Targeting β-Amyloid Pathology in Alzheimer's Disease with Aβ Immunotherapy

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Summary: More than 10 clinical trials of Aβ immunotherapy are currently underway in patients with Alzheimer's disease (AD). The aim is to identify safe approaches for the efficacious antibody-mediated removal of brain β-amyloid or its neurotoxic oligomeric precursors consisting of aggregated amyloid β-peptide (Aβ). Initial experimental and neuro-pathological evidence for clearance of brain β-amyloid in response to Aβ immunotherapy is associated with structural and functional rescue of neurons, as well as initial signs of clinical stabilization and reduced rates of dementia progression. For the next steps in the future improvement of Aβ immunotherapy, major challenges in pharmacokinetics, safety, and tolerability need to be addressed. These include the low penetrations rates of IgG molecules through the blood-brain barrier, possible reductions in brain volume, the possibility of autoimmune disease related to unwanted cross-reactivity with endogenous antigens on physiological structures, micro-hemorrhages related to cross-reaction with pre-existing vascular amyloid pathology, possible relocalization of Aβ from β-amyloid plaques to brain blood vessels resulting in increased amyloid angiopathy, and the lacking activity of Aβ antibodies on pre-existing neurofibrillary tangle pathology, as well as the lacking molecular identification of the forms of Aβ to be therapeutically targeted. The solutions to these problems will be guided by the fine lines between tolerance and immunity against physiological and pathological structures, respectively, as well as by the understanding of the pathogenic transition of soluble Aβ into toxic oligomeric aggregation intermediates in the dynamic equilibrium of β-amyloid fibril assembly. Provided that the ongoing and planned clinical trials address these issues in a timely manner, there is a good chance for Aβ immunotherapy to be one of the first disease-modifying therapies of Alzheimer's disease to be introduced into clinical practice. **Key Words:** Humanized monoclonal antibody, neurodegeneration, vaccination, clinical trial, APP, passive immunization.

PROOF OF CONCEPT IN EXPERIMENTAL ANIMALS

The concept of Aβ immunotherapy was first introduced by Schenk et al.[1] who showed that vaccination with Aβ1-42 and Freund's adjuvant prevented β-amyloid formation in brains of young transgenic mice and removed β-amyloid in older mice with pre-existing pathology. Subsequent active vaccinations in transgenic mice, nonhuman primates, and other species confirmed these findings[2–6] (For review, see references[7–10]). Moreover, the passive transfer of antibodies against Aβ can efficiently reduce β-amyloid pathology.[11–19] The immunotherapy-mediated clearance of β-amyloid pathology is paralleled by the recovery of neuronal and cytoskeletal morphology,[20,21] by the rescue of synaptic electrophysiological functions and neurotransmission,[22–24] and by signs of neuroprotection,[25] as well as by restored behavioral functions.[2,26–28]

While many antibodies show signs of efficacy in experimental animals, there are also striking differences among different antibodies, both with respect to mechanism of action as well as to potential safety considerations. For example, an antibody against the central domain of Aβ is efficacious in preserving synapses and behavior without clearing β-amyloid plaques,[13] an activity consistently demonstrated with other antibodies directed either against the N-terminal or the C-terminal domains.[11,12,16,17] On the other hand, the antibody against the central domain of Aβ did not cause microhemorrhages, and activity observed with antibodies against N-terminal or C-terminal domains.[16,29,30] These differences may be related to different mechanisms of action within brain or plasma. To date, at least five, mutually nonexclusive, mechanisms of immunotherapy are discussed. These include clearance of β-amyloid by

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phagocytosis, antibody-mediated shift of the equilibrium toward Aβ monomers favoring clearance and degradation, neutralization of toxic oligomeric Aβ species, peripheral Aβ sink and efflux of soluble Aβ species from brain, and antibody-independent, cell-mediated clearance of β-amyloid plaques (for review, see references[10,31]).

