Developing RNAi Therapeutics Targeting Huntingtin with Direct CNS Delivery
Presenter Disclosures

Martin Goulet

The following personal financial relationships with commercial interests relevant to this presentation existed during the past 12 months:

Alnylam Pharmaceuticals
Medtronic
The information provided by speakers in workshops, forums, sharing/networking sessions and any other educational presentation made as part of the 2011 HDSA convention program is for informational use only.

HDSA encourages all attendees to consult with their primary care provider, neurologist or other healthcare provider about any advice, exercise, medication, treatment, nutritional supplement or regimen that may have been mentioned as part of any presentation.
Who Are We?

- Alnylam Pharmaceuticals
  - Biopharmaceutical company developing novel therapeutics based on RNA interference, a recently discovered naturally occurring process of gene regulation

- CHDI
  - Not-for-profit virtual biotech company that is exclusively dedicated to rapidly discovering and developing therapies that slow the progression of Huntington’s disease

- Medtronic, Inc.
  - Medical technology company that develops and markets therapies and devices (such as pacemakers and insulin pumps) for chronic diseases
Our Mission

Develop a treatment for Huntington’s Disease that uses a pump to deliver, directly into the person’s brain tissue, a drug that reduces the amount of the disease-causing protein in the brain.

Let’s look at each of these items, then you’ll understand what we’re doing....
Develop a treatment for Huntington’s Disease that uses a pump to deliver, directly into the person’s brain tissue, a drug that reduces the amount of the disease-causing protein in the brain.

*Let’s look at each of these items, then you’ll understand what we’re doing....*
Understanding the Disease-causing Protein...
DNA is copied to make RNA that is used for instructions on how to make Protein.

Proteins form structures, or do work, in our bodies.

e.g. letters “CAG” mean “add the amino acid ‘glutamine’”.

A gene = a section of DNA.

messenger RNA
(Part of) the HD Gene DNA sequence –
Too Many CAG’s → Too Many Glutamines In Protein → Causes The Disease

