Regulation of anti-viral B cell responses
Use of virus-like particles for therapeutic vaccination against addiction and other chronic diseases

Neue Möglichkeiten der Immuntherapie
Martin Bachmann, Immunologie Bern
History of Vaccinology

- Variolation (pre-1700)
- Vaccinia (1796)

- Variolation (pre-1700)
- Vaccinia (1796)

- Rabies (1885)
- Cholera (1896)
- Allergy (1911)
- Tuberculosis (1921)
- Diphtheria (1923)
- Tetanus (1926)
- Yellow Fever (1935)
- Polio (1952)
- Measles (1958)
- Mumps (1967)
- Rubella (1970)
- HBV subunit (1982)
- Conjugate (Hib) (1987)
- Genetically-modified pertussis (1995)

Europe: 1800
(India, China: 1000)

1900
2000

Specificity and safety

Risk Factors in the 21st Century

Number of people aged 60 and over

Source: UN, 2002
Risk Factors in the 21st Century

- **Hypertension**
  - 40%-60% patients non-compliant within 1 year

- **Hypercholesterinemia**
  - 45% non-compliant within 1 year
  - 60% non-compliant within 18 months

- **Asthma**
  - 50% non-compliant within year

Sources:  
Drugs treating risk factors have to be:

- Convenient, to maximize
- Compliance

→ Vaccines are convenient since they have a long-lasting effect
Hypertension Vaccine

- ACE inhibitors
- AIIR blocker
- Renin inhibitors

Diagram showing the modulatory effect of these inhibitors on Angiotensin II production and action.
Hypertension

- Compliance: An estimated 50-80% do not take all of the prescribed medication
- Morning pressor surge: Pharmacokinetic profile of current inhibitors of the RAS may limit efficacy in early morning hours

Vaccine targeting angiotensin II may address these two issues, due to long-lived antibody responses

Antigen Organization drives B cell responses

<table>
<thead>
<tr>
<th>Organization:</th>
<th>high</th>
<th>low</th>
<th>absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody response</td>
<td>+++</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Induction of auto-antibodies</td>
<td>+++</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Science 262, 1448-1451*
Why is epitope repetitiveness so important?

Our immune system exploits **structural features** for the discrimination of **self and foreign**.

Viruses have small genomes and therefore consist of a small number of proteins.

Viruses cannot help but have to express highly repetitive and highly ordered arrays of antigens on their surface.

→ **Antigen Organisation** is a geometric **PAMP** (Pathogen associated molecular pattern)
Active immunization: Mechanism

Bachmann & Dyer  Nat Rev Drug Discov. 2004
No IgG antibodies in the absence of T help

T help provided by the carrier is required for antibody production

\[ \rightarrow \] Hence no boosting of the response by endogenous cytokine

Bachmann & Dyer  Nat Rev Drug Discov. 2004
Carrier-specific help bypasses Th cell tolerance

Bachmann & Dyer Nat Rev Drug Discov. 2004
Vaccine Design

- Non-replicating
- Contains RNA as natural TLR7/8 ligand
- Very stable
- Economic production in bacteria
- 2 g/l bacterial culture of GMP grade material
High Antibody Titers in Mice and Men

**Mouse**

Antibody titer (OD$_{50}$)

- Peptide
- Peptide
- Peptide-Protein Carrier
- Peptide-VLP

**Factor 100**

**Human**

Antibody titer (Endpoint)

- 10μg i.m.
- 50μg i.m.
- 10μg s.c.
- 50μg s.c.

**Adjuvants**

- none
- Alum
- Alum
- none

*J Allergy Clin Immunol. 117:1470*
Vaccine Design

Qbeta virus-like particle

CGGDRVYIHPF

Angiotensin II

30 nm

CYT006-AngQb
Strong antibody responses against Angiotensin II

ELISA titer (OD50%) vs Days after immunization

- d7
- d14
- d21
- d28

J Hypertens 25:63-72
Reduction of blood pressure in rats

![Graph showing blood pressure and antibody titers over time.](image)

- Systolic BP (mm Hg) vs. Days
- Anti Ang II titer (OD50%)

- Lines for Titer CYT006-AngQb, BP VLP, and BP CYT006-AngQb

- Statistical significance indicated: \( p < 0.05 \)
### Study Outline (1)