INITIAL CLINICAL TRIALS

Together, these findings led to initial phase I and phase II clinical trials with aggregated Aβ42 and the Th1 response-activating adjuvant QS-21 (Elan/Wyeth AN1792 trial).[32] During the later stages of the phase I trial, the emulsifier polysorbate 80 was added to the active vaccine, a change to the formulation after which the observed immune responses shifted from a predominantly Th2-biased response to a pro-inflammatory Th1 response.[33] Following the formulation change, an initial case with meningoencephalitis and vascular T-lymphocyte infiltrations occurred,[34] and in the subsequent phase II study, 18 further patients, or 6% of the patients treated with the active vaccine, developed forms of subacute aseptic meningoencephalitis after having received mostly two doses, and in some cases one or three of the initially planned six doses of the active vaccine.[35] Besides the T-cell reaction, the active vaccination also led to a humoral immune response in a subpopulation of the vaccinated patients with significantly increased IgG and IgM titers,[36–38] but antibody titers were unrelated to the occurrence or the severity of meningoencephalitis.[35] There was even an individual case with severe meningoencephalitis, but with no detectable antibody titers indicating that the humoral immune response was not required to cause meningoencephalitis.

Long-term follow-up of the Zurich cohort of actively vaccinated patients revealed increased titers of antibodies reacting with brain β-amyloid plaques[36]; these patients had significantly slower decline of cognitive functions and daily living capacities as compared to the nonresponders.[38] Likewise, immune responders with high antibody titers in the multicenter cohort scored significantly better in composite scores of memory functions as compared with low-responders and nonresponders, or compared with the placebo group of patients.[39] Despite these consistent findings of titer-dependent effects on cognition, overall comparisons of the active vaccine group *versus* the placebo group failed to show significant differences among groups.[37] This could have been related to the fact that there was little decline in ADAS-cog-related cognitive functions in the placebo group.[37,40] In the same study, decreased CSF levels of tau protein, an intraneuronal microtubule-associated protein, were found, possibly as a correlate of slowed neuronal degeneration.[37] Histopathological examinations of autopsy tissues obtained from individual patients who died from unrelated causes revealed patchy patterns of β-amyloid plaque clearance in association with increased antibody titers, with some regions virtually free of β-amyloid plaque pathology.[34,41–43] In several cases, reductions in β-amyloid plaques were associated with increased brain tissue concentrations of water and detergent-soluble forms of Aβ,[44] suggesting biological plaque-clearing activity of antibodies generated as a result of vaccination. No β-amyloid clearance was observed in a single case without detectable antibody titers.[44] Together these observations suggest biologically relevant activities of Aβ antibodies on β-amyloid plaque pathology in patients with Alzheimer's disease (AD).

SECOND-GENERATION ACTIVE VACCINES

These combined data obtained in the pre-clinical experiments and the observations in the initial clinical active vaccination trials led to the development of a second generation of presumably safer active vaccines with less strong Th1-cell activating formulations and with C-terminally truncated Aβ fragments, as the Aβ C-terminus contains T-cell activating epitopes (Table 1). Several active vaccination approaches are currently tested in clinical trials (www.clinicaltrials.gov) including the Merck V950 trial, the Novartis/Cytos CAD-106 trial using a virus-like particle-linked N-terminal Aβ peptide fragment, as well as the Affiris Affitope AD01 and AD02 active vaccination trials with Aβ peptide mimetics. The Elan/Wyeth ACC-001 phase II active vaccination trial with an N-terminal Aβ peptide fragment conjugated to a carrier protein was recently suspended because of transient skin lesions in a single patient who participated in the study. Studies to determine the underlying pathophysiology of the transient skin lesions are ongoing (www.clinicaltrials.gov).

