques (Solomon, Koppel, Frankel, & Hanan-Aharon, 1997).

The third theory is referred to as the peripheral sink hypothesis. Dematttos et al. (2001) developed this theory after studying the effects of peripheral administration of a monoclonal antibody against Aβ in transgenic mice that have Aβ plaques confined to the central nervous system (CNS). What they found was that the monoclonal antibody did *not* bind to Aβ plaques in the brain, however, the plaques were significantly reduced in amount and deposition in the CNS. They proposed that the peripheral administration of antibodies altered the equilibrium of Aβ between the CNS and plasma, with the antibodies in the plasma acting as a sink, pulling the Aβ fibrils from the CNS into circulation and thus neutralizing them. Although these three theories are most popular in the mechanism of action of AD vaccination, none are mutually exclusive or perfectly understood. They are, however, valuable in refining the current vaccines being developed and improving the understanding of both passive and active immunization.

Passive Immunization

The most promising immunization technique appears to be administration of anti-β amyloid via passive immunization. Bard et al. (2000) studied the effects of peripheral administration of antibodies against amyloid β-peptide for the reduction of amyloid burden in AD induced mice. An initial concern was whether the antibodies would have the ability to cross the blood-brain barrier; however, it became clear that amyloid plaques in the brain were covered with the exogenous antibodies.

One of the studies performed by Bard et al. (2000) was to determine whether the peripherally administered antibodies solely cleared the preexisting amyloid or if they prevented the formation of additional new amyloid plaques. They found that 60% of the small plaques and diffuse amyloid had been eliminated, and new plaques were not observed after 32 days of treatment with the antibodies. This finding is significant in showing that the peripheral administration of antibodies could be effective in AD-ravished human brains with large amyloid burden already causing cognitive effects in the patient. Therefore, the immunization would not necessarily have to be used as preventative treatment against AD, but could be used in those already suffering from the disease. Bard et al. (2000) also found, in a study done *in vivo*, that the antibodies introduced to the mouse models had high affinity and efficacy against deposited Aβ but not for the benign soluble Aβ. They concluded that the effect the antibodies have on deposited Aβ is more clinically relevant and efficacious than using the antibodies for prevention of amyloid plaques, as the Aβ has not yet deposited. This further showed that these antibodies may be more useful in the direct treatment of AD rather than its prevention.

To determine the mechanism by which the antibodies cleared out the pre-existing amyloid plaques, Bard et al. (2000) used confocal microscopy to visualize microglial cells and

Aβ plaques in mouse brain tissue. In the control study, the microglial cells were in a completely different plane than the plaques. With administration of the antibodies, almost all of the Aβ plaque was shown to be localized *within* the microglial cells. This response showed that the clearance of the plaques occurred via Fc receptor-mediated phagocytosis initiated by the antibodies. This finding demonstrates the effectiveness of the antibodies in inducing an immune response within the AD brain. This known mechanism of action of the peripheral introduction of Aβ antibodies allows more studies to be done to further improve their function and change their configuration to possibly be more useful in prevention. The fact that monoclonal antibodies can successfully enter the CNS and induce a phagocytic response has significant, positive implications for the use of vaccines in AD patients.

Bapineuzumab

A vaccine currently in Phase III of clinical trials is showing promise in human subjects using passive immunization. This drug, bapineuzumab, is a humanized monoclonal antibody against a terminal portion of the Aβ plaques in the brains of AD patients. The preclinical phase of the pharmaceutical study by Elan and Wyeth used the mouse model of bapineuzumab, called 3D6. 3D6 is an immunoglobulin G (IgG) 2b monoclonal antibody selective for a specific chain of amino acids at the N-terminus of the amyloid-β involved in Alzheimer's disease (Bacskai et al., 2002). Bacskai et al. (2002) reported that amyloid plaques were decreased by 48% in transgenic mice with APP. They used 3D6 lacking the Fc portion to show that the mechanism of action is most likely due to direct binding of the antibody to the Aβ plaques and the peripheral sink mechanism rather than microglial activation. The success of 3D6 on mouse models allowed the Phase I trials to begin with the humanized version of 3D6, bapineuzumab. This phase tested the safety and tolerability of bapineuzumab for it to be approved for subsequent phases. The

results from Elan corporation showed that the most serious side effects included retinal vascular disorder and cerebral vasogenic edema in the highest dose group of 5mg/kg. This dosage of bapineuzumab was discontinued for use in the study. With Phase 1 approval for safety and tolerability, the study continued on to Phase II.

Phase II was a randomized, double-blind, placebo-controlled, multiple, ascending dose study to assess the dosing, safety, efficacy, tolerability, pharmacokinetics, pharmacodynamics, and immunogenicity of bapineuzumab in patients with mild to moderate Alzheimer's disease. Participants totaled 240, and the dosing period lasted 65 weeks. The primary efficacy endpoints were changes in screening scores on the Alzheimer's Disease Assessment Scale, Cognitive Subscale, and Disability Assessment Scale (ADAS-Cog) for Dementia. Other screening tools used were the Neuropsychological Test Battery, Mini Mental Status Examination, Clinical Dementia Rating – Sum of Boxes, and Dependence Scale. Phase II results reported no statistically significant difference on Alzheimer's disease screening scores between bapineuzumab and control groups in the total study population. The results did show, however, that within the population of AD patients who do *not* carry the ApoE4 allele, there were statistically significant differences in the Alzheimer's Disease Assessment Scale, Neuropsychological Test Battery, Mini Mental Status Exam, and Clinical Dementia Rating – Sum of Boxes. Researchers also found that the ApoE4 allele non-carriers showed less brain atrophy than the placebo group and less likelihood of demonstrating the adverse effect of vasogenic edema. While ventricular volume paradoxically increased in ApoE4 carriers, it decreased in non-carriers. Vasogenic edema occurred in ten ApoE4 carrier patients and only two non-carriers. The differences reported between ApoE4 carriers and non-ApoE4 carriers have shaped the design of the third phase of the bapineuzumab study (Elan & Wyeth, n.d.). The

results of the Phase II trial of the bapineuzumab study further confirm that beta amyloid is a crucial component of AD. Although its removal has not been shown to entirely reverse the cognitive and functional impairment of the disease, it is enough evidence to continue on to Phase III of the study.

The third phase of the bapineuzumab study is intended to study the monoclonal antibody in a large scale clinical setting to determine whether bapineuzumab can be approved for use as a treatment for mild to moderate AD patients. The study began in May of 2007 and is a randomized, double-blind, placebo-controlled, parallel group consisting of 4,100 participants lasting for 18 months. Patients will receive intravenous (IV) infusions once every 13 weeks. They are divided into four groups, two of which are ApoE4 carriers and two non-carrier groups. Primary efficacy tests include the Neuropsychological Test Battery and the Disability Assessment Scale for Dementia, and the other screening tools will be treated as secondary endpoints. Imaging studies are also being performed to measure whole brain volume, ventricular volume and ventricular boundary shift integral (Elan & Wyeth, n.d.). The progress and results of the study have not been released; however, no setbacks have forced Elan and Wyeth to suspend the study.

LY2062430

Another passive immunization drug, LY2062430, has entered Phase III of the drug approval trials. This is also a monoclonal antibody like bapineuzumab, but its mechanism of action appears to be solely that of the peripheral sink as demonstrated by mouse models in previous studies. This theory is based on the equilibrium of soluble beta amyloid between the cerebrospinal fluid (CSF) and plasma. Ghersi-Egea et al. (1996) studied the movement of Aβ between these two mediums. They compared the movement of a 40 amino acid length amyloid

β-peptide to a reference material. Within minutes, 30% of the Aβ was found in plasma whereas none of the reference material was found in plasma, showing that there is movement between CSF and plasma. To determine the mechanism by which the Aβ moved, they radiolabeled soluble Aβ and found that after injection of the peptide into the CSF, the increased concentration allowed almost half of the substance to cross via simple diffusion and the remaining majority via a protein mediated mechanism. This study had implications for further research into the question of a possible decrease in the transport and clearance mechanism of Aβ in Alzheimer's patients.

