Progress in Immunotherapy for Treating Alzheimer’s Disease

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Course Objectives

• Identify Alzheimer’s disease therapeutic targets
• Provide an introduction to how immunotherapy works
• Discuss how immunotherapy reduces amyloid plaques in the brain
• Introduce the concepts of active versus passive immunotherapy for Alzheimer’s disease
• Explain the difference between protein and DNA vaccines
• Provide a summary of the human immunotherapy clinical trial results
• Discuss the potential advantages of early intervention with immunotherapy in Alzheimer’s patients
Overview

• Very brief introduction to Alzheimer’s disease (AD), neuropathology, & rationale for anti-Aβ therapies
• Brief review of immunotherapy (IT) for AD
• Pros and Cons from immunotherapy Clinical Trials
• Rationale for an Epitope Vaccine
• Evolution of the DNA Epitope Vaccine
• B & T cell epitope selection & optimization
• Potential for Using Memory T Helper Cells to Enhance the antibody Response to a DNA Epitope Vaccine
• Experiments in mice, rabbits, dogs & non-human primates
• Summary of immunotherapy Clinical Trials
How Alzheimer Disease Changes the Brain
Alzheimer’s Disease Neuropathology

Amyloid Precursor Protein and β-Amyloid

MUTATIONS → FAD Swedish
β1
α extracellular
γ membrane
β42 intracellular

SECRETASES → β

6E10 Prefrontal  6E10 small vessels  6E10 large vessels
6E10 leptomenin  AB40 small vessels  AB40 large vessels
Evidence in support of the Amyloid Hypothesis

- Familial AD – all mutations (presenilins, APP) lead to increased Aβ1-42 and AD
- Trisomy 21 or Down syndrome – overexpression of APP
- French & Dutch families with duplication of APP
- Toxicity (assembly states) – Aβ is toxic to neurons in culture and if injected into rodents
- Correlation with cognition (yes – there are lots of studies that show this!)
- Mouse models – overexpress mutant human genes and develop plaque pathology and behavioral dysfunction
Anti-\( \text{A} \beta \) Therapeutic Strategies for the Treatment or Prevention of AD

- Reduce the production of A\( \beta \) (secretase inhibitors, statins, NSAIDs, antioxidants)
- Prevent A\( \beta \) aggregation (antioxidants, chelators)
- Enhance degradation and clearance

**Immunotherapy!**
Active Immunization with Fibrillar Aβ & Alum in Aged Canines

Immunization Protocol

Dogs were 8.4-12.4 years old at the beginning of the study, and 10.5-14.5 years old at the end.

Monthly injections. Blood were drawn same day as injection every month for 6 months and every 6 months after that.

- Group 1. Fibrillar Aβ_{42} formulated in Alum (n=9)
- Group 2. Oligomeric Aβ_{42} on a gold particles (n=9)
- Group 3. Control group with Alum injections only (n=6)
- Group 4. Control group with PBS injections (n=5)
Reduction of amyloid plaques in immunized canines

Mechanism of Vaccine-Mediated Clearance of Amyloid Plaques from the Brain

**Intracellular**

- CNS

**Extracellular**

- Blood Brain Barrier

- Blood

- Monomeric
- ADDLs
- Soluble
- Protofibrils
- Insoluble
- Fibrils
- Plaques

**Anti-αβ antibody**
Subacute meningoencephalitis in a subset of patients with AD after Abeta42 immunization.

Elan and Wyeth Immunotherapy AN-1792
Clinical Trial for AD

AN-1792 Vaccine
- Multiple Injections in AD Patients

Fibrillar Aβ1-42 peptide (Antigen)
QS21 (Strong adjuvant to boost the immune response)
Polysorbate 80 reformulation

Immune Response to Aβ

T-Cell Response
- Activated T-Cells
- Inflammatory Cytokines
- Cells that attack Aβ

B-Cell Response
- Antibodies specific for Aβ

Adverse Autoimmune Response???

Clearance of Toxic Aβ

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ALZHEIMER’S DISEASE RESEARCH CENTER
INSTITUTE for MEMORY IMPAIRMENTS and NEUROLOGICAL DISORDERS
UNIVERSITY of CALIFORNIA - IRVINE
Potential problems and solutions for anti-Aβ immunotherapy

**Potential Problems:**

- Th1-mediated autoimmune response to Aβ-immunization
- Anaphylactic response to adjuvant combination in AN1792
- Complement activation by anti-Aβ-antibody-Aβ/APP immune complexes
- Immune response induced hemorrhages at sites of cerebrovascular amyloid angiopathy

