Antisense Oligonucleotides to Delay or Prevent Onset of Prion Disease in Mice

Byron Caughey
Slides for CJD Foundation Meeting July 2016
Outline

• What is a prion, and how can we stop it?
• Why target the healthy protein?
• Why antisense technology?
• Progress so far
• Plans for preclinical studies
What is prion disease?

A healthy protein that your body normally produces

PrP<sup>c</sup>

A misfolded protein that kills brain cells

PrP<sup>Sc</sup>
Why do we want to reduce PrP$^C$ levels?

Effect of mouse PrPC expression level on disease progression in mice

- **Time to terminal disease**
- **Time to first symptoms**
Why do we want to reduce PrP$^C$ levels?

Effect of mouse PrPC expression level on disease progression in mice

- **Time to terminal disease**
- **Time to first symptoms**

Mice that produce more than the normal amount of PrP$^C$ get sick and die sooner.
Why do we want to reduce PrP\textsuperscript{C} levels?

Effect of mouse PrPC expression level on disease progression in mice

- Mice that produce less than the normal amount of PrP\textsuperscript{C} stay healthy longer.
- Mice that produce more than the normal amount of PrP\textsuperscript{C} get sick and die sooner.

(days post-infection vs. PrPC expression level (fold wild-type))
Why do we want to reduce PrP<sub>C</sub> levels?

Effect of mouse PrPC expression level on disease progression in mice

- Mice that produce **no** PrP<sub>C</sub> **never** get prion disease.
- Mice that produce **less** than the normal amount of PrP<sub>C</sub> stay healthy **longer**.
- Mice that produce **more** than the normal amount of PrP<sub>C</sub> get sick and die **sooner**.
Is it safe to reduce PrP\textsuperscript{C} levels?

• Mice engineered to produce \textbf{no} PrP\textsuperscript{C} have only mild health issues (Bueler et al 1992, Bremer et al 2010)

• Mice engineered to produce \textbf{half} the normal amount of PrP\textsuperscript{C} are indistinguishable from normal mice (Bremer et al 2010)

• Humans with only 1 functional copy of the prion protein gene, instead of 2, are healthy (Minikel et al 2016)
Therapeutic hypothesis

• If we can make people produce less PrPC, they will stay healthy longer
Therapeutic hypothesis

- If we can make people produce less PrP<sub>C</sub>, they will stay healthy longer

- …and how can we do that?
Antisense targets RNA before the protein is produced
What is antisense technology and how does it work?

- An **antisense oligonucleotide (ASO)** is a 20-mer of chemically modified DNA.
- It is complementary to 20 bases of RNA sequence.
- Binds to the RNA and causes the enzyme RNase H to break down the RNA.
- Reduces the amount of a specific RNA, thereby reducing the amount of a specific protein.
- Different ASOs have been developed against many different RNAs for many different diseases.
Antisense is already in clinical trials for other neurological diseases

- ASOs for brain disorders are usually dosed into intrathecal space (base of spine) once per four months
- Patient's experience is similar to undergoing a lumbar puncture (e.g. at Michael Geschwind's study at UCSF)
- Phase 1 trial in spinal muscular atrophy (nusinersen / SMNRx) found IT delivered ASOs to be safe and found preliminary evidence of efficacy (Chiriboga et al 2016), program is currently in phase 3
- Phase 1 trial in Huntington's disease launched last fall
  - Aims to reduce huntingtin RNA levels, similar to our goals for PrP
Antisense meets all the requirements to be a sound therapeutic strategy