To determine, more specifically, the mechanism by which soluble Aβ transported between CSF and plasma, Shibata et al. (2000) studied the peptide's clearance in a mouse model study. They found that when they inserted antibodies against LDL receptor-related protein-1 (LRP-1) and α_2-macroglobulin (α_2M) in the CSF of the healthy mouse brains, the movement of beta amyloid was slowed down, indicating that these two proteins are involved in moving soluble Aβ out of CSF. They also found that in mice lacking apolipoprotein E, clearance of beta-amyloid was reduced up to 55%. The percent cleared progressively dropped as the mice aged. This result demonstrates that proper function and amount of apolipoprotein E is vital to removing Aβ from the CNS where it can aggregate and form plaques. Shibata et al. (2000) concluded that "if the levels of Aβ in brain extracellular space exceed the transport capacity of the clearance mechanism across the blood brain barrier (BBB), or if the vascular transport of the peptide were impaired, for example by downregulation of LRP-1, this would result in accumulation of Aβ in the brain, and possibly formation of amyloid plaques" (p. 1498). These findings overall were of great significance in developing immunization strategies involving the peripheral sink mechanism theory because if an antibody can be administered peripherally and "draw out" the

Aβ fibrils and oligomers from the brain extracellular space, amyloid plaques could hypothetically be reduced and/or prevented.

To test the sink mechanism, DeMattos et al. (2001) used the monoclonal antibody m266 (the mouse version of LY2062430), and found that this antibody shows significant action in Aβ clearance from the central nervous system to the plasma. They first used an in vitro dialysis system to determine how well m266 sequestered Aβ compared to other established Aβ clearing agents like IgG, bovine serum albumin (BSA), and ApoE4. Human CSF was placed above a bottom chamber containing these various binding proteins and separated by a dialysis membrane. It was observed that IgG, BSA, and ApoE4 had a significant effect on Aβ getting drawn into the bottom chamber. Monoclonal antibody 266, however, drew in the beta-amyloid almost 20-fold more than the other binding proteins, and 50% of the Aβ was cleared to the bottom chamber with m266. The effectiveness of m266 in vitro stimulated the study of its effects in vivo. APP transgenic mice were administered a peripheral solution of m266, and plasma levels of Aβ were measured. DeMattos et al. (2001) found that when all of the m266 complexed with Aβ was removed, the levels of Aβ in the plasma were virtually undetectable, meaning the Aβ sequestered by m266 was almost all bound to the monoclonal antibody. They then measured the amount of Aβ sequestered in the plasma and found that there was a 1,000 fold increase of Aβ in the plasma bound to m266 compared to untreated mice. This finding strongly suggested that m266 can act as a sink to draw out Aβ from the CNS.

Interestingly, CSF Aβ levels increased as well. Upon further testing the researchers found that peripheral administration of m266 will cause an efflux of beta-amyloid peptide from brain interstitial spaces (where it can aggregate and cause most injury to brain tissue) into brain extracellular space mostly occupied by CSF. Since another part of the study determined that

only an insignificant amount of m266 crossed from plasma into the CSF, the findings show that presence of the antibody in the periphery alone could account for the increase of soluble Aβ in the CSF, and thus a decrease in brain interstitium beta-amyloid. This study became the precursor to the development of the humanized version of monoclonal antibody 266, called LY2062430.

Eli Lilly and Company is entering Phase III of clinical trials for its monoclonal antibody, LY2062430. This antibody targets the central domain of beta amyloid, working by the same mechanism as m266. The progression from Phase I to Phase II of the study indicates that the safety of LY2062430 was established, important due to the serious adverse effects previously shown with passive immunization methods (Nitsch & Hock, 2008). Phase II results were recently released by Eli Lilly and Company (Eli Lilly, 2008) indicating that they were ready to move on to Phase III of the trial. Phase II was a randomized, controlled trial in which both healthy volunteers and mild to moderate AD patients were given intravenous LY2062430. Using information obtained from previous studies involving m266, the levels of beta amyloid were measured in the participants' plasma and CSF. These levels would indicate how well the monoclonal antibody is sequestering Aβ from the interstitial space of the brain. Increased levels of the peptide in plasma and CSF would mean that LY2062430 is indirectly drawing the amyloid beta out of the brain where it can cause damage and into the CSF and plasma where it can be cleared away in its soluble form. The study lasted 12 weeks and 52 AD patients were given 100mg or 400mg infusions once a week or once every four weeks. Magnetic resonance imaging (MRI), CSF examinations, and single photon emission tomography (PET) were performed to assess drug performance. Cognitive scores were also assessed, however, due to the extremely short duration of the study, researchers correctly predicted that no positive change would be observed (Eli Lilly, 2008).

The outcome of the Phase 2 trial showed a significant increase in both blood and cerebrospinal fluid levels of beta amyloid, along with an increase in two other types of amyloid beta protein thought to only exist in the pathologic amyloid plaques of Alzheimer's patients. The beta amyloid can then be cleared rather than deposit as plaques in the brain tissue. These increases were observed for several weeks, so researchers predict with longer term treatment, reversal and dissolution of amyloid plaques in the brain may occur and be associated with improvement in cognitive and functional status. Another important observation was the absence of brain inflammation or bleeding in study participants as indicated by MRI and CSF evaluations. No other side effects were noted in association with the treatment. These findings allowed for further progression to Phase III of the LY2062430 drug trial which will begin this year (Eli Lilly, 2008).

IVIG

Newer research and developing human trials are becoming established for the use of intravenous immunoglobulins (IVIG) for the treatment and prevention of Alzheimer's disease. IVIG is presently very widely used for the treatment of a variety of immunologic disorders, however, it has not been approved for AD. The difference between the use of IVIG and the other immunization therapies discussed is that IVIG is a *polyclonal* antibody solution whereas all of the other strategies have involved *monoclonal* antibody solutions (Relkin et al., 2008). Since monoclonal antibodies are selective for a very specific ligand, it was expected that they would be more effective in targeting Aβ fibrils and oligomers. Studies have shown, however, that the polyclonal antibodies exert very similar effects on Aβ and the interaction of the innate human antibodies against Aβ with the other components of a polyclonal antibody solution may contribute to the positive results observed in trials (Relkin et al., 2008).

It has been found that naturally occurring antibodies against amyloid beta peptides appear in human CSF and plasma, and people with Alzheimer's disease have significantly reduced levels of these innate antibodies (Du et al., 2001). Since IVIG is procured through collection of nonselective antibodies from various healthy human patients, researchers have been studying the administration of IVIG in AD patients to potentially increase their levels of naturally occurring anti-amyloid antibodies. In 2002, Dodel et al. devised a human study to investigate the effects of commercially available IVIG products on human serum and CSF Aβ levels. While looking for the effect of IVIG on beta amyloid already present in serum and CSF, they observed a statistically significant decrease in levels of beta-amyloid peptides in the CSF of the patients following treatment. Increased levels of anti-amyloid antibodies were found in the CSF as well, providing an explanation for the decreased amounts of Aβ. They also found that Aβ and Aβ antibody levels increased in serum, but not significantly. Dodel et al. (2002) hypothesized that this increase may be due to the CSF Aβ crossing over into the plasma and getting locally metabolized there.

In 2004, Dodel et al. performed another small human study using IVIG. They administered IVIG monthly in five patients with clinically probable or clinically possible Alzheimer's disease and measured CSF and serum Aβ levels along with performance on cognitive tests. No control group was utilized in this study. Similar results to their previous studies were attained in that CSF Aβ levels decreased and serum levels increased but not significantly. Anti-Aβ antibodies increased in both locations. They did find, however, that patients improved by a mean of 3.7 ± 2.9 points on the Alzheimer's Disease Assessment Scale-cognitive subscale. Although these patients were currently taking cholinesterase inhibiters as their AD treatment, the cognitive improvement was significant because cholinesterase inhibitors

have been found to correspond with a decrease in decline from an untreated seven to eleven points per year to an average of 6.6 points per year with the drug (Rogers & Friedhoff, 1998). Since IVIG showed an improvement in cognitive score, rather than a decreased slope in decline, use of this treatment for AD looked promising.