<table>
<thead>
<tr>
<th>Indication</th>
<th>Mild to moderate hypertension (systolic 140–179mmHg; diastolic 90–109mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Design</td>
<td>Double-blind, randomized, placebo-controlled, sequential two-dose comparison study in 72 patients</td>
</tr>
<tr>
<td></td>
<td>Study period: 4 months plus 8 months safety follow-up</td>
</tr>
<tr>
<td>Endpoints</td>
<td>Safety, tolerability, and exploratory efficacy (change from baseline blood pressure)</td>
</tr>
</tbody>
</table>
Two Dose Levels vs. Placebo

N=24

100µg AngQb

N=12

placebo

24h ambulatory blood pressure measurement

Injection


N=24

300µg AngQb

N=12

placebo

safety follow up
Evidence for Affinity Maturation

Antibody Responses in Study 01

ELISA titer

weeks

300 µg AngQb
100 µg AngQb
Placebo

Evidence for Affinity Maturation
### Day-time blood pressure

<table>
<thead>
<tr>
<th></th>
<th>-9.0 / -4.0 mm Hg</th>
<th>p=0.015 / p=0.064</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day-time Blood Pressure</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>at 8am</strong></td>
<td>-25 / -13 mm Hg</td>
<td>p&lt;0.0001 / p=0.0035</td>
</tr>
</tbody>
</table>
24 Hours Measurement

Conclusion

- Immunization against angiotensin II can significantly reduce blood-pressure in hypertensive individuals
- Regimen and dosing, however, needs optimization
Vaccination against Parkinson`s disease

Martin Bachmann, Jenner Institute, University of Oxford, UK
Parkinson’s Disease (PD)

Neurodegenerative disease
Loss of dopaminergic neurons
Leading to motor control disorder
Characterised by Lewy bodies = protein aggregates in the brain

Mis-folded / aggregated disease-specific proteins common feature of neurodegenerative diseases
PrP, Kreuzfeld-Jakob disease, etc
Amyloid-β (Aβ), tau - Alzheimer’s disease (AD)
α-syn – Parkinson’s disease (PD)
Huntingtons, ALS
**α-Synuclein**

- α-synuclein is expressed in various tissues and adopts a monomeric but largely unfolded structure (J Biol Chem. 2012 May 4;287(19):15345-64.).
- α-synuclein undergoes heavy posttranslational modification, including phosphorylation, nitration and sometimes aggregation (Nat Rev Neurosci. 2013 Jan;14(1):38-48)
- Mild overexpression (2-fold) but also single point mutations may cause familial Parkinson`s disease (Lancet 364(9440):1167–1169.; Science 276: 2045)
- Aggregated α-synuclein is thought to be central for Parkinson`s disease development
Parkinson’s Disease (PD)

Nature; 501(7465):45-51
Antibodies may stop propagation

Small aggregates / oligomers neutralised by antibodies

Protection of degenerative pathology

Hope for approach to stop progression and spread of proteo-pathological disorders
Passive Immunization Reduces Behavioral and Neuropathological Deficits in an Alpha-Synuclein Transgenic Model of Lewy Body Disease

Eliezer Masliah¹,²*, Edward Rockenstein¹, Michael Mante¹, Leslie Crews², Brian Spencer¹, Anthony Adame¹, Christina Patrick¹, Margarita Trejo¹, Kiren Ubhi¹, Troy T. Rohn³, Sarah Mueller-Steiner⁴, Peter Seubert⁴, Robin Barbour⁴, Lisa McConlogue⁴, Manuel Buttini⁴, Dora Games⁴, Dale Schenk⁴

¹ Department of Neurosciences, University of California San Diego, La Jolla, California, United States of America, ² Department of Pathology, University of California San Diego, La Jolla, California, United States of America, ³ Department of Biology, Boise State University, Boise, Idaho, United States of America, ⁴ ELAN Pharmaceuticals, South San Francisco, California, United States of America
Active versus passive vaccination

• mAb therapy is highly effective, but
  → cost-intensive
  → many patients develop anti-antibody responses which neutralize the therapeutic potential of mAbs

• Increasingly older populations pose a pharmacoeconomic threat to health-care systems resulting in downward pressure on drug-costs

• Innovative and cost-effective therapies are needed

• Vaccination addresses these issues

Drug Discov Today. 2006 Nov;11(21-22):1028-33
Vaccine. 2013 Apr 3;31(14):1777-84
1) Induction of antibodies recognizing oligomeric and aggregated \( \alpha \)-synuclein

2) Oligomers should be recognized preferentially over monomeric \( \alpha \)-synuclein (even though native \( \alpha \)-synuclein is intracellular and not obviously accessible to antibodies).