HUMANIZED MONOCLONAL ANTIBODIES

The passive transfer of therapeutic antibodies can circumvent unwanted T-cell responses associated with active vaccination, while maintaining important biological activities involved in efficacy. As a consequence, several clinical trials with humanized monoclonal antibodies directed against a large variety of different linear and conformational Aβ epitopes are ongoing. For example, Elan/Wyeth is testing Bapineuzumab, a humanized monoclonal antibody against the N-terminus of Aβ, thereby targeting a wide range of soluble and aggregated forms of Aβ including β-amyloid plaques and possibly even N-terminally truncated forms of Aβ assembled within Aβ aggregates. The Bapineuzumab development program is currently entering phase III in large patient cohorts, some of which are stratified according to the ApoE genotype (www.clinicaltrials.gov). In addition, a phase I study

Table 1. *Selected Ongoing Clinical and Preclinical Developments of Aβ Immunotherapy for the Treatment of Alzheimer's Disease*

Company	Approach	Aβ Epitope	Biological	Stage
Elan/Wyeth	Passive	N-terminus	Bapineuzumab	Phase III
Elan/Wyeth	Active	N-terminus	ACC-001	Phase II
Eli Lilly	Passive	Central domain	LY2062430	Phase II
Baxter/Cornell	Passive	IVIg – mix	Gammaguard	Phase II
Novartis	Active	N-terminus	CAD106	Phase I
Roche	Passive	N-terminus + central domain	R1450	Phase I
Pfizer	Passive	C-terminus	PF04360365	Phase I
Merck	Active		V950	Phase I
GlaxoSmithKline	Passive		GSK933776A	Phase I
Elan/Wyeth	Passive	N-terminus	Bapineuzumab s.c.	Phase I
Affiris	Active	Aβ mimetic	Affitope AD1 and AD2	Phase I
Abbott	Passive			Preclinical
Elan/Wyeth	Passive	Conformational	AAB-002	Preclinical
Genentech/ACImmune	Passive	Conformational		Preclinical
BiogenIdec/Neurimmune	Passive			Preclinical
Eisai/BioArctic	Passive	Protofibrils		Preclinical
Boehringer/Ablynx	Nanobodies	Aβ		Preclinical

testing subcutaneous Bapineuzumab formulations is being pursued (www.clinicaltrials.gov). Ely Lilly (Indianapolis, IN) is testing LY2062430, a humanized monoclonal antibody against the central domain of Aβ. This antibody may utilize a different mechanism of action because it does not directly bind to and clear brain β-amyloid plaques, but it reverses behavioral deficits and prevents further amyloid build-up in experimental animals, presumably via a peripheral Aβ sink mechanism followed by efflux of soluble forms of Aβ.[13] Reported phase I data indicated safety of LY2062430, which is now being tested in a phase II trial (www.clinicaltrials.gov). Pfizer is testing the Rinat RN-1219/PF-04360365 humanized monoclonal antibody directed against the C-terminal domain of Aβ in a phase I trial. While murine antibodies against a similar C-terminal epitope cleared brain β-amyloid deposits and reversed behavioral deficits in transgenic mouse models, they also increased vascular amyloid and microhemorrhage,[16,17] activities that were effectively prevented by using the de-glycosylated forms of the antibodies.[45] In a phase I study, Hoffmann-La Roche (Basel, Switzerland) is testing the humanized monoclonal antibody R1450 against combined central- and N-terminal domains in several European countries (www.roche-trials.com). R-1450 was selected to cross the blood brain barrier (BBB) to bind to both soluble Aβ and β-amyloid deposits with high affinity (Bohrmann et al.,10th International Hong Kong/Springfield Pan-Asian Symposium on Advances in Alzheimer Therapy, Hong Kong, 2008). In addition, intravenous IgG preparations containing low concentrations of naturally occurring Aβ antibodies have shown signs of pilot efficacy in a phase II trial,[46] and a phase III trial was recently announced by Baxter BioScience (Deerfield, IL) (Table 1). The results will provide important insights into the mechanisms of Aβ immunotherapy, as well as on safety profiles of both active *versus* passive approaches and different Aβ-related epitopes targeted by the different antibodies.