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<tr>
<th>Requirement</th>
<th>Why ASOs</th>
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<td>Safe for chronic use</td>
<td>• There is one ASO drug against a different RNA already approved and marketed (Kynamro, for high cholesterol)</td>
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<td>• Preliminary data from ASO trials for spinal muscular atrophy indicate ASOs are well-tolerated in the human brain</td>
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<td>Gets into brain</td>
<td>• ASOs are stable for multiple months and can be injected into base of spine, directly into cerebrospinal fluid, a few times per year</td>
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<td>• Monkeys treated with ASOs for Huntington's disease have reduced huntingtin levels across many brain regions</td>
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<td>• Preliminary data from spinal muscular atrophy trials indicate good ASO brain distribution in humans</td>
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<td>Likely to translate from mouse studies to</td>
<td>• By targeting RNA, ASOs slow the disease process upstream of species- and strain-specific problems</td>
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<td>humans</td>
<td>• Should be effective in multiple species and against all subtypes of prion disease</td>
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Previous work on ASOs for prion disease

- In addition to targeting RNA, some ASOs happen to also interfere directly with prion formation in cells and in mice (Kocisko et al 2006, Karpuj et al 2007)
- An ASO against PrP RNA was moderately effective at delaying disease in mice (Nazor-Friberg et al 2012)
- Improvements in ASO chemistry and sequence screening offer opportunities for more effective, longer-lasting, and safer ASOs.
Goals of our preclinical study

- Develop ASOs against the mouse prion protein RNA
- Dose ASOs directly into mouse brain (intraventricular injection)

- **Potency**: How much we can reduce PrP levels in the mouse brain
- **Efficacy**: How much can ASOs extend the survival time of mice infected with prions?
- **Safety**: Are ASOs well tolerated in these mice?
- **Biomarkers**: Establish whether we can measure PrP levels and prion seeding activity levels in mouse brain as a predictor of efficacy.

- **Ultimate goal**: establish a proof of principle so that we can develop an ASO against the human prion protein RNA and get it into clinical trials
Identification of potent well-tolerated ASOs for use in preclinical studies

- 450+ ASOs screened in Hepa1-6 cells
- 26 of the most potent ASOs selected for large-scale synthesis and \textit{in vivo} testing
- 11 of the most \textit{in vivo} potent ASO selected for high dose, long-term tolerability screening
- The two most potent, with the longest duration of action and well-tolerated ASOs chosen for additional testing

\textit{In vitro} dose response of 2 lead mouse \textit{Prnp} ASOs
Two lead *in vivo* active rodent *Prnp* ASOs selected

- Study #1: Single 300μg ICV bolus injection in C57bl6 mice of ASO or PBS and tissue collection 2 weeks post-treatment

![Graphs showing Prnp mRNA (% PBS) in Cortex and Spinal cord for PBS, ASO 1, and ASO 2.](image)
**Lead in vivo ASOs are active, well-tolerated and have long duration of action**

- **Study #2:** Single 700μg ICV bolus injection in C57bl6 mice of ASO or PBS. Animals weighed and subject to neurological exam weekly. Tissue collection 8 weeks post-treatment.

**RNA 8 week post-injection**

- **No microglial activation**
- **No body weight change**

**No adverse events on neurological exams and no histopathological findings**
# Plans for preclinical studies

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<tr>
<th>Question</th>
<th>Experiments planned</th>
<th>Progress</th>
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<td><strong>Potency</strong></td>
<td>Screen 100s of ASOs in cells.</td>
<td>Complete</td>
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<tr>
<td></td>
<td>Test 26 ASOs in mice.</td>
<td>Complete</td>
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<td>Re-test best ASOs for 4 or 8 weeks in two different varieties of mice to identify single most potent ASO.</td>
<td>In progress</td>
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<td><strong>Safety and efficacy</strong></td>
<td>Inject mice at 4 different timepoints at 5 different doses to identify highest tolerated dose.</td>
<td>Planned</td>
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<td>Dose prion-infected mice with ASOs at four different timepoints: prophylactic, immediately after infection, early infection, and late stages. Determine survival times and tolerability.</td>
<td>Planned</td>
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<td><strong>Biomarkers</strong></td>
<td>Determine reduction in PrP levels in mouse brain</td>
<td>Planned</td>
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<td>Quantify prion seeding activity (RT-QuIC) in brains of mice treated or not treated with ASO</td>
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<td>Establish ability to measure PrP levels in human cerebrospinal fluid as a potential biomarker</td>
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Collaborators

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