With the information gathered, Weill Cornell Institutional Review Board and the Scientific Advisory Committee of the Weill Cornell General Clinical Research Center approved a Phase 1 trial to begin for IVIG therapy in AD. Relkin, Szabo et al (2008) headed the study which consisted of eight mild AD patients who received IVIG treatment for six straight months, discontinued for three months, then resumed treatment for the following nine months. Patients were randomly assigned to receive IV infusions every one, two, or four weeks and then all patients received varying doses of IVIG every two weeks beginning at month ten. Aβ and anti-amyloid antibody levels were measured in the CSF, and plasma and cognitive tests were performed throughout the course of the study. Three patients had measurable, but very low, titers of anti-Aβ antibodies, and five patients had no measurable anti-Aβ in their serum. Antibody titers after infusion were much higher in the three patients who had measurable levels before administration. Both groups had significantly increased their antibody levels, appearing to double their counts even with the lowest possible dose of IVIG. CSF levels of Aβ significantly dropped after six months of therapy, returned to baseline levels during the discontinuation, then dropped again during the subsequent nine month treatment period. Serum Aβ increased in plasma, but not significantly, indicating that Aβ was mobilized from CSF to plasma. Since CSF Aβ levels returned to their baseline levels during the three month discontinuation phase, IVIG must be given on a consistent, continuous schedule. However,

plasma antibody levels increased with each consecutive IVIG treatment, indicating that a longer study must investigate this further.

Relkin et al. (2008) also proposed that since the polyclonal antibodies from IVIG seem to exhibit very similar effects to peripheral monoclonal antibodies, some interaction may exist between the various antibody components of IVIG allowing it to be effective in AD treatment. A large caveat to the recent studies using IVIG is the use of very small sample sizes. It is hard to determine the effects of IVIG on the general population based on a sample of five to eight participants. No serious adverse events were reported with the IVIG infusions, although mild symptoms were reported that are common in all populations of patients receiving IVIG. One of the largest detriments to using IVIG is most likely its high cost and low availability, a problem encountered with the monoclonal antibodies as well. Safety and efficacy must still be established, so the trial progressed to Phase 2 in order to incorporate more AD patients and study IVIG effects on a larger AD population.

The Phase 2 trial for IVIG treatment was a placebo-controlled, randomized, double-blind clinical study for the treatment of mild to moderate AD (U.S. National Institute of Health, 2006a). The study involved 24 patients, 16 of whom received IVIG infusions for six months and then discontinued for six weeks. Different doses were administered to evaluate which dosage is optimal for clinical improvement. The other eight received a placebo. Aβ levels in plasma and CSF were measured, with the primary outcome measures to be the ADAS-Cog and AD Cooperative Study - Clinical Global Impression of Change (ADCS-CGIC) changes. Since researchers are hoping to use IVIG as an "add-on" treatment, they included only participants who were currently taking AD medications or had contraindications to taking the oral medications (U.S. National Institute of Health, 2006a).

Results of the Phase 2 study report that IVIG administration is successful in improving cognitive and functional skills deteriorated by AD (Results of 9-month phase II study of Gammagard intravenous immunoglobulin, 2008). The length of time for IVIG infusions was extended from six months to nine months. Statistically significant differences were observed in ADCS-CGIC scores with an average 1.2 points increase compared to placebo at six months, and 1.4 point increase at nine months. The ADAS-Cog reached statistically significant improvements in the treatment group at three and nine months. No statistically significant differences were reported for patients' ADLs. Some patients declined in their ADLs whereas others improved, so no correlation could be made. However, it should be noted that the placebo group appeared to drop more points on the ADL scale. The results of the CSF and serum tests have not yet been reported. The lack of adverse events and presence of significant improvements in cognitive test scores have allowed the study to continue on to Phase 3. Since the Phase 2 study only consisted of 24 patients, the Phase 3 portion will incorporate many more patients across the nation, giving a better indication of the efficacy of using IVIG in Alzheimer's disease treatment (Results of 9-month phase II study of Gammagard intravenous immunoglobulin, 2008).

Active Immunization

Active immunizations are now emerging as potential treatment options for Alzheimer's disease. When the use of vaccination for Alzheimer's disease was first proposed, active immunization seemed to be the most viable option creating much excitement among both the Alzheimer's and medical communities as preventing or treating AD seemed conceivable. Schenk et al. (1999) tested a vaccination strategy against Aβ. They used transgenic mice predisposed to overproducing the amyloid precursor protein, thus making them more likely to overproduce Aβ-42, the beta-amyloid fragment that is prone to aggregate and form the plaques associated with AD. They vaccinated these mice directly with Aβ-42 at six weeks, to analyze preventative potential of the vaccine, and at eleven months, to observe treatment efficacy of already manifested AD. Seven out of the nine mice that were immunized at six weeks had no evidence of beta-amyloid deposits in their CNS, indicating the vaccination prevented its deposition. The older mice that already had existing beta-amyloid deposits had 81% less Aβ at 15 months of age compared to untreated mice of the same age, indicating active removal of the plaques by the antibodies induced by the vaccination. Schenk et al. (1999) concluded that immunization with Aβ-42 "either prevents deposition and/or enhances the clearance of Aβ from the brain" (p. 175). This study was one of the first to describe immunization potential for AD.

Following the Schenk et al. study, researches began questioning the cognitive effects that the reduction in beta-amyloid has on mouse models with AD. In order to determine these behavioral changes in mice immunized with Aβ-42, Janus et al. (2000) used transgenic mice over-expressing APP with spatial and learning deficits associated with the increase in Aβ. These mice were immunized with Aβ-42 at 6, 8, 12, 16, and 20 weeks while being monitored for their anti-Aβ antibody titers. They found that the immunization caused a two to three fold increase in

antibody titers by 23 weeks and observed a "50% reduction in the number and size of Aβ-positive dense-cored plaques." Janus et al. (2000) then had the mice perform the Morris water maze test at 11, 15, 19, and 23 weeks. They reported that the immunized mice performed statistically significantly better than non immunized mice at every age. These results gave evidence that Aβ-42 immunization can either reverse or slow the cognitive decline typical of Alzheimer's disease.

Morgan et al. (2000) elicited a very similar finding in transgenic mice with excess APP and learning deficits. These mice were immunized with Aβ-42 and had to perform the radial-arm water maze testing at 11.5 months and at 15.5 months. At 11.5 months, when the untreated mice did not yet exhibit memory deficits common to AD mice, the immunized mice performed statistically significantly better on the memory testing than non-immunized mice. At 15.5 months, when untreated mice had significant memory loss, the immunized mice performed just as well as the mice that were *not* genetically predisposed to learning and memory deficits. These findings led to the development of the human vaccine developed for Phase I clinical trials.

AN1792

The Aβ-42 vaccination, AN1792, was approved to be tested for safety and tolerability in humans in the Phase 1 of trials. The vaccination was given in combination with QS-21 which is an adjuvant compound intended to stimulate and enhance the body's natural immune response against the antigen present within the vaccine (Antigenics, 2008). The Phase 1 trials indicated the proper dosage of both AN1792 and QS-21 necessary to elicit an adequate antibody response along with maintaining its safety in a small sample of patients (Gilman et al., 2005).

These results allowed investigators to move on to the Phase 2a of the trial. Gilman et al. (2005) described the study as a double-blind, placebo-controlled, multicenter study intended to

determine the safety and tolerability of AN1792, along with initial observations of efficacy. Primary endpoints were initially set to observe changes in whole brain volume by MRI and cognitive changes by the ADAS-Cog. Three hundred and seventy-two patients were enrolled in the study; 300 received AN1792 and 72 received a placebo. The suspension was administered via a single intramuscular (IM) injection, intended to be given on the first day of the study and months 1, 3, 6, 9, and 12. Unfortunately, the study was cut short as a result of 6% of the patients developing meningoencephalitis, and a new primary endpoint was set to determine safety alone. The patients continued to be followed nine months after discontinuation of treatment. The truncated trial resulted in patients receiving between one and three injections, rather than the original six injections planned. Using the limited information available, researchers found that about 20% of those immunized developed sufficiently high antibody titers to be considered responsive to the treatment, consistent with their hypothesis. The most common adverse side effects to treatment were meningoencephalitis, headache, and confusion, occurring mainly in the AN1792 treated group. The meningoencephalitis occurred solely in the AN1792 treated group.

In terms of cognition, researches found that there were *no* differences between the treatment groups in any of the cognitive function tests. The patients who were labeled as antibody responders appeared to have greater improvement on the scores although not significantly. At month 12 (at least six months after the study was cut short) the composite Neuropsychological Test Battery (NTB) z-score showed statistically significantly less worsening in the patients with antibody response compared to placebo. The finding that 20% of the patients became antibody responders and at 12 months showed cognitive improvement after only one to three injections indicated that the vaccine did partially achieve the original goals set by the

researchers. These significant results prompted scientists to investigate the cause of meningoencephalitis so that the vaccine research could continue forward.