BLOOD-BRAIN BARRIER PASSAGE

With a few exceptions of small antibody fragments, most current approaches of passive antibody transfer rely on intact IgG molecules, but several of the proposed mechanisms of action—phagocytosis, disaggregation, and neutralization—rely on the presence of therapeutically relevant IgG concentrations within the CNS. Antibody transport across the BBB is thus a critical issue for both active and passive Aβ immunotherapy relying on any of the previously mentioned mechanisms. Despite the large molecular mass of IgG, a small fraction can penetrate the BBB by passive diffusion or by leakage. Available data from the initial active vaccination trial suggests that an estimated fraction of 0.1 to 1% of plasma IgG antibodies against β-amyloid were present within the CNS,[36,38] and immunohistological analyses of autopsy tissues obtained from participants of the AN-1792 study indicated the presence of IgG on the remaining β-amyloid plaques.[34] Within the CNS, antibodies are stabilized by binding to the high local concentrations of target epitopes in β-amyloid fibrils, and the result is very slow off-rates of the IgG-Aβ interaction. As a result, antibodies can accumulate at brain β-amyloid deposits, and even small amounts of antibodies penetrating the BBB may be sufficient to cause therapeutically relevant brain concentrations over time. As an alternative, peripheral mechanisms of action as provided by the Aβ sink hypothesis and efflux of Aβ species from brain- and IgM-mediated Aβ hydrolysis does not require the presence of

antibody within the CNS;[13,31] for these approaches, antibody-Aβ interactions in plasma are sufficient.

CONGOPHILIC AMYLOID ANGIOPATHY AND MICROHEMORRHAGES

Studies in transgenic mice indicated the possibility of antibody treatment-related microhemorrhages associated with pre-existing congophilic amyloid angiopathy (CAA).[16,29] These may be related to interactions of Aβ antibodies with the vascular amyloid causing structural fragility of degenerated smooth muscle and endothelial cells, whereas other antibodies that do not act centrally do not cause microhemorrhages.[30] Antibody-mediated increases in microhemorrhages can also be related to Fc effector functions as de-glycosylated IgG without effector functions have a significantly reduced ability to induce microhemorrhages in transgenic mice.[16,45] Clinical studies will show whether this concern is relevant for safety of Aβ immunotherapy in human patients. In fact, 3 of 22 patients who had received Bapineuzumab in the initial phase I study had transient subclinical MRI signs develop that were consistent with vasogenic edema.[39] On the other hand, Aβ antibodies can also prevent the deposition of vascular amyloid,[47] and they may contribute to vascular repair following clearance of vascular amyloid (Games et al., 10th International Hong Kong/Springfield Pan-Asian Symposium on Advances in Alzheimer Therapy, Hong Kong, 2008).

A related concern is that CAA could increase as a result of clearance of β-amyloid from the neuropil, followed by increased transport of Aβ to the vasculature and accumulation at the blood vessel walls. This concern was raised by severe CAA in initial autopsy findings in patients with AD who received the AN1792 active Aβ vaccination.[34,41,42] Likewise, active vaccination of transgenic mice increased vascular amyloid while decreasing parenchymal β-amyloid plaque pathology.[19] Such increases in CAA, however, are not observed with all Aβ antibodies,[30] and they were not observed in AN1792 study participants who survived the initial vaccination for a longer time period, suggesting a time-dependent course of initial accumulation of vascular amyloid followed later by clearance.

REDUCTIONS OF BRAIN VOLUME

Morphometric analyses of brain volumes suggested reduced brain volume in actively vaccinated patients as compared with the placebo-treated control group.[48] Although these findings are matter of serious concerns, they do not necessarily reflect accelerated tissue loss and accelerated neurodegeneration. Clearance of large amounts of β-amyloid may be associated with volume reductions, and they may be associated with local decreases related to water content, and accompanied by reductions in glial cell volume. Initial long-term observations in individual patients appear to support this hypothesis (Nitsch, 10th International Hong Kong/Springfield Pan-Asian Symposium on Advances in Alzheimer Therapy, Hong Kong, 2008).

