PRION DISEASE

A review of experimental drug treatments
Normal prion protein: (PrP-sen or PrP<sup>C</sup>)

- Sensitive to proteases
- Soluble in detergents
- In diverse tissues, cell types
- Apparent cellular roles:
  - Adhesion
  - Differentiation
  - Neuritogenesis
  - Synaptogenesis
  - Cell survival, apoptosis
  - Resistance to oxidative stress
  - Metal binding
  - Hemin binding

Essential for TSE diseases
TSE-associated prion protein:  
(PrP-res, PrP\textsuperscript{Sc}, PrP\textsuperscript{CJD}, PrP\textsuperscript{BSE}, PrP\textsuperscript{CWD}, etc.)

- Resistant to proteases
- Form insoluble aggregates, polymers  
  - e.g. amyloid fibrils, plaques
- Nervous tissue, lymphoid tissue
- Co-localized with neuropathology
- Associated with infectivity
- Covalent structure indistinguishable from normal PrP

Main difference appears to be conformational

PrP\textsuperscript{Sc} = red

Valerie Sim, unpublished data

B. Chesebro et al,  
Science 308:1435 (2005)
Therapeutic targets – where to begin?

- **PrP-res**
  - Directly block conversion
    - Binding of PrP\(^C\) and PrP-res
    - Redistribute or sequester PrP\(^C\) to a location incompatible with conversion
    - Suppress PrP\(^C\) expression
  - Indirectly block conversion
    - Interfere with accessory molecules or pathways required for conversion and/or pathology
  - Enhance clearance

- **Neuroprotection**

- **Combination therapy**
Therapeutic targets – when to begin?

Before exposure: prophylaxis / decontamination
- Familial CJD, GSS, FFI
- Blood transmission of vCJD, surgical instruments
- Reduce spread of BSE, CWD, scrapie

Neuroinvasion (symptomatic)
- Sporadic / genetic diseases
- Requires bypassing blood-brain barrier
- Combined approach?
  - PrP-res inhibition, clearance
  - Block neurotoxicity or promote recovery

Peripheral replication
- Orally-acquired diseases
  - vCJD, BSE, CWD, scrapie
- Immunotherapies, chemotherapies
- Requires early diagnosis
  - PMCA-based methods (including rPrP-PMCA, QuIC)
Therapeutic targets – how to begin?

• **In vivo**
  – Slow, costly, impractical for screening
  – Dosage and route considerations (intraventricular)

• **In vitro**
  – Infected cell culture models
    • Allow high-throughput screening
      – scrapie N2a (mouse) [Kocisko et al, J Virol 2003]
      – scrapie fibroblasts (mouse) [Vorberg et al, JID 2004]
      – scrapie SN56 (mouse) [Baron et al, J Virol 2006]
      – scrapie Rov9 (sheep) [Kocisko et al, Neurosci Lett 2005]
      – MDB^{CWD} (deer) [Raymond et al, J Virol 2006]
    • Allow investigation of underlying mechanisms
  – Non-cell based models
    • Competitive binding of PrP^C and PrP-res
    • Prevention of amyloid fibril formation
    • Computer-based predictions of PrP binding partners
Results so far

- Thousands of compounds tested in vitro
- Hundreds of new inhibitors identified
- Some protect rodents against peripheral scrapie inoculation
- Some prolong the lives of rodents with established CNS infections
- Two inhibitors (at least) are being tested in CJD patients
  - Pentosan polysulphate
  - Quinacrine (PRION1 trial)
Targetting PrP conversion

- **Binding PrP<sup>C</sup> and / or PrP-res**
  - Polyanions, sulphated glycans
    - Pentosan polysulphate, phosphorothioated oligonucleotides
  - Sulphonated dyes (may mimic polyanions by stacking)
    - Congo red, suramin, curcumin
  - Cyclic tetrapyrroles
    - Porphyrins, phthalocyanines, hemin
  - Lysosomotropic factors
    - Quinacrine
  - Tetracyclics
    - Tetracycline, doxycycline
PrP-res inhibitors: a common mechanism of action?