**Potential Solutions:**

- Convert the immune response to Aβ-immunization to a Th2 phenotype
- Immunize with chimeric antigens that contain the predominate Aβ-B cell epitope
- But lack the Aβ-T cell epitope
- Immunize before vascular deposition of Aβ
Sequences of overlapping 15mers for fine epitope mapping of Aβ₁-₄₂

<table>
<thead>
<tr>
<th>Aβ₁-₄２</th>
<th>DAEFR₅HDSGY₁₀EVHHQ₁₅KLVFF₂₀AEDVG₂₅SNKGA₃₀IIGLM₃₅VGGVV₄₀IA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptide A (1-15)</td>
<td>DAEFR₅HDSGY₁₀EVHHQ₁₅</td>
</tr>
<tr>
<td>Peptide B (6-20)</td>
<td>HDSGY₁₀EVHHQ₁₅KLVFF₂₀</td>
</tr>
<tr>
<td>Peptide C (11-25)</td>
<td>EVHHQ₁₅KLVFF₂₀AEDVG₂₅</td>
</tr>
<tr>
<td>Peptide D (16-30)</td>
<td>KLVFF₂₀AEDVG₂₅SNKGA₃₀</td>
</tr>
<tr>
<td>Peptide E (21-35)</td>
<td>AEDVG₂₅SNKGA₃₀IIGLM₃₅</td>
</tr>
<tr>
<td>Peptide F (26-42)</td>
<td>SNKGA₃₀IIGLM₃₅VGGVV₄₀IA</td>
</tr>
</tbody>
</table>
Fine epitope mapping of B and T cell epitopes of Aβ₁₋₄₂
AD Epitope Vaccines (AD EV)

Peptide AD EV
Multiple Antigen Peptide (MAP)

- Lys
- Lys - Lys - Lys

Key:
- Universal Th epitope
- Multiple copies of Aβ_{1-11}

Requires formulation in conventional adjuvant, for examples:
- Th1 type (pro-inflammatory) QS21
- Th2 type (anti-inflammatory) Alum

Cribbs et al., 2003
Ghochikyan et al., Vaccine 2006
Petrushina et al., J. Neurosci 2007

May or may not require fusion with molecular adjuvant, for examples:
- Th1 type (pro-inflammatory) chemokine, IFNγ
- Th2 type (anti-inflammatory) cytokine, MDC, IL4

Ghochikyan et al., 2003
Mamikonyan et al., 2007
Movsesyan et al., 2008

DNA AD EV
DNA Plasmid

Molec. Adjuvant
B cell epitope
Th epitope
Non-self T-cell epitopes for anti-Aβ immunotherapy

Substitution of non-self T cell epitopes for the Aβ T cell epitope in the immunogen will:
• Generate T cells that support the production of antibodies that recognize the Aβ B-cell epitope
• Generate T cells that don’t recognize native Aβ or APP

PADRE: a promiscuous synthetic foreign T cell epitope.
• PADRE was engineered to provide a T cell helper epitope that is able to generate effective helper T cell responses in the general human population.
• This epitope is synthetic, non-natural pan HLA DR-binding epitope that binds with high affinity to 15 of 16 of the most common HLA-DR.
• PADRE sequence: aKXVAAWTLKAAa
Advantages of DNA vaccines as a potential therapeutic approach for Aβ immunization

• Expression of intracellular and secreted immunogens.
• Targeted B- and T cell responses.
• Inexpensive and stable.
• Can be easily engineered to incorporate molecular adjuvants, such as cytokine cassettes to enhance the immune response.
Construction of Aβ40 and Aβ42 DNA immunogens
The Helios Gene Gun by BioRad

TriGrid Delivery System (TDS) by Ichor Medical Systems

**Bio**-**l**istic DNA Delivery
The Helios gene gun system is a handheld bio**l**istic device that fires nucleic acid directly into eukaryotic and prokaryotic cells. An adjustable helium pulse of 100–600 psi propels gold microparticles that can be coated with DNA, RNA, and other biomaterials. Cartridge “bullets” act as carriers for 0.5 mg of microparticles and are easily prepared using the tubing prep station. The target area is 2 cm².

**Outline of Helios Gene Gun Operation**
- DNA (or other biomaterial) of interest is coated onto gold microparticles
- Coated microparticles are loaded into the cartridge holder (loading differently coated microparticles permits codeelivery)
- The operator pulls the trigger; the helium pulse propels the microparticles into target cells
- Nucleic acid coated onto the microparticles is taken up by the genome of target cells

**GFP DNA**
**GFP DNA + EP**

**Ichor Electroporation Device**
Ichor holds multiple patents that protect the innovative technology embodied in our TriGrid™ Delivery System and DNA drugs. The multidisciplinary research program undertaken by Ichor's team of scientists and engineers has led not only to the development of innovative solutions for DNA drug delivery, but also to the devices and procedures necessary for their safe, effective, and reproducible implementation in the clinic.
Comparison of anti-Aβ antibody response to Gene Gun versus electroporation with TriGrid™ Delivery System