NEUROFIBRILLARY TANGLE PATHOLOGY

Besides β-amyloid, the neuropathology of AD is characterized by the formation within neurons of neurofibrillary tangles. These consist of abnormally phosphorylated tau proteins that aggregate to form paired helical filaments in the cell bodies and dendrites where they disrupt cytoskeletal functions leading to the degeneration of neurons with the neurofibrillary tangle remaining deposited within the neuropil at the end of this process. Autopsy findings in patients who had participated in the initial active vaccination trial consistently show the persistence of neurofibrillary tangles, in particular also in regions with signs of β-amyloid clearance indicating that Aβ immunotherapy is not effective in clearing pre-existing neurofibrillary tangles.[34,41,42,49] On the other hand, because β-amyloid or the related toxic aggregation intermediates can trigger the formation of neurofibrillary tangles,[50–54] therapeutic reduction of β-amyloid or its related toxic oligomers may well be effective in preventing the formation of further downstream neurofibrillary tangle pathology, as suggested by data in transgenic mice, in which Aβ antibodies reduced both β-amyloid and early, but not late forms of hyperphosphorylated tau.[21] Therefore, Aβ immunotherapy could be active in preventing neurofibrillary tangle formation without affecting numbers or the morphology of pre-existing neurofibrillary tangles.

STRUCTURE OF THE THERAPEUTIC TARGET

Accumulating experimental evidence indicates toxicity of oligomeric assemblies, including Aβ*56[55] and yet smaller oligomers including dimers can attain toxic functions (for review, see[56–58]). Early intracellular Aβ aggregates associated with impaired neuronal functions and dendritic structures in transgenic mouse models can precede β-amyloid plaque deposition in the neuropil by several months.[23,59] Neuropathology in autopsy samples shows dystrophic neurites surrounding β-amyloid plaques, as well as gradients of neurite pathology, where the degree of pathology decreases with increasing distance from the β-amyloid plaque.[60] Local concentration gradients of toxic oligomeric Aβ aggregation intermediates in the immediate surroundings of, and within, β-amyloid plaques may explain this finding. The further development of Aβ immunotherapy will focus on approaches

that selectively target pathogenic and toxic structures with little or no cross-reactivity with APP derivatives occurring during physiological proteolytic processing.

OUTLOOK

Despite the fact that one of the initial Aβ immunotherapy trials failed because of autoreactive T-cell responses in 6% of the vaccinated patients, optimism regarding the development of Aβ immunotherapy AD prevails. A wealth of information derived from ongoing active vaccination and passive immunotherapy approaches with humanized monoclonal antibodies is foreseeable for the near future. After removal of Aβ-mediated toxicity, cognitive rehabilitation and recovery of neuronal functions lost to neurodegeneration will be an important future goal. Likewise, preclinical diagnosis and prevention via surrogate markers, risk factors, and β-amyloid imaging[61] will be a future direction in the joint global struggle against dementia.