**Small molecules:** porphyrins, phthalocyanines, sulphonated dyes

**Large polymers:** sulphated glycans, phosphorothioate-oligonucleotides (PS-ONs)

- Bind PrP\(^C\) (but not necessarily PrP-res)
- Compete for the same, or overlapping, binding sites in the amino-terminal half of the molecule
- Induce clustering of PrP\(^C\) and internalization (?) from cell surface
  (Harris, Schätzl, Winklhofer, Tatzelt, Caughey labs)

Phthalocyanine Tetrasulphonate (PcS\(_4\))  Iota-carrageenan (sulphated glycan)

His globular domain (residues ~126-231) Trp octapeptide repeats

sulphated glycans or PS-ONs

Non-ionic core

sulphonated (-) porphyrins, phthalocyanines or dyes

flexible domain (residues 23-~125) Lys105

PrP C

Model of inhibitor interactions with PrP C

proteoglycan
GAG chains
PrP
PrP Sc
endosomes
lysosomes
Inhibition
No inhibition
inhibition
lysosomes
degradation
A role for PrP<sup>C</sup> in heme binding, sensing, transport, signal transduction, detoxification?

- 10-20 hemins per PrP via flexible N-terminal domain
- PrP<sup>C</sup> clustering on cell surface
- PrP<sup>C</sup> internalization
- PrP<sup>C</sup> degradation
- altered redox activity of hemin
- inhibition of PrP-res formation

Hemin interactions with PrP<sup>C</sup>

Kil Sun Lee et al., JBC. 2007
Phosphorothioate oligonucleotides (PS-ONs) as PrP^{Sc} inhibitors

- CpG PS-ONs (20-mers) have prophylactic anti-scrapie activity attributed to stimulation of innate immunity through TLR-9 receptor (Sethi et al. Lancet. 2002)
- Repeated CpG-PS-ON treatments are highly destructive to lymphoid organs (Heikenwalder et al. Nature Med. 2004)

What about non-CpG PS-ONs with little or no immunostimulatory activity?

Degenerate, randomized mixtures (Randomers) or uniform homopolymers
Prophylactic anti-scrapie activity of degenerate PS-ON 40-mers in Tg7 mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Survival time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>subcutaneous mock</td>
<td>87 ± 6</td>
</tr>
<tr>
<td>subcutaneous PS-ON</td>
<td>218 ± 33</td>
</tr>
<tr>
<td>i.p. mock</td>
<td>80 ± 8</td>
</tr>
<tr>
<td>i.p. PS-ON</td>
<td>329 ± 8</td>
</tr>
</tbody>
</table>

Days post infection

-7 0 28

i.p. infection (~10^4 LD_{50})

s.c. or i.p. drug doses

Phosphorothioate-oligonucleotides (non-CpG)

• Potently inhibit PrP-res in scrapie-infected cell cultures
  • size dependent (best at ≥25 bases)
  • independent of base composition
  • dependent on phosphorothioation

• Bind to PrP\(^\text{C}\) with nM affinity

• Bind to PrP\(^\text{C}\) on cell surface, causing clustering and internalization.

• Quadruple \textit{in vivo} survival times after i.p. scrapie challenge

• Increase survival times with one-time mixing with i.c. scrapie brain inoculum, as if scrapie was diluted >1000 X

• Neutralizes blood-borne hamster scrapie

• Lower anticoagulation activity than PPS

A new class of anti-TSE compound with no apparent CpG-ON-like toxicity

Porphyrrins: Sulphonated (TSP) & Anilinium (TAP)

• **Prophylactic activity**
  FeTAP quadruples survival time after i.p. scrapie challenge (~10^4 LD<sub>50</sub>)

• **Decontaminating activity**
  FeTAP & NiTAP increase survival time ~40% when mixed with i.c scrapie inocula (~10^6 LD<sub>50</sub>), as if inocula were diluted 1,000-10,000-fold.

• **Effects against established brain infections**
  FeTSP increases survival times by ~50% when treatment is begun 2 wks after i.c. scrapie inoculation (~10^6 LD<sub>50</sub>), if drug is injected directly into the brain.

  * Similar to effects of pentosan polysulphate infusions into the brain (Doh-Ura et al.)