Figure 1. Concentrations of anti-Aβ antibodies in sera of immunized mice after Gene Gun vs TDS-IM delivery (n=8/per group).
DNA vaccine encoding 3 copies of Aβ B cell epitope induced stronger anti-Aβ antibody response than the vaccine encoding 1 copy of Aβ_{1-11}

Concentration of anti-Aβ antibody ug/ml

~Titer 1:180,000

~Titer 1:2,500
**Schematic presentation of 3rd generation of Th-EV construct**

<table>
<thead>
<tr>
<th>Th epitope</th>
<th>Origin</th>
<th>aa sequence and position</th>
</tr>
</thead>
<tbody>
<tr>
<td>P23 TT</td>
<td>Tetanus Toxin</td>
<td>1084 – VSIDKFRIFCKANPK - 1099</td>
</tr>
<tr>
<td>P32 TT</td>
<td>Tetanus Toxin</td>
<td>1174 – LKFIKRYTPNEIDS - 1189</td>
</tr>
<tr>
<td>P21 TT</td>
<td>Tetanus Toxin</td>
<td>1064 – IREDNNTLKLDRCNN - 1079</td>
</tr>
<tr>
<td>P30 TT</td>
<td>Tetanus Toxin</td>
<td>947 – FNNFTVSFWLRVPKVSVASHLE - 967</td>
</tr>
<tr>
<td>P2 TT</td>
<td>Tetanus Toxin</td>
<td>830 – QYIKANSKFIGITE - 843</td>
</tr>
<tr>
<td>HBVnc</td>
<td>HBV nuclear capsid</td>
<td>50 – PHHTALRQAILCWGELMTLA - 69</td>
</tr>
<tr>
<td>HbsAg</td>
<td>HBV surface Antigen</td>
<td>19 – FLLLTRILTPQSLD - 33</td>
</tr>
<tr>
<td>MT</td>
<td>Influenza matrix</td>
<td>17 – YSGPLKAEIQRLEDV - 31</td>
</tr>
</tbody>
</table>
Titers of anti-Aβ antibody

Vector optimization. Vectors with a free N-terminus along with PADRE, Thep or PADRE plus Thep sequences were evaluated in rabbits.
DNA epitope vaccine induces anti-Aβ antibodies in rabbits (A) and monkeys (B).

Concentration of anti-Aβ antibodies, µg/ml

Immunizations

DNA epitope vaccine induces anti-Aβ antibodies in rabbits (A) and monkeys (B). Concentrations of anti-Aβ Ab in sera of individual rabbits (n=4) were calculated using a calibration curve generated with rabbit anti-Aβ (1-17) polyclonal antibody (Genscript, NJ). Because monkey anti-Aβ antibody was not available, endpoints titers were detected in the sera of individual monkeys (n=5).

1U44 NS065518 Claire Evans (PI) 05/01/09 – 04/30/14

(Consortium Head: David H. Cribbs)

A Therapeutic DNA Epitope Vaccine for Alzheimer's Disease

Ichor Medical Systems proposes to use intramuscular electroporation of a novel DNA vaccine, ADepVac, for the disease-modifying treatment of Alzheimer’s disease.
Schematic Presentation of Epitope Vaccines with Multiple Foreign T cell Epitopes: (i) promiscuous synthetic Th epitope, PADRE; (ii) P23, P32, P21, P30, P2, P7, P17 and P28 from Tetanus Toxin; (iii) HBVnc and HBsAg from Hepatitis B and (iv) MT from Influenza virus

Evans C, Davtyan et al., Alz and Dementia, 2013
Davtyan et al., submitted, 2013
Comparison of humoral immune responses in mice of H2b haplotype immunized with AV-1955 and AV-1959 DNA epitope vaccines

A

B cell epitopes

String of T helper cell epitopes (MultiTEP)

AV-1955

AV-1959

C57BL/6 mice
Anti-Aβ Epitope Vaccine Summary

- DNA vaccine technology offers advantages over conventional protein/peptide approaches
- Electroporation devices such as the TriGrid Delivery System (TDS) by Ichor Medical Systems provide efficient transfection and strong immune responses in large animals and humans
- Rationale, evolution & optimization of the DNA epitope vaccine
- Potential for using memory T helper cells to enhance the antibody response to a DNA epitope vaccine (Overcoming immunosenescence in the elderly)

Anti-Aβ Protein Epitope Vaccine

3rd Generation anti-Aβ DNA Epitope Vaccine

Lundbeck Clinical Trial 2013

Approved for Year 4 U44 5-U44-NS065518-03 funding
July 24, 2012 — Pfizer Inc has announced topline results of a phase 3 trial of bapineuzumab showing treatment failed to meet the co-primary endpoint of change in cognitive or functional performance versus placebo in patients with mild to moderate Alzheimer's disease (AD) who are positive for the apolipoprotein E4 (ApoE4) risk allele.