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REFERENCES

1. Schenk D, Barbour R, Dunn W, et al. Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. Nature 1999;400:173–177.
2. Janus C, Pearson J, McLaurin J, et al. A beta peptide immunization reduces behavioural impairment and plaques in a model of Alzheimer's disease. Nature 2000;408:979–982.
3. Weiner HL, Lemere CA, Maron R, et al. Nasal administration of amyloid-beta peptide decreases cerebral amyloid burden in a mouse model of Alzheimer's disease. Ann Neurol 2000;48:567–579.
4. Lemere CA, Maron R, Selkoe DJ, Weiner HL. Nasal vaccination with beta-amyloid peptide for the treatment of Alzheimer's disease. DNA Cell Biol 2001;20:705–711.
5. Sigurdsson EM, Scholtzova H, Mehta PD, Frangione B, Wisniewski T. Immunization with a nontoxic/nonfibrillar amyloid-beta homologous peptide reduces Alzheimer's disease-associated pathology in transgenic mice. Am J Pathol 2001;159:439–447.
6. Lemere CA, Beierschmitt A, Iglesias M, et al. Alzheimer's disease abeta vaccine reduces central nervous system abeta levels in a non-human primate, the Caribbean vervet. Am J Pathol 2004;165:283–297.
7. Weiner HL, Frenkel D. Immunology and immunotherapy of Alzheimer's disease. Nat Rev Immunol 2006;6:404–416.
8. Schenk D, Hagen M, Seubert P. Current progress in beta-amyloid immunotherapy. Curr Opin Immunol 2004;16:599–606.
9. Solomon B. Antibody-mediated immunotherapy for Alzheimer's disease. Curr Opin Investig Drugs 2007;8:519–524.
10. Brody DL, Holtzman DM. Active and passive immunotherapy for neurodegenerative disorders. Annu Rev Neurosci 2008 Mar 19; [Epub ahead of print].
11. Bard F, Cannon C, Barbour R, et al. Peripherally administered antibodies against amyloid beta-peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease. Nat Med 2000;6:916–919.
12. Bacskai BJ, Kajdasz ST, Christie RH, et al. Imaging of amyloid-beta deposits in brains of living mice permits direct observation of clearance of plaques with immunotherapy. Nat Med 2001;7:369–372.
13. DeMattos RB, Bales KR, Cummins DJ, Dodart JC, Paul SM, Holtzman DM. 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. Proc Natl Acad Sci U S A 2001;98:8850–8855.
14. Bacskai BJ, Kajdasz ST, McLellan ME, et al. Non-Fc-mediated mechanisms are involved in clearance of amyloid-beta in vivo by immunotherapy. J Neurosci 2002;22:7873–7878.
15. Das P, Howard V, Loosbrock N, Dickson D, Murphy MP, Golde TE. Amyloid-beta immunization effectively reduces amyloid deposition in FcRgamma-/- knock-out mice. J Neurosci 2003;23:8532–8538.
16. Wilcock DM, Rojiani A, Rosenthal A, et al. Passive amyloid immunotherapy clears amyloid and transiently activates microglia in a transgenic mouse model of amyloid deposition. J Neurosci 2004;24:6144–6151.