Nicoll et al. (2003) determined that the meningoencephalitis incurred by one of the patients in the trial was a T-cell mediated meningoencephalitis. This prompted researchers to determine what could have induced a T-cell response from the vaccination (which should induce a humoral response). They found that the T-cell response was most likely due to the change in formulation of the vaccine in the middle of the Phase 1 trial. The new formulation added polysorbate-80 to keep the vaccine from precipitating out of solution (Gilman et al., 2005). The T-cell response against this new polysorbate may have caused the meningoencephalitis because no cases of meningoencephalitis were reported before it was added to the solution. Thus, researchers believe that it was the T-cell response and not the antibodies themselves that caused this serious adverse event (Pride, Black, & Hagen, 2003). The cognitive benefit exhibited by the AN1792, even though interrupted, gave significant incentive to reconfigure the vaccine to prevent the T-cell response for further research.

ACC-001

A new vaccine was created based on the original design of AN1792. The intention of this new vaccine is to eliminate the T-cell response that caused meningoencephalitis but retain the antibody response against Aβ-42 (Pride et al., 2008). Pride et al. created ACC-001 which is a vaccine consisting of only the N-terminal fragment of Aβ. This fragment is not long enough to bind to the molecules that would induce a T-cell response. The lack of T-cell response and successful anti-Aβ-42 antibody response in mouse and computerized models have allowed ACC-001 to advance to preclinical trials (Pride et al., 2008).

ACC-001 conjugated with QS-21 entered Phase 1 of human clinical trials in the fall of 2005 under the sponsorship of Elan and Wyeth pharmaceuticals. Patients with a diagnosis of mild or moderate AD were administered a single dose of ACC-001 to determine the safety and tolerability of the redesigned active immunization vaccine (Alzheimer Research Forum, 2008). The success of Phase 1 allowed the vaccine to continue on to Phase 2 of drug trials. Elan and Wyeth began recruiting participants in October of 2008 for a "multicenter, randomized, third-party unblended, multiple ascending dose, safety, tolerability, and immunogenicity trial of ACC-001 in subjects with mild to moderate Alzheimer's disease"(U.S. National Institute of Health, 2009). Wyeth has announced that the primary measurement will be safety, whereas cognitive and functional measures will be secondary. Participants receive ACC-001 with QS-21, ACC-001 without QS-21, a placebo consisting of only QS-21, and a placebo of phosphate buffered saline. The companies hope to finish the trial in 2012 with ambitions to move on to Phase 3.

CAD106

Other researches have been studying alternative ways to avoid the T-cell response that induced meningoencephalitis in a portion of the AN1792 drug trial. Chackerian et al. (2001) found that conjugating antigens at a specific density and arranged in a specific pattern to the surface of non-infectious virus like particles (VLPs) can induce a high and clinically relevant antibody titer against the antigen. In 2006 they tested this with the Alzheimer's disease specific antigen Aβ (Chackerian, Rangel, Hunter, & Peabody). They determined this active immunization technique would be useful in AD because it would build up an adequate antibody response against Aβ but would avoid the T-cell response against Aβ. Instead of the T-cell immunity building up directly against the Aβ structure, it would instead be aimed at the VLP that is conjugated to the Aβ, avoiding the adverse events caused by Aβ T-cell response. Since some

T cell activation is necessary to build a humoral response against an antigen, the T-cell response against VLPs would be adequate for B-cell activation, retaining the ability to induce a large antibody titer post vaccination. This antibody response would be large enough to eliminate the need of adding an adjuvant, such as QS-21, used in the AN1792 study.

Chackerian et al. (2006) tested their Aβ conjugated to VLPs vaccine in a mouse model. Their results showed that an adequate antibody response was achieved, whether an adjuvant was used or not. They then used a bacteriophage called Qβ as the VLP to be conjugated to the antigen Aβ. The researchers indicated that this bacteriophage is a useful VLP to use as its structure and function has been very well established. They found that a very large antibody response was induced with immunization using Aβ linked to the bacteriophage Qβ. Another portion of their study showed that when the immunized mice were restimulated with the antigen Aβ there was a negligible, or no T-cell response toward it. The only strong T-cell response was against the VLP itself. They described the T-cell response as a one less inflammatory in nature, determined by the specific IgG antibodies that were elicited by the T-cell activation. The findings of this report were very suggestive of a new technique in active vaccination and provided promising results that led to this mouse model's modification to human trials.

Novartis Institutes for BioMedical Research developed CAD106, which is a beta-amyloid fragment coupled to the virus-like particle, Qβ (Staufenbiel et al., n.d.). They found that using the fragment consisting of the first six amino acids of the beta-amyloid (Aβ1-6) in mouse models would avoid a T-cell response due to its short length and the T-cells' inability to bind to such a short segment. Attaching it to Qβ would further ensure that a T-cell response would not be activated specifically against Aβ but towards the virus-like particles. In their animal models, Staufenbiel et al. (n.d.), found that 100% of those immunized developed an antibody response,

and brain studies showed that the antibodies attacked the plaques typical of AD. These mouse models also showed that immunization both prevented amyloid deposits in APP transgenic mice and reduced amyloid deposits in AD manifested APP transgenic mice. The success against amyloid deposits and reduction of a T-cell response allowed Novartis to progress on to Phase 1 studies of CAD-106.

Phase 1 of the study for CAD-106 as an Alzheimer's treatment and prevention drug was a 52-week, multi-center, randomized, double-blind, placebo-controlled study in 60 patients with mild to moderate Alzheimer's disease to investigate the safety, tolerability, and Aβ-specific antibody response following three subcutaneous injections of CAD-106. The primary measures were to assess the safety and tolerability of the drug, however, they also involved measuring the antibody response following the injections in the participants. Secondary measures included cognitive assessments of the participants (U.S. National Institute of Health, 2006b). Phase 1 was completed in 2008. Due to a positive antibody response and lack of serious adverse events, CAD 106 was able to enter Phase 2 of the drug trial (U.S. National Institute of Health, 2008).

Novartis is currently enrolling participants to enter Phase 2 of the CAD-106 drug trials. This study will also last 52 weeks, but will include only patients with mild AD and will allow participation of only 30 individuals. Safety and tolerability will again be assessed and the immune response and cognitive and functional changes will be included as secondary measures. The researchers want to focus on the response of participants to multiple injections of the CAD-106 vaccine. This study is planning its completion in 2010 and from there will determine if the drug will enter Phase 3 (U.S. National Institute of Health, 2008).

V950

Another active immunization drug, V950, has reached Phase 1 human trials. Rush University Medical Center is currently enrolling patients with mild to moderate Alzheimer's disease into the study. They will receive either V950 or the placebo drug (Lee, 2008). Merck has sponsored the Phase 1 effort, and four different arms of the study were created to test V950's effectiveness if given with the adjuvant, Iscomatrix. V950 is an N-terminal fragment of Aβ (Lee, 2008) and its link with Iscomatrix is predicted to induce a large titer of antibodies against the N-terminal of Aβ associated with AD. Approximately 70 participants will either receive V950 at increasing doses alone, or V950 at increasing doses with increasing doses of Iscomatrix. They will receive a total of six injections per year over a total of four years. Safety and tolerability will be measured along with the immune response after each dose. The study is projected to be completed in October of 2013 and from there will advance to Phase 2 if deemed appropriate (U.S. National Institute of Health, 2007)

Other Therapies

Neural Stem Cells

Although immunizations appear to be at the forefront of the development of advances in AD treatment and prevention, there are several other approaches being investigated. Much media attention has focused on the use of neural stem cells for use in AD. Although no human trials are currently in progress using stem cell therapy, new technology and scientific discoveries have allowed this research to advance exponentially. Sugaya and Merchang (2008) state that neuroreplacement therapy would involve producing neurons *in vitro* from human neural stem cells (HNSCs) obtained from both fetal and *adult* human central nervous systems and then transplanting them into brain tissue. They discuss the success of this strategy in Parkinson's disease (PD), reporting that most of the research done on NSCs for AD is being mimicked from PD. For example, dopaminergic cells are produced from HNSCs and transplanted into the basal ganglia of PD brains. These newly functioning neurons aid in the release of increased levels of dopamine for symptomatic relief of PD. Their study involving the transplantation of HNSCs into aged rat models indicated an improvement in behavior and functioning. In some instances this improvement was more dramatic in the aged, memory impaired mice than the normal functioning mice.