CAG repeat region (here, 19 repeats)  NM_002111

```
/gene="HD"
CAG, CAG, CAG, .... CAG
```

```
1 gctgccccgga cgggtccaag atggacgggcc gctcaggttcc tgcttttacc tgccggccag
gctgccccgga cgggtccaag atggacgggcc gctcaggttcc tgcttttacc tgccggccag
61 agcccccattc attgccccggg tgctgagggg ccgccgaggt ctggcccgaggg cttcccgggga
agcccccattc attgccccggg tgctgagggg ccgccgaggt ctggcccgaggg cttcccgggga
121 ccggccgtgccc ggccggagaga ccgcccagggc gaccctggaaga aagctgctga aaggctttcga
ccggccgtgccc ggccggagaga ccgcccagggc gaccctggaaga aagctgctga aaggctttcga
181 ctccctcaag tccttcacgc agcagcagca gcaagcagcag cagcagcagcag cagcagcagcag
tccttcacgc agcagcagca gcaagcagcag cagcagcagcag cagcagcagcag cagcagcagcag
241 gcagcagcag cagcAGACAG cgcaccagcccg gccgcaggcccg cgcgcgcctc ctcagctttcc
gcagcagcag cagcAGACAG cgcaccagcccg gccgcaggcccg cgcgcgcctc ctcagctttcc
301 tcagccgcgcg ccgcaggcagc agccgctgtgct gcctcagggc cagccccccc ccgcccggccg
301 tcagccgcgcg ccgcaggcagc agccgctgtgct gcctcagggc cagccccccc ccgcccggccg
361 ccggccgcaga ccgggcggcgg ccggtggctga ggagccggctg caccggacaa agaagaagact
ccggccgcaga ccgggcggcgg ccggtggctga ggagccggctg caccggacaa agaagaagact
421 ttcagctacc aaggaagacc gttgtgaattca ttgtctgaaca aatacgtaaa cagctagttgccc
421 ttcagctacc aaggaagacc gttgtgaattca ttgtctgaaca aatacgtaaa cagctagttgccc
481 acagttcgtgc acaaatatctt cagaaatttcc aaaaatcttct ggcatcgtc agataaattcattttc
481 acagttcgtgc acaaatatctt cagaaatttcc aaaaatcttct ggcatcgtc agataaattcattttc
541 tctgctgtgtcc agtgaagactg cagactcaga tgctgagagtg tggtgctgagc aatgcctcaa
541 tctgctgtgtcc agtgaagactg cagactcaga tgctgagagtg tggtgctgagc aatgcctcaa
601 caaagtttac caaagctttgaa tggatcttaa tctccaaaggttatcagtcg agcttctaa
601 caaagtttac caaagctttgaa tggatcttaa tctccaaaggttatcagtcg agcttctaa
661 ggaattaa aagatgggtg cccctcggag tttgcgctgtg gccctgtgga gttggctgat
661 ggaattaa aagatgggtg cccctcggag tttgcgctgtg gccctgtgga gttggctgat
721 gctggctcacc cttgtgtcggc ctctgaatatg cagccctttc ctcggtgagacc ctcctgcgtgc
721 gctggctcacc cttgtgtcggc ctctgaatatg cagccctttc ctcggtgagacc ctcctgcgtgc
781 cctgactcag acaaggaaga gaccgcagga atcagttcag gagacgcttgc tgcagctgtgt
781 cctgactcag acaaggaaga gaccgcagga atcagttcag gagacgcttgc tgcagctgtgt
841 tccccaaatt atggctctcct ttggaatatt tcgcacagtac aatgaaatta agggttttgaattt
841 tccccaaatt atggctctcct ttggaatatt tcgcacagtac aatgaaatta agggttttgaattt
901 aaagcncttc atagccgaacc gaggagtcaag cttccccacc attcggcggag caggcgtcgg
901 aaagcncttc atagccgaacc gaggagtcaag cttccccacc attcggcggag caggcgtcgg
961 atcagcacgtg agcatctggcc agcartcaag aagagacaa taatcttata gttggctact
961 atcagcacgtg agcatctggcc agcartcaag aagagacaa taatcttata gttggctact
1021 aatgtgtgtc ttaggcttac tcgttctctgt cgaggatgaa cacctccacttc tgcgtattct
1021 aatgtgtgtc ttaggcttac tcgttctctgt cgaggatgaa cacctccacttc tgcgtattct
1081 cggtgtgtgctg ctccaccctgg ccgtaattggt gcacgtggtcg tcaaggacac
How Does The Expanded Huntingtin Protein Cause Disease?

• The expanded protein
  – Aggregates (clumps) of protein accumulate
  – Affects the way other genes in the cell are used...
    • “Transcriptional dysregulation”
  – Changes ability of the cell to produce energy (metabolism)
  – Makes the neuron more susceptible to being injured by too much exposure to certain neurotransmitters
What If We Just “Turn Off” Production of Expanded Protein?

- 2000 – Ai Yamamoto, Columbia University
  - Conditional transgenic HD mouse
    - Expanded huntingtin gene artificially put into mouse, but in a form that only turns on when mouse fed a particular antibiotic
  - Turn on expanded protein → mouse develops disease
  - Turn off expanded protein → mouse recovers
    - Suggests that symptoms are not due just to loss of brain cells, but dysfunction of neurons before cell death…
    - … Neurons can recover when mutant protein is shut off…
    - … Sufficient neurons remain for a time after disease onset to allow some recovery
What If We Just “Turn Off” Production Of Expanded Protein?

• Mice
  – Mutant mice expressing half the amount of huntingtin are normal
    • 1995 – Mabel P. Duyao, Massachusetts General Hospital
    • 1995 – Scott Zeitlin, Columbia University

• Human
  – An individual with half the amount of huntingtin is normal
    • 1994 – Christine M. Ambrose, Massachusetts General Hospital
  – Suggest that lowering huntingtin is safe
Our Mission

Develop a treatment for Huntington’s Disease that uses a pump to deliver, directly into the person’s brain tissue, a drug that reduces the amount of the disease-causing protein in the brain.

*Let’s look at each of these items, then you’ll understand what we’re doing....*
RNA Interference

- Normally, DNA → mRNA → protein
RNA Interference

- Normally, DNA → mRNA → protein → ribosome → protein

Natural Way To Reduce Production Of Protein
“siRNA Therapeutic”

RNA interference targeting a specific mRNA in cells

• RNA Interference
  – Fundamental mechanism built into all our cells:
  – Short, double-stranded molecule of RNA causes cell to “silence” its corresponding gene.

• Normal function:
  – major player in development, gene regulation; some viral defense
  – Operates very specifically (targets specific gene)
  – Can be induced in cells
    • Synthetically”
    • Small interfering RNA (siRNA)
Develop a treatment for Huntington’s Disease that uses a pump to deliver, directly into the person’s brain tissue, a drug that reduces the amount of the disease-causing protein in the brain.

Let’s look at each of these items, then you’ll understand what we’re doing....
Finding The Drug
Alnylam’s Screening Program

Starting Pool of Possibilities for siRNA against Huntingtin
(all short DNA / RNA sequences along the HD gene)