17. Wilcock DM, Rojiani A, Rosenthal A, et al. Passive immunotherapy against Abeta in aged APP-transgenic mice reverses cognitive deficits and depletes parenchymal amyloid deposits in spite of increased vascular amyloid and microhemorrhage. J Neuroinflammation 2004;1:24.
18. Boyett KW, DiCarlo G, Jantzen PT, et al. Increased fibrillar beta-amyloid in response to human clq injections into hippocampus and cortex of APP+PS1 transgenic mice. Neurochem Res 2003;28:83–93.
19. Wilcock DM, Jantzen PT, Li Q, Morgan D, Gordon MN. Amyloid-beta vaccination, but not nitro-nonsteroidal anti-inflammatory drug treatment, increases vascular amyloid and microhemorrhage while both reduce parenchymal amyloid. Neuroscience 2007;144:950–960.
20. Lombardo JA, Stern EA, McLellan ME, et al. Amyloid-beta antibody treatment leads to rapid normalization of plaque-induced neuritic alterations. J Neurosci 2003;23:10879–10883.
21. Oddo S, Billings L, Kesslak JP, Cribbs DH, LaFerla FM. Abeta immunotherapy leads to clearance of early, but not late, hyperphosphorylated tau aggregates via the proteasome. Neuron 2004;43:321–332.
22. Klyubin I, Walsh DM, Lemere CA, et al. Amyloid beta protein immunotherapy neutralizes Abeta oligomers that disrupt synaptic plasticity in vivo. Nat Med 2005;11:556–561.
23. Knobloch M, Farinelli M, Konietzko U, Nitsch RM, Mansuy IM. Abeta oligomer-mediated long-term potentiation impairment involves protein phosphatase 1-dependent mechanisms. J Neurosci 2007;27:7648–7653.
24. Bales KR, Tzavara ET, Wu S, et al. Cholinergic dysfunction in a mouse model of Alzheimer disease is reversed by an anti-A beta antibody. J Clin Invest 2006;116:825–832.
25. Mohajeri MH, Saini K, Schultz JG, Wollmer MA, Hock C, Nitsch RM. Passive immunization against beta-amyloid peptide protects central nervous system (CNS) neurons from increased vulnerability associated with an Alzheimer's disease-causing mutation. J Biol Chem 2002;277:33012–33017.
26. Morgan D, Diamond DM, Gottschall PE, et al. A beta peptide vaccination prevents memory loss in an animal model of Alzheimer's disease. Nature 2000;408:982–985.
27. Sigurdsson EM, Knudsen E, Asuni A, et al. An attenuated immune response is sufficient to enhance cognition in an Alzheimer's disease mouse model immunized with amyloid-beta derivatives. J Neurosci 2004;24:6277–6282.
28. Chen G, Chen KS, Kobayashi D, et al. Active beta-amyloid immunization restores spatial learning in PDAPP mice displaying very low levels of beta-amyloid. J Neurosci 2007;27:2654–2662.
29. Pfeifer M, Boncristiano S, Bondolfi L, et al. Cerebral hemorrhage after passive anti-Abeta immunotherapy. Science 2002;298:1379.
30. Racke MM, Boone LI, Hepburn DL, et al. Exacerbation of cerebral amyloid angiopathy-associated microhemorrhage in amyloid precursor protein transgenic mice by immunotherapy is dependent on antibody recognition of deposited forms of amyloid beta. J Neurosci 2005;25:629–636.