The success of NSC transplantation in transgenic mice shows much promise for stem cell technology in treatment and prevention of AD; however, it has not been established how these stem cells will react in a human AD brain. Although many similarities exist between the human brain and mouse model, the human AD brain environment may alter the adaptation of the neural stem cell biology, making it difficult to begin human trials in this research.

Antibiotics

Some studies have offered evidence for a role of antibiotics in the treatment and prevention of Alzheimer's disease. Balin et al. (1998) have completed post-mortem studies of the brains of patients with late-onset AD and analyzed them for the presence of *Chlamydia pneumoniae*. This bacteria is present in many older individuals, manifesting as a respiratory pathogen in pneumonia, sinusitis, and bronchitis. Evidence has shown that the inflammatory response induced by *Chlamydia pneumoniae* infection may occur in the same areas of neurologic damage seen in AD brains. *C. pneumoniae* was isolated from AD damaged brains in 17 out of 19 Alzheimer's patients, while 18 out of 19 control patients revealed no presence of the organism. Balin et al. suggest that "*C. pneumoniae* is present, viable, and transcriptionally active in areas of neuropathology in the AD brain, suggesting that the infection may be a risk factor for AD" (p. 23). It is still unclear whether the bacteria has a causal relationship with AD, or if it is simply an opportunistic invader of an already damaged organ (Balin et al., 1998). The presence of bacteria in AD brains has stimulated research in the use of antibiotics for AD.

Several researchers have studied the potential of using rifampicin for the use in AD treatment and prevention. Rifampicin is an antibiotic typically used in the treatment of leprosy. The use of this antibiotic is based on the finding that non-demented elderly patients suffering from leprosy who were treated with rifampicin had a significant *absence* of senile plaques in their brains (Namba, Kawatsu, Izumi, Ueki, & Ikeda, 1992). Rifampicin inhibits Aβ1-40 peptide aggregation and neurotoxicity in a concentration dependent manner in-vitro. Its activity appears to involve the naphthohydroquinone structure of the antibiotic which carries anti-free radical function (Tomiyama et al., 1996). This finding supports the potential of using antibiotics in AD therapy.

Other antibiotics studied for their potential in Alzheimer's disease are the tetracyclines. The tetracyclines, tetracycline and doxycycline, were chosen for research due to their favorable central nervous system profile. It was found that both tetracyclines reduced amyloid fibril formation, enzymatically degraded Aβ1-42, and exerted a de-fibrillogenic effect against pre-formed beta-amyloid fibrils in in-vitro studies. These findings were compared to other antibiotics, like gentamicin, which exhibited none of the above effects (Forloni, Colombo, Girola, Tagliavini, & Salmona, 2001). Doxycycline and rifampicin were used in conjunction in a human trial for the uses of these antibiotics in AD therapy. The outcome measured was the Standardized Alzheimer's Disease Assessment (SADA). At six months after a three month course with these antibiotics, there was significantly less worsening in the SADA cognitive subscale score compared to placebo. These researchers believe that their effect appears to lie in anti-inflammatory and anti-neurofibrillary tangle activity (Loeb et al., 2004). These two drugs show promise in their use to reverse some of the cognitive changes that appear in AD patient brains.

Secretase inhibitors and activators

Gamma-secretase, the enzyme that cleaves amyloid precursor protein into the Aβ peptide, has been the focus of many emerging therapies as well. The intention is to block the activity of gamma-secretase to subsequently block the production of Aβ peptide. A gamma-secretase inhibitor has been developed by Eli Lilly, called LY450139. After trials were successful in mouse models with Alzheimer's disease, the effects of the inhibitor were studied in humans. Seventy patients with mild to moderate AD received six weeks of therapy with LY450139. They found that plasma Aβ was reduced by an average of 38.2%, with the lowest concentration found at three hours post administration and a return to baseline after seven hours. The reduction in

CSF Aβ was not found to be significant, drawing concern about the drug's ability to cross the blood brain barrier. Later studies will examine the cognitive changes that this reduction in plasma Aβ induce in AD patients (Siemers et al., 2006).

In addition to inhibiting gamma-secretase, researchers are determining whether it would be therapeutic to *activate* alpha-secretase. This enzyme is considered anti-amyloidogenic, because it cleaves the amyloid precursor protein into two fragments that do not form Aβ oligomers, and thus prevents formation of amyloid plaques. Unfortunately, it is easier to block an enzyme with a medication than to activate an enzyme through a stimulation of gene transcription and translation. Mouse models have shown promise, however, the use in human models will require more extensive study (Lichtenthaler & Haass, 2004).

Chelators

An innovative development using metal chelators has progressed to human clinical trials in the treatment of AD. It has been found that the beta-amyloid plaques found in patients with AD contain accumulations of both copper and zinc molecules. The effect of metal chelators, which bind and inactivate copper and zinc, on beta-amyloid accumulation in the brain was studied (Cherny et al., 2001). Clioquinol, an antibiotic, was chosen as a chelator because of its extensive use until the 1970's and its ability to cross the blood brain barrier. After mouse models with AD were shown to inhibit and possibly reverse accumulation of Aβ after nine weeks of treatment (Cherny et al., 2001), human trials were initiated. In a Phase II clinical trial, 36 AD patients were administered clioquinol for 36 weeks. Aβ42 levels decreased significantly after week 20, while cognitive improvement, or slowed decline, was significant in only the most severely affected subjects. While these findings showed promise, many side effects exist with

the metal chelators, and the benefit-risk ratios must still be investigated further to analyze effectiveness in AD treatment and prevention (Ritchie et al., 2003).

Testosterone

Previous studies have shown that low testosterone levels correlate with AD development in men independent of health status, age, or education (Moffat et al., 2004). To investigate the effect of testosterone replacement, Cherrier et al. (2005) executed a human study in which nineteen men, between the ages of 63 and 85, with AD or mild cognitive impairment, received weekly injections of Testosterone enanthate. Cognitive measurements were done before initial injection, at three weeks, and at six weeks. Spatial memory and constructional ability significantly improved, and verbal memory showed a trend toward improvement. As these abilities can be some of the most devastating effects of AD, these improvements could greatly improve the quality of life of men suffering form Alzheimer's disease. The brevity of the study does not provide information on the long term effects of testosterone supplementation, therefore, long term studies must be performed. As many men tend to have decreased levels of testosterone as they age, this new therapy may have a significant impact in the AD community.

Insulin

Another study by Regar et al. (2008) tested the effects of intranasal insulin administration in Alzheimer's disease or MCI patients. Insulin has been shown to have an effect on brain signaling in the CNS. Intranasal administration of insulin was found to be the most effective and safest mode of entry into the CNS, preventing dramatic decreases in blood sugar through intravenous administration. Thirteen patients received 20 units twice a day of intranasal insulin. The patients treated with insulin experienced an increased proportion of verbal information retained after a delay period, improved attention, and improved functional status. The change in

functional status was most therapeutic to AD patient's caregivers, showing the importance of not only cognitive improvement, but functionality in treatment of Alzheimer's disease (Reger et al., 2008). This study offers a gateway into further investigation of the usefulness of insulin in prevention and treatment of AD.

Conclusion

Current ethical limitations exist in Alzheimer's disease research, minimizing the available study population. When considering the development of preventative treatments for AD, the most reasonable cohort of patients to be invited for research would be those who have not yet manifested cognitive impairment but are predisposed to progressing to that level. The question remains of how researchers can screen for this particular population. With genetic screening becoming more widely available, testing patients for familial AD (those with mutations in PSEN1, PSEN2 and/or APP) has become a reality. Unfortunately, most of the immunization studies have found that vaccinations are not as effective in patients with early onset familial AD. In addition, this form of AD occurs in only 5-10% of cases. The identification of ApoE4 as a genetic biomarker for development of late-onset Alzheimer's disease has offered a screening tool for late onset AD, however, this marker does not guarantee that the individual will develop Alzheimer's nor can it predict the severity of the disease. For this reason, it is difficult to choose patients to participate in preventative studies and measure the outcomes, because it is not certain that patients will develop AD. In order for the vaccine to enter the public market, a target population must be established. More research must be done to determine the characteristics of that population in order for the vaccinations to be most effective. As screening tools improve for AD, the usefulness of vaccines as a preventative therapy will become clearer.