Bapineuzumab is an investigational monoclonal antibody that targets amyloid-ß (Aß) under development by the Alzheimer's Immunotherapy Program (AIP), a partnership between

The findings would appear to mirror phase 2 study results with bapineuzumab that had missed the same primary endpoints but showed a suggestion of benefit only in ApoE4 noncarriers.

"The lack of clinical efficacy in the ApoE4 carriers at the stage of dementia is very disappointing, but perhaps not surprising, given the significant pathology by this stage of the disease in this genetic risk group," Reisa Sperling, MD, professor of neurology at Harvard Medical School, and director, Center for Alzheimer Research and Treatment at Brigham and Women's Hospital.
Safety and changes in plasma and cerebrospinal fluid amyloid beta after a single administration of an amyloid beta monoclonal antibody in subjects with Alzheimer disease.

Objective: Active and passive immunization strategies have been suggested as possible options for the treatment of Alzheimer disease (AD). LY2062430 (solanezumab) is a humanized monoclonal antibody being studied as a putative disease-modifying treatment of AD.

Conclusion: A single dose of solanezumab was generally well tolerated, although infusion reactions similar to those seen with administration of other proteins may occur with higher doses. A dose-dependent change in plasma and CSF Abeta was observed, although changes in cognitive scores were not noted. Further studies of solanezumab for the treatment of AD are warranted.
Intravenous immunoglobulin* (IVIG/Gammagard, Baxter)

- **First report of long-term stabilization of Alzheimer's in 3 year extension of Phase II IVIG trial**
- Intravenous immunoglobulin* (IVIG/Gammagard, Baxter) is being studied as an immunotherapy for Alzheimer's. Positive results of a placebo controlled **Phase 2 study** in mild to moderate Alzheimer's were previously reported. The 24 participants in that study received six months of treatment followed by a 12-month open-label extension where all subjects received IVIG. Several doses were tested.
- To evaluate the long term effects of IVIG, participants were offered additional IVIG treatment at a single standardized dose (0.4mg/kg every 2 weeks) for an additional 18 months. Sixteen of the originally enrolled subjects received treatment through month 36, including five originally given placebo and 11 treated with various doses of IVIG.
- The researchers found that:
  - Study participants who were treated with IVIG 0.4g/kg every two weeks for the full 36 months (n=4) had the best outcome, with no decline on several standard measures of cognition, memory, daily functioning and mood (ADAS-Cog, CGIC, 3MS, ADCS-ADL, NPI, QOL) at the three year endpoint.
  - **As a group, the 11 participants who received IVIG for the full 36 months had favorable outcomes in terms of their thinking abilities, behavior and daily function.**
  - The five participants who were initially treated with a placebo and then switched to IVIG declined while on placebo but experienced less rapid decline while receiving a uniform dose of IVIG.
  - "Alzheimer's disease progresses over many years," said study leader Norman Relkin, MD, PhD, of Weill Cornell Medical College, New York City. "It is crucial that we find effective, long-term treatments."
Neuropathology and Clinical Stages in Alzheimer’s disease
The Dominantly Inherited Alzheimer Network – Therapeutic Trials Unit (DIAN)

- The Dominantly Inherited Alzheimer Network (DIAN) – Therapeutic Trials Unit*** is preparing to launch prevention trials in people with young-onset genetic Alzheimer's.
- *** The DIAN Study itself is not designed to test any treatments or preventive strategies; rather, it is designed to collect information about the changes in the brain that precede the development of disease symptoms. However, because biochemical changes can be detected and measured many years before symptoms develop, it may be possible for researchers to develop treatments that halt or slow the biological processes that cause those biochemical changes, potentially arresting the disease process before brain function is impaired.
- The DIAN Therapeutic Trials Unit (DIAN-TTU) was developed to pursue this possibility. The Alzheimer's Association is the lead funder of the project, accounting for 56% of its initial funding, with the DIAN Pharma Consortium, a pioneering consortium of 10 pharmaceutical companies providing the balance.
- Recently announced, study leaders from the Banner Alzheimer's Institute chose crenezumab (Genentech), a humanized monoclonal antibody against beta amyloid, as the therapeutic agent for this trial. The API trial will test the drug on 300 individuals from the Columbian kindred, and about two dozen people in the U.S., who have younger-onset Alzheimer's-causing gene mutations, but do not yet show symptoms of the disease.
- An IgG4 subclass was selected to reduce the risk of Fcγ receptor-mediated over-activation of microglia.
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