Determining the Therapeutic Potential of Anti-PrP Nanobodies

Jiyan Ma
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<table>
<thead>
<tr>
<th>Humans</th>
<th>Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sporadic</strong></td>
<td><strong>Scrapie</strong> – sheep, goats</td>
</tr>
<tr>
<td>Sporadic Creutzfeldt-Jakob disease (sCJD)</td>
<td></td>
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<tr>
<td>Sporadic fatal insomnia</td>
<td></td>
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<tr>
<td>Variably Protease-sensitive prionopathy (VPSPr)</td>
<td><strong>Chronic Wasting Disease (CWD)</strong></td>
</tr>
<tr>
<td><strong>Inherited</strong></td>
<td>– deer, elk</td>
</tr>
<tr>
<td>Gerstmann-Sträussler-Scheinker syndrome (GSS)</td>
<td><strong>TME</strong> – mink</td>
</tr>
<tr>
<td>Fatal familial insomnia (FFI)</td>
<td><strong>BSE</strong> – cattle</td>
</tr>
<tr>
<td>Familial Creutzfeldt-Jakob disease (fCJD)</td>
<td><strong>FSE</strong> – domestic cats, captive wild cats</td>
</tr>
<tr>
<td><strong>Acquired</strong></td>
<td><strong>Exotic Ungulate Spongiform Encephalopathy</strong></td>
</tr>
<tr>
<td>Iatrogenic Creutzfeldt-Jakob disease (iCJD)</td>
<td>– exotic zoo ruminants of the family Bovidae (kudu, elands, etc)</td>
</tr>
<tr>
<td>Kuru</td>
<td><strong>TSE in non-human primates</strong> – captive</td>
</tr>
<tr>
<td>Variant Creutzfeldt-Jakob disease (vCJD)</td>
<td>lemurs, Rhesus macaque</td>
</tr>
</tbody>
</table>
Prion Protein (PrP)

Aguzzi and Heikenwalder *Nature Reviews Microbiology* 4, 765–775
**PrP<sub>C</sub>**
- 42% α-helix, 3% β-sheet
- Soluble in mild detergents
- Sensitive to protease digestion
- Sensitive to PI-PLC digestion

**PrP<sub>Sc</sub>**
- Almost all β-sheet
- Insoluble in mild detergents
- Resistant to protease digestion
- Resistant to PI-PLC digestion
Prion infectivity: Seeded conversion

Prion strains
Cell membrane

Changes in the cell

Toxicity to neurons

Targets
- \( \text{PrP}^\text{Sc} \)
- \( \text{PrP}^\text{C} \)
- Cellular changes

Agents
- Small molecules
- Antibodies
PrPSc

Advantage
• Disease specific

Potential pitfalls
• Prion strains
• Difficult to generate PrPSc-specific reagents

Cellular changes

Advantage
• Independent of strains

Potential pitfalls
• Affecting other cellular processes

IND24
• Significantly increase the life span of mouse prion infected mice
• Ineffective against human prions

GSK2606414
• penetrates the blood-brain barrier
• prevents clinical disease in prion-infected mice
• but severe side effects (toxicity)
**PrPC**

**Advantage**
- Independent of strains
- Bind to a larger region of a protein

**Potential pitfalls**
- Potential toxicity due to PrP binding
- Difficult to cross the blood brain barrier (BBB).

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Thus far, PrPC appears to be a good target and the therapeutic effects of anti-PrP antibodies are better than small molecules.

But, crossing the blood brain barrier appears to be a major obstacle for diseases already reached central nervous system.

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Monoclonal antibodies inhibit prion replication and delay the development of prion disease

*Monoclonal antibodies inhibit prion replication and delay the development of prion disease*

Anthony R. White*, Perry Enever*, Mourad Tayebi†, Rosey Mushens†, Jackie Linehan‡, Sebastian Brandner‡, David Anstee‡, John Collinge‡ and Simon Hawke*  


i.p. injection of anti-PrP antibody
- Prevents prion disease in mice received intraperitoneal prion infection (> 500 dpi)
- No effect against mice received intracerebral prion infection.
Encoded by two different genes

Encoded by one gene
Adeno-associated Virus (AAV)

- A replication-defective virus found in humans (It requires co-infection of other virus, such as adenovirus or herpesviruses, for its replication).
- ~ 80 - 90% of adults are positive with AAV, but it is not associated with any symptoms or disease.
- In human cells, it preferentially integrate into the AAVS1 region, ~ 2Kb region on the long arm of human chromosome 19.
- Gene therapy treatment of spinal muscular atrophy (SMA) with AAV vector has been approved by FDA.
- Scientists are actively searching for AAVs that can cross the BBB. In C57BL mice, the newly identified AAV-PHP.eB is able to cross BBB.
Recombinant PrP
Probing the N-Terminal β-Sheet Conversion in the Crystal Structure of the Human Prion Protein Bound to a Nanobody

Romany N. N. Abshkaron, Gabriele Giachin, Alexandre Wohlkonig, Sameh H. Soror, Els Pardon, Giuseppe Legname, and Jan Steyaert

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**Graph:**
- MoPrP
- MoPrP•Nb484
- Nb484

**Table:**
<table>
<thead>
<tr>
<th>Nb484 (µM)</th>
<th>0</th>
<th>0</th>
<th>0.75</th>
<th>1.75</th>
<th>3.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>PK -</td>
<td>ScGT1 -</td>
<td>GT1 -</td>
<td>ScGT1 -</td>
<td>ScGT1 -</td>
<td>ScGT1 +</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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</tbody>
</table>

**Image:**
- Western blot showing actin bands.
AAV delivery to central nervous system

Control

Exposure time: 3s
Exposure time: 200ms

14 days

AAV-GFP

Exposure time: 200ms

Higher Amount

Exposure time: 200ms

Lower Amount

Exposure time: 200ms

21 days

Anti-GFP

GFP | Nb1 | Nb2
---|---|---
L | H | L | H | L | H

Anti-Flag
Summary

• We identified nanobodies that bind to not only PrP expressed in bacteria, but also the fully modified PrP expressed in mammalian cells.

• These anti-PrP nanobodies are able to inhibit prion replication in vitro, and do not show any neurotoxicity.

• The anti-PrP nanobodies have been packaged into AAV and successfully expressed in the central nervous system.

• The study of the potential therapeutic effect of expressing anti-PrP nanobodies by AAV in mice is underway.
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- Jan Steyaert
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- Els Pardon

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