Research and drug trials require extremely large amounts of money. The larger the studies and the more expensive the involved drugs are, the more money is necessary to fund the research. It appears that most of these studies result in improvement of one aspect of AD rather than general improvement with clinical significance. Since definitive and clear results have yet

to be established from previous research, pharmaceutical companies are weary of funding follow up investigations. Not only are pharmaceutical companies hesitant, but it is difficult to convince the public of the safety of these new research products. The AN1792 research trials were made very public in the media, and many people had high hopes for the drug. Unfortunately, the complications of the study and unanticipated side effects were also readily publicized and the public understandably does not have the same interest and expectations for a new "so-called" vaccine. More significant evidence for benefit of immunization must be shown to receive the money and public support necessary to continue AD vaccine research.

Alzheimer's disease immunization is becoming a very tangible reality as human trials continue to progress. Many trials have shown impressive results that have the potential to alleviate many of the burdens of AD to both patient and caregiver. The success in reducing the β-amyloid burden in the CNS has provided more information on the pathophysiology of AD along with an understanding of where the therapeutic focus should be for treatment and prevention. A reduction in Aβ plaques secondary to vaccination has shown cognitive benefits and in some cases reversal of cognitive deterioration based on very specific cognitive scoring systems. Unfortunately, although these are significant findings to researchers and scientific minds, patients and caregivers commonly do not notice these effects in their daily lives. Caregivers and patients are not as concerned with an extra point on a memory test or a percentage decrease in Aβ plaques as they are with an improvement in basic functional performance in activities of daily living. If certain levels of improvement on tests were better correlated with improved functional status, more specific set points could be established for the various research trials.

It would also be useful to find out which functional defect in the AD patient is most detrimental and burdensome to both caregiver and patient. Research could then be more focused on a certain functional improvement. Since functional improvement is very related to statistical, pathologic improvement in the AD brain and CNS, it is still crucial to see these significant data increases after immunization. However, this only shows that more extensive research must be done to more fully understand the correlation between statistical significance and functional significance.

The clinical trials have not spanned an adequate amount of time to determine how effective treatments are long-term in patients and if they would only delay the onset of severe cognitive decline or offer significant lifelong results. Many trials have spanned as little as six months, which does not appear very long when researching a disease that people can suffer from for almost thirty years. Although statistically significant improvements are noted after vaccination studies, the short study intervals cannot determine the length of time the improvements last, the quality of the improvements, and the differing effect that varying doses of drugs may have. Longer studies with post-mortem analysis may offer answers to these crucial questions.

In light of the uncertainties that still exist in the investigation of new therapies for the treatment and prevention of Alzheimer's disease, vaccinations and other new modalities of treatment can only offer temporary cognitive improvement that has yet to show significant impact in the daily lives of AD patients and caregivers. This being said, any improvement in cognitive function in AD is a significant accomplishment, and any research that allows for a better understanding of the disorder is a forward step. Out of all the treatments that exist, research shows that vaccinations are promising to be very effective in ameliorating the

deleterious effects of Alzheimer's disease. As research and technology progress, AD immunization may be the answer to preventing one of the most prevalent and destructive diseases in humans.

The primary care Physician Assistant will undoubtedly encounter Alzheimer's disease in practice, whether it is a patient suffering from the illness or a caregiver dealing with the projected stress of the disease. Patients and caregivers usually only understand AD from the media's negative portrayal of the disease or stories and observations from friends and family who have encountered the illness. The primary care PA must be able to explain AD to the patient along with offering current information on drugs and therapies available. This clinical review allows the PA to understand the various types of vaccines being researched. This will offer hope to the patient and caregiver that research *is* progressing and improvements have been seen. Also, by recognizing the immunizations and other therapies that are currently being researched, the PA may be able to direct the patient to participate in various research studies, possibly providing the patient with relief or a breakthrough in research. Alzheimer's disease is an extremely frightening and stressful illness to cope with, and the Physician Assistant must be a reliable source of information, hope, and guidance.

References

Alzheimer Research Forum. (2008). Drugs in clinical trials: ACC-001. Retrieved January 12, 2009, from http://www.alzforum.org/drg/drc/detail.asp?id=102

Antigenics. (2008). QS-21 immune adjuvant. Retrieved January 21, 2009, from http://www.antigenics.com/products/tech/qs21/

Bacskai, B. J., Kajdasz, S. T., McLellan, M. E., Games, D., Seubert, P., Schenk, D., et al. (2002). Non-Fc-mediated mechanisms are involved in clearance of amyloid-beta in vivo by immunotherapy. *Journal of Neuroscience, 22*(18), 7873-7878.

Balin, B. J., Gerard, H. C., Arking, E. J., Appelt, D. M., Branigan, P. J., Abrams, J. T., et al. (1998). Identification and localization of Chlamydia pneumoniae in the Alzheimer's brain. *Medical Microbiology and Immunology, 187*(1), 23-42.

Bard, F., Cannon, C., Barbour, R., Burke, R. L., Games, D., Grajeda, H., et al. (2000). Peripherally administered antibodies against amyloid beta-peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease. *Nature Medicine, 6*(8), 916-919.

Bertram, L., & Tanzi, R. E. (2004). The current status of Alzheimer's disease genetics: what do we tell the patients? *Pharmacological Research, 50*(4), 385-396.

Brookmeyer, R., Gray, S., & Kawas, C. (1998). Projections of Alzheimer's disease in the United States and the public health impact of delaying disease onset. *American Journal of Public Health, 88*(9), 1337-1342.

Brookmeyer, R., Johnson, E., Ziegler-Graham, K., & Arrighi, H. M. (2007). Forecasting the global burden of Alzheimer's disease. *Alzheimer's and Dementia, 3*(3), 186-191.

Chackerian, B., Lowy, D. R., & Schiller, J. T. (2001). Conjugation of a self-antigen to papillomavirus-like particles allows for efficient induction of protective autoantibodies. *Journal of Clinical Investigations, 108*(3), 415-423.

Chackerian, B., Rangel, M., Hunter, Z., & Peabody, D. S. (2006). Virus and virus-like particle-based immunogens for Alzheimer's disease induce antibody responses against amyloid-beta without concomitant T cell responses. *Vaccine, 24*(37-39), 6321-6331.

Cherny, R. A., Atwood, C. S., Xilinas, M. E., Gray, D. N., Jones, W. D., McLean, C. A., et al. (2001). Treatment with a copper-zinc chelator markedly and rapidly inhibits beta-amyloid accumulation in Alzheimer's disease transgenic mice. *Neuron, 30*(3), 665-676.

Cherrier, M. M., Matsumoto, A. M., Amory, J. K., Asthana, S., Bremner, W., Peskind, E. R., et al. (2005). Testosterone improves spatial memory in men with Alzheimer disease and mild cognitive impairment. *Neurology, 64*(12), 2063-2068.

DeMattos, R. B., Bales, K. R., Cummins, D. J., Dodart, J. C., Paul, S. M., & Holtzman, D. M. (2001). Peripheral anti-A beta antibody alters CNS and plasma A beta clearance and decreases brain A beta burden in a mouse model of Alzheimer's disease. *Proceedings of the National Academy of Sciences United States of America, 98*(15), 8850-8855.

Du, Y., Dodel, R., Hampel, H., Buerger, K., Lin, S., Eastwood, B., et al. (2001). Reduced levels of amyloid beta-peptide antibody in Alzheimer disease. *Neurology, 57*(5), 801-805.

Elan, & Wyeth. (n.d.). Bapineuzumab (AAB-001) backgrounder: Understanding the clinical potential and research program. Retrieved January 12, 2009, from http://www.elan.com/Images/Bapineuzumab%20_AAB-001_%20Backgrounder%20Final_tcm3-20147.pdf

Eli Lilly. (2008). Phase II data on Lilly's antibody show it affects amyloid beta, a protein believed to be associated with Alzheimer's disease. Retrieved January 22, 2009, from http://newsroom.lilly.com/releasedetail.cfm?releaseid=325343

Forloni, G., Colombo, L., Girola, L., Tagliavini, F., & Salmona, M. (2001). Anti-amyloidogenic activity of tetracyclines: studies in vitro. *Federation of European Biochemical Societies Letters, 487*(3), 404-407.