3) Specific T cells should be avoided (see ELAN and their AD vaccine) \( \rightarrow \) no strong adjuvants and peptide epitopes
Vaccine Design

- Non-replicating
- Contains RNA as natural TLR7/8 ligand
- Very stable
- Economic production in bacteria
- 2 g/l bacterial culture of GMP grade material

Bachmann&Jennnings
Nature reviews Immunology 10:787-796
CAD106: A Vaccine for Alzheimer`s now in Phase III based on the same principle

\[
\begin{align*}
\text{A}_\beta_{1-6} & \quad \text{DAEFRHG} \\
\text{A}_\beta_{1-42} & \quad \text{DAEFRHDSGYEVHHQKVFFAEDVGSNKGAIIGLMVGGVIA}
\end{align*}
\]
- 3 peptides chosen for vaccine design: MDVFMKGL, KNEEGAPQ, EGYQDYEPEA
- Sequence comprised between 8-10 AA $\Rightarrow$ essentially no T cell epitopes
- C-term and N-term of proteins generally accessible in aggregates
Good response with all peptides

Figure 2. Peptide-specific antibody responses induced by the three vaccines and a biosimilar of CAD106 (a vaccine against Alzheimer’s disease currently developed by Novartis) (n=4 mice/group).
Recognition of aggregated α-synuclein (human brain tissue)

IgG from immunised mice (VLP-peptide 3) day 70

Figure 4. Immunohistochemistry on paraffin-embedded post-mortem brain tissue from a PD patient, Braak stage 4. Purified IgGs (1 mg/mL) used at 1:1000. Region sampled, substantia nigra. Scale bar, 50 µm. Lewy bodies (white arrows), Lewy neurites (black arrows), Pale body (white arrowhead), intracellular aggregation (white dashed arrows).
Recognition of aggregated α-synuclein (human brain tissue)

Figure 3. Immunohistochemistry on paraffin-embedded post-mortem brain tissue from a PD patient, Braak stage 4. Purified IgGs (1 mg/mL) used at 1:1000. Region sampled, substantia nigra. Scale bar, 50 µm. Lewy bodies (white arrows), intracellular aggregation (white dashed arrows).
Mouse Model of Parkinson`s disease

Figure 1. A model of Parkinson`s disease in mice.

Figure 2. Workflow of the program.

Janezic et al., PNAS, 2013
α-syn is recognized in tg-mouse model
1) Induction of antibodies recognizing oligomeric and aggregated \( \alpha \)-synuclein

2) Oligomers should be recognized preferentially over monomeric \( \alpha \)-synuclein \( \rightarrow \) even though native \( \alpha \)-synuclein is intracellular and not obviously accessible to antibodies.

3) Specific T cells should be avoided (see ELAN and their AD vaccine) \( \rightarrow \) no strong adjuvants and peptide epitopes
Soluble proteins versus aggregates

Bivalent Binding → „avidity“

Monovalent Binding → „affinity“

→ Low affinity antibodies recognize aggregates but not soluble proteins
α-synuclein specific Abs have low affinity

<table>
<thead>
<tr>
<th>Affinity of immune sera</th>
<th>Day 14</th>
<th>Day 70</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptide 1</td>
<td>200 nM</td>
<td>190 nM</td>
</tr>
<tr>
<td>Peptide 3</td>
<td>30 nM</td>
<td>35 nM</td>
</tr>
</tbody>
</table>
Efficacy Experiment Ongoing

**Vaccination of young mice (6 week-old)**

VLP-peptide (20 μg), s.c. administration (every 2 weeks for 1 month, then monthly)

0 7 14 21 28 (1) (2) (3) (4) (5) (6) (12) (18)  

Experimental day (month)

6 wks 10 wks 14 wks (3.5 mo.)  12 mo.  18 mo.

Age of animal

Study 1

1. Biochemistry: assess α-synuclein protein burden (ELISA, WB)

Study 2

2. a. Dopamine neurotransmission (FCV)

Controls:
- SNCA-OVX mice: administration of non-coupled VLPs
Acknowledgements

Cytos Biotechnology, Zürich
Gunther Spohn
Patrik Maurer
Gary Jennings

University Hospital Zürich
Franziska Zabel
Antonia Fettelschoss
Thomas Kündig

University of Oxford
Aadil El-Turabi
Marika Doucet
Richard Wade-Martins

BRSC Riga
Paul Pumpens
Andris Zeltins
Andris Dishlers

Hypertension
Robert Sabat, Berlin
Frank Wagner, Berlin
Jürg Nussberger, Lausanne