Eliminate the ones that might also suppress other proteins beyond Huntingtin

Stability / Efficacy in cell experiments

Chemical enhancements

Distribution, Efficacy in Animals

Lead Candidate

1
Candidate siRNAs
Screening in Cell Experiments

Normalized Huntingtin mRNA level (%)

- screen 1
- screen 2

cells with various candidate siRNAs

cells without anti-huntingtin siRNA
Finding the Most Potent siRNA In Cell Experiments: How Much Suppression, With How Much siRNA?

siRNA #1
IC50 ~ 0.3 nM

siRNA #2
IC50 ~ 0.08 nM
siRNA Reduce Amount of Expanded Protein (As Well As Normal Length Protein) In Cells From HD Patients

GM04281 line

GM04719 line
siRNA Can Reduce Amount of Huntingtin mRNA In Brains Of Laboratory Rats

Intrastriatal Infusion (siHtt at 2, 4, 8 and 15mg/mL and siLUC at 15mg/mL)

Ipsilateral Striatum for bDNA

Day 0

Day 7

Intrastriatal Infusion (siHtt at 2, 4, 8 and 15mg/mL and siLUC at 15mg/mL)

Ipsilateral Striatum for bDNA

Day 0

Day 7

Increasing concentrations of siRNA targeting Huntingtin mRNA (mg/mL)

Control (using an siRNA that targets an mRNA not found in the rat brain)

Normalized Htt/GAPDH (% siLUC)

**p<0.01; *** p<0.001 vs. siLUC (ANOVA, Tukey posttest)

Error bar: SE

SFN, Oct 2009
Our Mission

Develop a treatment for Huntington’s Disease that uses a pump to deliver, directly into the person’s brain tissue, a drug that reduces the amount of the disease-causing protein in the brain.

Let’s look at each of these items, then you’ll understand what we’re doing....
How Does siRNA Get Into Rat (Or Other) Brain? 
.... We Put It There, Directly.
Direct Infusion Into Brain Using Catheter (Tube)

Length = 5mm for Rhesus

Pump Catheter
Cranial Anchor
IPa Catheter
(Elastomeric polymer)

SFN 2009
Direct Infusion of siRNA into Brains of Monkeys
Distribution of siRNA and correlation with Htt mRNA suppression

<table>
<thead>
<tr>
<th>Tissue location</th>
<th>% Suppression of Htt mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>39%</td>
</tr>
<tr>
<td>2</td>
<td>28%</td>
</tr>
<tr>
<td>3</td>
<td>47%</td>
</tr>
<tr>
<td>4</td>
<td>51%</td>
</tr>
<tr>
<td>5</td>
<td>40%</td>
</tr>
</tbody>
</table>

Keystone: RNA Silencing., Jan 2010
Huntingtin mRNA Suppression and siRNA Distribution in Rhesus Monkey Brain Consistent Htt mRNA Suppression Across Punches and Animals

Outliers indicated in blue square box are from posterior slabs (4 mm posterior to infusion site)

% Htt mRNA Normalized to PBS

P. Buffered Saline

14C-siHtt, 8mg/mL

14C-siHtt, 12mg/mL

Individual monkeys

Keystone: RNA Silencing., Jan 2010
Why Can’t siRNA be a Pill, Or Given By Shot In Arm Or Needle In Vein?

- siRNA cannot readily survive the digestive tract.
- Enzymes in serum can degrade siRNA in blood very quickly (although chemical stabilization methods exist).
- Main reason:
  - Blood-brain barrier excludes large molecules such as siRNA from entering the central nervous system.

Plastic cast of all the blood vessels in the brain.

Magnified view (diagram) of a blood vessel in brain tissue.
How Will People Receive Long-term Treatment?

- Rat brain
- Rhesus monkey brain
- Human brain: Homo sapiens

[Image: Human brain from the Univ. of Wisconsin-Madison Brain Collection]
Develop a treatment for Huntington’s Disease that uses a pump to deliver, directly into the person’s brain tissue, a drug that reduces the amount of the disease-causing protein in the brain.

Let’s look at each of these items, then you’ll understand what we’re doing....
What will the therapy look like?

- Surgical placement of one or more catheters (soft tubes) in brain
- Pump implanted in abdomen (3.4” x 0.77”)
- siRNA delivered to brain (droplets per day) from implanted pump
- Pump periodically refilled through skin (e.g., by nurse, with a hypodermic needle)

Medtronic implantable pumps are already used to deliver drugs to spinal cord (in more than 100,000 in people worldwide) to treat chronic pain or severe spasticity.
So, Now You Understand Our Mission:

Develop a treatment for Huntington’s Disease that uses a pump to deliver, directly into the person’s brain tissue, a drug that reduces the amount of the disease-causing protein in the brain.
How Long Before This Therapy Is Available?
A While....We Have Lots More To Do, Yet.....

• So far, we have
  – Identified siRNA that works with direct delivery to brain
  – Developed new catheter for better direct delivery to brain
  – Optimized parameters for good drug distribution in brain

• Next steps
  – Complete studies required for Investigational New Drug (IND) application
    • Safety of siRNA
    • Catheter tests
  – Prepare for Phase I clinical trial to test for safety in small number of patients
The Progress We’ve Made So Far...

- Potent, selective siRNAs targeting Htt identified with bioinformatics (computers) and in vitro assays (cell experiments)
- Chemically-modified siRNAs targeting Htt reduce Htt mRNA in relevant parts of the brain in rats
  - ~50% Htt mRNA silencing in striatum of rat
  - Dose dependent silencing
  - Persistent Htt mRNA suppression, up to 14 days
- Direct delivery of Htt siRNA to the adult monkey brain by continuous infusion found to be effective in distributing the siRNA and suppressing Htt mRNA
  - ~40-45% Htt mRNA suppression throughout putamen
  - Significant reduction of Htt protein in the putamen
  - Both flow rate and siRNA concentration have been identified as important for maximizing the volume of efficacy
  - Well-tolerated for at least one month
- We’re now working towards our first human clinical trial, collecting data showing safety in animals needed to get FDA’s permission to start human trials.