31. Taguchi H, Planque S, Nishiyama Y, et al. Autoantibody-catalyzed hydrolysis of amyloid beta peptide. J Biol Chem 2008;283:4714–4722.
32. Bayer AJ, Bullock R, Jones RW, et al. Evaluation of the safety and immunogenicity of synthetic Abeta42 (AN1792) in patients with AD. Neurology 2005;64:94–101.
33. Pride M, Seubert P, Grundman M, Hagen M, Eldridge J, Black RS. Progress in the active immunotherapeutic approach to Alzheimer's disease: clinical investigations into AN1792-associated meningoencephalitis. Neurodegener Dis 2008;5:194–196.
34. Nicoll JA, Wilkinson D, Holmes C, Steart P, Markham H, Weller RO. Neuropathology of human Alzheimer disease after immunization with amyloid-beta peptide: a case report. Nat Med 2003;9:448–452.
35. Orgogozo JM, Gilman S, Dartigues JF, et al. Subacute meningoencephalitis in a subset of patients with AD after Abeta42 immunization. Neurology 2003;61:46–54.
36. Hock C, Konietzko U, Papassotiropoulos A, et al. Generation of antibodies specific for beta-amyloid by vaccination of patients with Alzheimer disease. Nat Med 2002;8:1270–1275.
37. Gilman S, Koller M, Black RS, et al. Clinical effects of Abeta immunization (AN1792) in patients with AD in an interrupted trial. Neurology 2005;64:1553–1562.
38. Hock C, Konietzko U, Streffer JR, et al. Antibodies against beta-amyloid slow cognitive decline in Alzheimer's disease. Neuron 2003;38:547–554.
39. Black RS SR, Kirby L, Safirstein B, Motter R, Pallay A. A single ascending dose study of bapineuzumab, a humanized monoclonal antibody to Aβ, in AD. Paper presented at: 9th International Symposium on Alzheimer Therapy; July 19, 2006; Geneva, Switzerland.
40. Harrison J, Minassian SL, Jenkins L, Black RS, Koller M, Grundman M. A neuropsychological test battery for use in Alzheimer disease clinical trials. Arch Neurol 2007;64:1323–1329.
41. Ferrer I, Boada Rovira M, Sanchez Guerra ML, Rey MJ, Costa-Jussa F. Neuropathology and pathogenesis of encephalitis following amyloid-beta immunization in Alzheimer's disease. Brain Pathol 2004;14:11–20.
42. Masliah E, Hansen L, Adame A, et al. Abeta vaccination effects on plaque pathology in the absence of encephalitis in Alzheimer disease. Neurology 2005;64:129–131.
43. Bombois S, Maurage CA, Gompel M, et al. Absence of beta-amyloid deposits after immunization in Alzheimer disease with Lewy body dementia. Arch Neurol 2007;64:583–587.
44. Patton RL, Kalback WM, Esh CL, et al. Amyloid-beta peptide remnants in AN-1792-immunized Alzheimer's disease patients: a biochemical analysis. Am J Pathol 2006;169:1048–1063.
45. Wilcock DM, Alamed J, Gottschall PE, et al. Deglycosylated anti-amyloid-beta antibodies eliminate cognitive deficits and reduce parenchymal amyloid with minimal vascular consequences in aged amyloid precursor protein transgenic mice. J Neurosci 2006;26:5340–5346.
46. Relkin NR. Beyond symptomatic therapy: a re-examination of acetylcholinesterase inhibitors in Alzheimer's disease. Expert Rev Neurother 2007;7:735–748.
47. Prada CM, Garcia-Alloza M, Betensky RA, et al. Antibody-mediated clearance of amyloid-beta peptide from cerebral amyloid angiopathy revealed by quantitative in vivo imaging. J Neurosci 2007;27:1973–1980.
48. Fox NC, Black RS, Gilman S, et al. Effects of Abeta immunization (AN1792) on MRI measures of cerebral volume in Alzheimer disease. Neurology 2005;64:1563–1572.
49. Nicoll JA, Barton E, Boche D, et al. Abeta species removal after abeta42 immunization. J Neuropathol Exp Neurol 2006;65:1040–1048.
50. Gotz J, Chen F, van Dorpe J, Nitsch RM. Formation of neurofibrillary tangles in P301l tau transgenic mice induced by Abeta 42 fibrils. Science 2001;293:1491–1495.
51. Ferrari A, Hoerndli F, Baechi T, Nitsch RM, Gotz J. beta-Amyloid induces paired helical filament-like tau filaments in tissue culture. J Biol Chem 2003;278:40162–40168.
52. King ME, Kan HM, Baas PW, Erisir A, Glabe CG, Bloom GS. Tau-dependent microtubule disassembly initiated by prefibrillar beta-amyloid. J Cell Biol 2006;175:541–546.
53. Lewis J, Dickson DW, Lin WL, et al. Enhanced neurofibrillary degeneration in transgenic mice expressing mutant tau and APP. Science 2001;293:1487–1491.
54. Oddo S, Caccamo A, Tran L, et al. Temporal profile of amyloid-beta (Abeta) oligomerization in an in vivo model of Alzheimer disease. A link between Abeta and tau pathology. J Biol Chem 2006;281:1599–1604.
55. Lesne S, Koh MT, Kotilinek L, et al. A specific amyloid-beta protein assembly in the brain impairs memory. Nature 2006;440:352–357.
56. Selkoe DJ. Soluble oligomers of the amyloid beta-protein impair synaptic plasticity and behavior. Behav Brain Res 2008.
57. Walsh DM, Klyubin I, Fadeeva JV, et al. Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. Nature 2002;416:535–539.
58. Walsh DM, Selkoe DJ. A beta oligomers - a decade of discovery. J Neurochem 2007;101:1172–1184.
59. Knobloch M, Konietzko U, Krebs DC, Nitsch RM. Intracellular Abeta and cognitive deficits precede beta-amyloid deposition in transgenic arcAbeta mice. Neurobiol Aging 2007;28:1297–1306.
60. Stern EA, Bacskai BJ, Hickey GA, Attenello FJ, Lombardo JA, Hyman BT. Cortical synaptic integration in vivo is disrupted by amyloid-beta plaques. J Neurosci 2004;24:4535–4540.
61. Klunk WE, Engler H, Nordberg A, et al. Imaging brain amyloid in Alzheimer's disease with Pittsburgh Compound-B. Ann Neurol 2004;55:306–319.