Ghersi-Egea, J. F., Gorevic, P. D., Ghiso, J., Frangione, B., Patlak, C. S., & Fenstermacher, J. D. (1996). Fate of cerebrospinal fluid-borne amyloid beta-peptide: rapid clearance into blood and appreciable accumulation by cerebral arteries. *Journal of Neurochemistry, 67*(2), 880-883.

Gilman, S., Koller, M., Black, R. S., Jenkins, L., Griffith, S. G., Fox, N. C., et al. (2005). Clinical effects of Abeta immunization (AN1792) in patients with AD in an interrupted trial. *Neurology, 64*(9), 1553-1562.

Goedert, M., & Spillantini, M. G. (2006). A century of Alzheimer's disease. *Science, 314*(5800), 777-781.

Grutzendler, J., & Morris, J. C. (2001). Cholinesterase inhibitors for Alzheimer's disease. *Drugs, 61*(1), 41-52.

Janus, C., Pearson, J., McLaurin, J., Mathews, P. M., Jiang, Y., Schmidt, S. D., et al. (2000). A beta peptide immunization reduces behavioural impairment and plaques in a model of Alzheimer's disease. *Nature Medicine, 408*(6815), 979-982.

Lee, K. (2008). Rush studies vaccine for Alzheimer's. Retrieved January 22, 2009, from http://media.www.chicagoflame.com/media/storage/paper519/news/2008/04/28/Pulse/Rush-Studies.Vaccine.For.Alzheimers-3352540.shtml

Lichtenthaler, S. F., & Haass, C. (2004). Amyloid at the cutting edge: activation of alpha-secretase prevents amyloidogenesis in an Alzheimer disease mouse model. *Journal of Clinical Investigations, 113*(10), 1384-1387.

Loeb, M. B., Molloy, D. W., Smieja, M., Standish, T., Goldsmith, C. H., Mahony, J., et al. (2004). A randomized, controlled trial of doxycycline and rifampin for patients with Alzheimer's disease. *Journal of the American Geriatric Society, 52*(3), 381-387.

Long, S. (2008). *Principles and Practice of Pediatric Infectious Diseases* (3 ed.). Philadelphia: Elsevier Inc.

Moffat, S. D., Zonderman, A. B., Metter, E. J., Kawas, C., Blackman, M. R., Harman, S. M., et al. (2004). Free testosterone and risk for Alzheimer disease in older men. *Neurology, 62*(2), 188-193.

Morgan, D., Diamond, D. M., Gottschall, P. E., Ugen, K. E., Dickey, C., Hardy, J., et al. (2000). A beta peptide vaccination prevents memory loss in an animal model of Alzheimer's disease. *Nature Medicine, 408*(6815), 982-985.

Namba, Y., Kawatsu, K., Izumi, S., Ueki, A., & Ikeda, K. (1992). Neurofibrillary tangles and senile plaques in brain of elderly leprosy patients. *Lancet, 340*(8825), 978.

Nicoll, J. A., Wilkinson, D., Holmes, C., Steart, P., Markham, H., & Weller, R. O. (2003). Neuropathology of human Alzheimer disease after immunization with amyloid-beta peptide: a case report. *Nature Medicine, 9*(4), 448-452.

Nitsch, R. M., & Hock, C. (2008). Targeting beta-amyloid pathology in Alzheimer's disease with Abeta immunotherapy. *Neurotherapeutics, 5*(3), 415-420.

Okura, Y., & Matsumoto, Y. (2007). Development of anti-Abeta vaccination as a promising therapy for Alzheimer's disease. *Drug News Perspectives, 20*(6), 379-386.

Pride, M., Seubert, P., Grundman, M., Hagen, M., Eldridge, J., & Black, R. S. (2008). Progress in the active immunotherapeutic approach to Alzheimer's disease: clinical investigations into AN1792-associated meningoencephalitis. *Neurodegenerative Diseases, 5*(3-4), 194-196.

Pride, M. W., Black, R. W., & Hagen, M. (2003). Evaluation of hte cellular immune response in AD patients after immunotherapy with AN1792(QS-21) [Poster 201.14]. New Orleans, LA.

Reger, M. A., Watson, G. S., Green, P. S., Wilkinson, C. W., Baker, L. D., Cholerton, B., et al. (2008). Intranasal insulin improves cognition and modulates beta-amyloid in early AD. *Neurology, 70*(6), 440-448.

Relkin, N. R., Szabo, P., Adamiak, B., Burgut, T., Monthe, C., Lent, R. W., et al. (2008). 18-month study of intravenous immunoglobulin for treatment of mild Alzheimer disease. *Neurobiology of Aging*.

Results of 9-month phase II study of Gammagard intravenous immunoglobulin. (2008). Results of 9-month phase II study of Gammagard intravenous immunoglobulin. Retrieved January 19, 2009, from www.sciencedaily.com/releases/2008/07/080730175522.htm

Ritchie, C. W., Bush, A. I., Mackinnon, A., Macfarlane, S., Mastwyk, M., MacGregor, L., et al. (2003). Metal-protein attenuation with iodochlorhydroxyquin (clioquinol) targeting Abeta amyloid deposition and toxicity in Alzheimer disease: a pilot phase 2 clinical trial. *Archeological Neurology, 60*(12), 1685-1691.

Rogers, S. L., & Friedhoff, L. T. (1998). Long-term efficacy and safety of donepezil in the treatment of Alzheimer's disease: an interim analysis of the results of a US multicentre open label extension study. *European Neuropsychopharmacology, 8*(1), 67-75.

Salloway, S., Mintzer, J., Weiner, M. F., & Cummings, J. L. (2008). Disease-modifying therapies in Alzheimer's disease. *Alzheimers Dementia, 4*(2), 65-79.

Sano, M., Grossman, H., & Van Dyk, K. (2008). Preventing Alzheimer's disease : separating fact from fiction. *Central Nervous System Drugs, 22*(11), 887-902.

Schenk, D., Barbour, R., Dunn, W., Gordon, G., Grajeda, H., Guido, T., et al. (1999). Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature Medicine, 400*(6740), 173-177.

Shibata, M., Yamada, S., Kumar, S. R., Calero, M., Bading, J., Frangione, B., et al. (2000). Clearance of Alzheimer's amyloid-ss(1-40) peptide from brain by LDL receptor-related protein-1 at the blood-brain barrier. *Journal of Clinical Investigations, 106*(12), 1489-1499.

Siemers, E. R., Quinn, J. F., Kaye, J., Farlow, M. R., Porsteinsson, A., Tariot, P., et al. (2006). Effects of a gamma-secretase inhibitor in a randomized study of patients with Alzheimer disease. *Neurology, 66*(4), 602-604.

Solomon, B., Koppel, R., Frankel, D., & Hanan-Aharon, E. (1997). Disaggregation of Alzheimer beta-amyloid by site-directed mAb. *Proceedings of the National Academy of Sciences United States of America, 94*(8), 4109-4112.

Staufenbiel, M., Wiederhold, K., Tissot, A., Frey, P., Fulurija, A., Hiestand, P., et al. (n.d.). Immunization with AB1-6 coupled to the virus-like particle QB (CAD106) efficiently removes B-amyloid without inducing AB-reactive T-cells. Retrieved January 22, 2009, from http://www.cytos.com/doc/CAD106_ICAD_abstract_160706.pdf

Sugaya, K., & Merchant, S. (2008). How to approach Alzheimer's disease therapy using stem cell technologies. *Journal of Alzheimers Disease, 15*(2), 241-254.

Tomiyama, T., Shoji, A., Kataoka, K., Suwa, Y., Asano, S., Kaneko, H., et al. (1996). Inhibition of amyloid beta protein aggregation and neurotoxicity by rifampicin. Its possible function as a hydroxyl radical scavenger. *Journal of Biological Chemistry, 271*(12), 6839-6844.

U.S. National Institute of Health. (2006a). Phase II study of intravenous immunoglobulin for alzheimer's disease. Retrieved January 16, 2009, from www.clinicaltrials.gov/ct2/show/NCT00299988

U.S. National Institute of Health. (2006b). Safety and tolerability study in patients with mild to moderate Alzheimer's disease. Retrieved January 22, 2009, from http://clinicaltrials.gov/ct2/show/NCT00411580?term=NCT00411580&rank=1

U.S. National Institute of Health. (2007). A study of V950 in people with Alzheimer's disease. Retrieved January 22, 2009, from http://clinicaltrials.gov/ct2/show/NCT00464334?term=NCT00464334&rank=1

U.S. National Institute of Health. (2008). Safety and Tolerability of Repeated Subcutaneous Injections of CAD106 in Mild Alzheimer's Patients. Retrieved January 22, 2009, from http://clinicaltrials.gov/ct2/show/NCT00733863?term=NCT00733863&rank=1

U.S. National Institute of Health. (2009). Study evaluating ACC-001 in mild to moderate Alzheimer's disease subjects. Retrieved January 21, 2009, from http://clinicaltrials.gov/ct/show/NCT00498602

Vellas, B., Coley, N., & Andrieu, S. (2008). Disease modifying trials in Alzheimer's disease: perspectives for the future. *Journal of Alzheimers Disease, 15*(2), 289-301.