Targetting PrP conversion

- **Redistribution or sequestration of PrP**
  - Simvastatin (cholesterol depletion?)
    - crosses blood-brain barrier
    - high dose delays disease when given 100 days after i.c. inoc. ([Mok et al. Biochem Biophys Res Commun. 2006](#))
    - may be secondary to anti-inflammatory effects
  
- Polyene antibiotics (alter raft domains)
  - Amphotericin B, MS-8209
    - some effect in late treatment of i.c. inoc. mice ([Demaimay et al. J Gen Virol. 1994; Demaimay JV. 1997](#))
Targetting PrP conversion

- **Suppress PrP<sup>C</sup> expression**
  - May reverse neuropathology and neurological signs
    (Mallucci et al. Science. 2003; Mallucci et al. Neuron. 2007)
  - Use siRNA, lentivirus vector delivery
Other approaches

- **Target accessory molecules and pathways**
  - Laminin receptor and precursor
    - some effect on peripheral phase (Zuber *et al.* Mol Immunol. 2008)
  - Tyrosine kinase inhibitors (Imatinib mesylate)
    - delayed neuroinvasion (Yun *et al.* J Neurovirol. 2007)
  - Follicular dendritic cells
    - cleared spleen PrP^res^ and prevented neuroinvasion

- **Enhance PrP-res clearance?**
  - Cationic polyamines (phosphorus dendrimers, gen 4.0)
    - reduce spleen PrP^Sc^ in i.p. inoc. mice (Solassol *et al.* J Gen Virol. 2004)
  - Antibodies...
**Antibodies**

- **Active immunization**
  - Self-tolerance to PrP\(^c\) makes generation of high titres difficult
    - Specific PrP\(^\text{res}\) antibodies eg. Tyr-Tyr-Arg ([Paramithiotis et al. Nat Med. 2003](#))
  - Must avoid widespread cellular lysis
    - Alzheimer Abeta vaccine caused meningoencephalitis ([Orgogozo et al. Neurology. 2003](#))
    - Avoid T-cell responses by using a PrP sequence inserted into a bovine papillomavirus 1 protein carrier ([Handisurya et al. 2007](#))
Antibodies

- **Passive immunization**
  - Immediate post-exposure or early treatment
    - can prolong incubation times in i.p. inoc. mice
  - Blood-brain barrier
    - may limit effectiveness after neuroinvasion
- **Side effects**
  - Hippocampal injection of high concentrations of PrP antibodies (against sequence 95-105) led to degeneration of hippocampal and cerebellar neurons (Solforosi et al. Science. 2004)
  - Fab fragments may avoid degeneration secondary to cross-linking of PrPc
• **Neuroprotection**

  – Analgesics (flupirtine maleate)
    • human trial, only mild cognitive benefit *(Otto et al. Neurology. 2004)*

  – Cannabis (cannabinol)
    • early treatment benefit in i.p. inoculated mice *(Dirikoc et al. J Neurosci. 2007)*

  – Anti-oxidants?

  – Bilobalide (derived from Gingko biloba)
    • not effective in i.c. inoculated mice *(Sim, Morrey, Caughey, unpublished data)*
Combination Therapy?
Enhanced anti-scrapie effect using **combination** treatment:

Intracerebral injection of pentosan polysulphate (PPS) and a porphyrin (FeTSP) beginning 2-4 wks after intracerebral scrapie inoculation of Tg7 mice

i.c. infection (~10^6 LD<sub>50</sub>)

Kocisko *et al.* Antimicrob Agents & Chemother. 2006
Drug combinations with more than additive effects

Demonstrated *in vivo* effects:

- Pentosan polysulphate and FeTSP (effect decreases late in incubation period)  

*In vitro* data:

- Quinacrine and simvastatin  
  *(Klingenstein et al. J Neurochem. 2006)*

- Quinacrine and desipramine (new drug created: quipramine)  
  *(Klingenstein et al. J Neurochem. 2006)*

- Quinacrine and rPrP-Q218K  
  *(Kishida et al. Amyloid. 2004)*
Inhibition of PrP$_{Sc}$ accumulation is important, but may not be sufficient in the clinical phase.

Combining conversion inhibitors with neuroprotective agents or those that enhance PrP$_{Sc}$ clearance may be more successful...
Coworkers: Inhibitor studies

**Rocky Mountain Laboratories**
Byron Caughey
David Kocisko
Kil Sun Lee
Rick Race
Winslow Caughey
Lynne Raymond
Emily Olsen
Lauren Kett
Brianna Schoen
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Intramural Research Program of the National Institute for Allergy and Infectious Diseases (NIAID) and the Department of Defense
I hope you and your team will eventually find a way to prevent, cure, and eradicate this horribly cruel disease that leaves a family to watch their loved one suffer until death with hands tied.

I have never wished for cancer before in my life.

At least there is hope.

- Daughter of a patient diagnosed with CJD