Wilcock, D. M., Munireddy, S. K., Rosenthal, A., Ugen, K. E., Gordon, M. N., & Morgan, D. (2004). Microglial activation facilitates Abeta plaque removal following intracranial anti-Abeta antibody administration. *Neurobiological Disorders, 15*(1), 11-20.

Figure 1. Mechanisms of amyloid reduction with a vaccine treatment. From "Development of anti-AB vaccination as a promising therapy for Alzheimer's disease," by Y. Okura, & Y. Matsumoto, 2007, Drug News Perspectives, 20(6), p. 381. Reprinted with permission

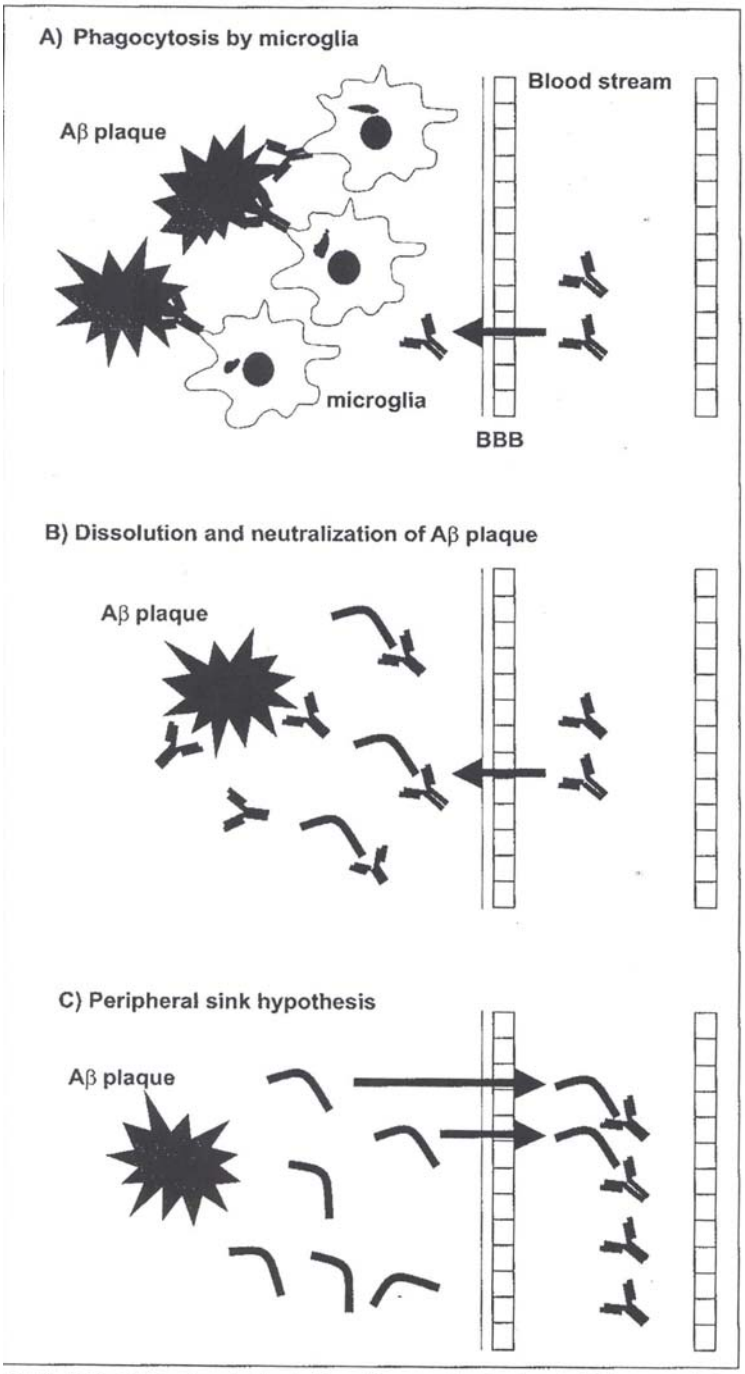
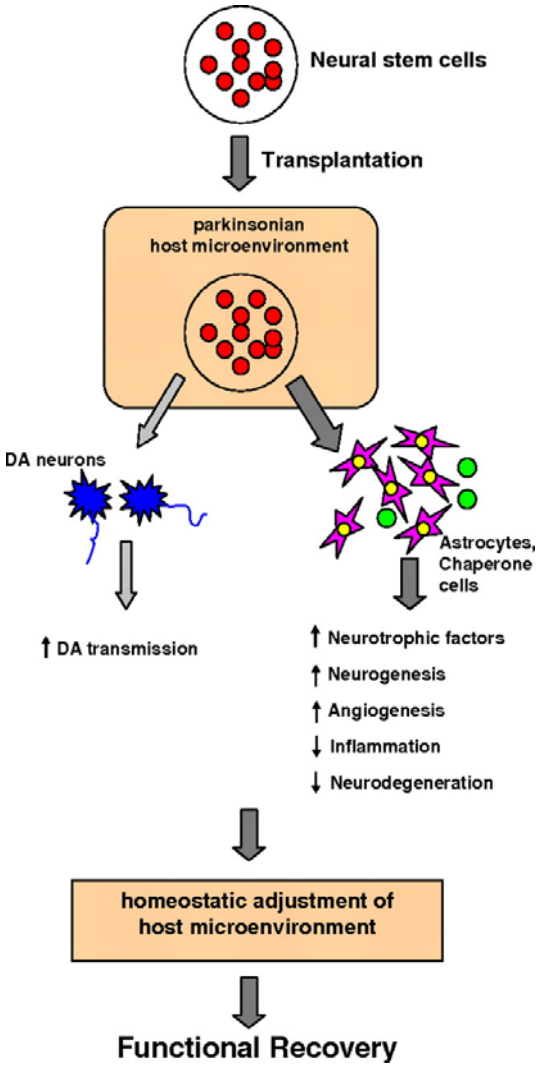


Fig. 2. Mechanisms of amyloid reduction with a vaccine treatment. A) Phagocytosis by microglia: anti-Aβ antibodies traverse blood–brain barrier (BBB) and attach to Aβ deposits, which leads to Fc receptor-mediated phagocytosis by microglia. B) Dissolution and neutralization of Aβ plaque: antibodies bind N-terminal end of Aβ depositions and dissolve amyloid fibrils or neutralize Aβ oligomers. C) The peripheral sink hypothesis: anti-Aβ antibodies in the circulation induce a net efflux of Aβ from the brain to the plasma.

Figure 2. Use of neural stem cells in Parkinson's disease. From "Neural stem cells for Parkinson's disease: to protect and repair," by P. Sanberg, 2007, Proceedings of the National Academy of Sciences United States of America, 104(29), p. 11869. Reprinted with permission.



Abstract

Objective: The objective of this paper was to review the immunizations and emerging therapies currently being researched to treat and/or prevent Alzheimer's disease.
Methods: Databases used to conduct the research included MEDline and PubMed. An initial broad search was conducted using the phrase "Alzheimer's disease/prevention and control." More narrow searches were then conducted using the search terms "Alzheimer vaccines," "Alzheimer stem cell," "Alzheimer antiamyloid," and "Alzheimer neuroprotection." Current drug trials were found at www.clinicaltrials.gov and newspaper articles via Internet search engines such as Google.
Results: Clinical trials are currently in progress for both passive (bapineuzumab, LY2062430, IVIG), active immunization (AN1792, ACC-001, CAD106, V950), and other therapies (neural stem cells, antibiotics, secretase inhibitors and activators, chelators, testosterone, and insulin).
Conclusion: The immunizations entering final clinical trial phases appear to delay the onset or improve symptom control in Alzheimer's disease, but do not seem to provide a cure at this time.