



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:

**Anylam Pharmaceuticals
Medtronic**

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Who Are We?



- Anylam 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



**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.**

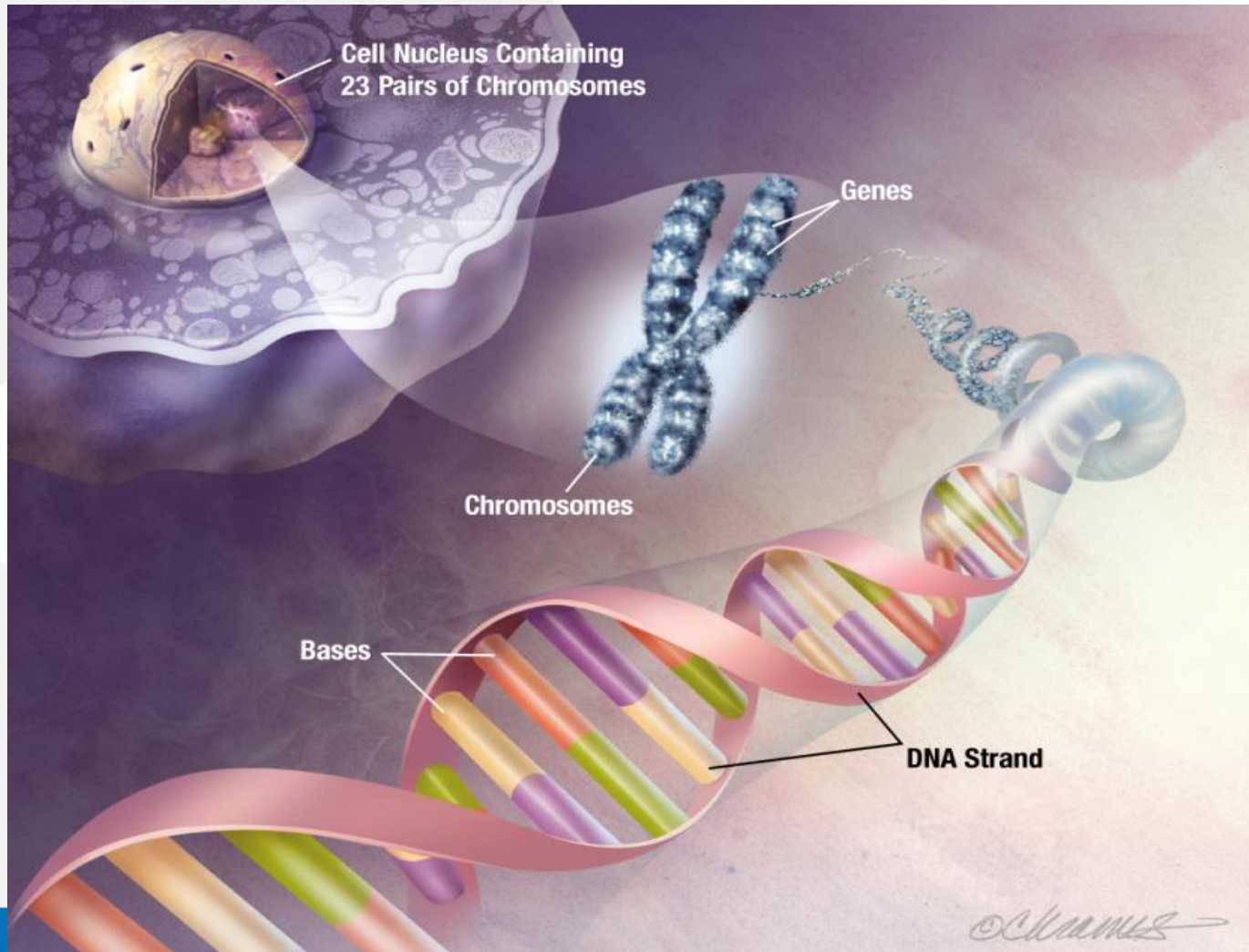
Let's look at each of these items, then you'll understand what we're doing....

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.

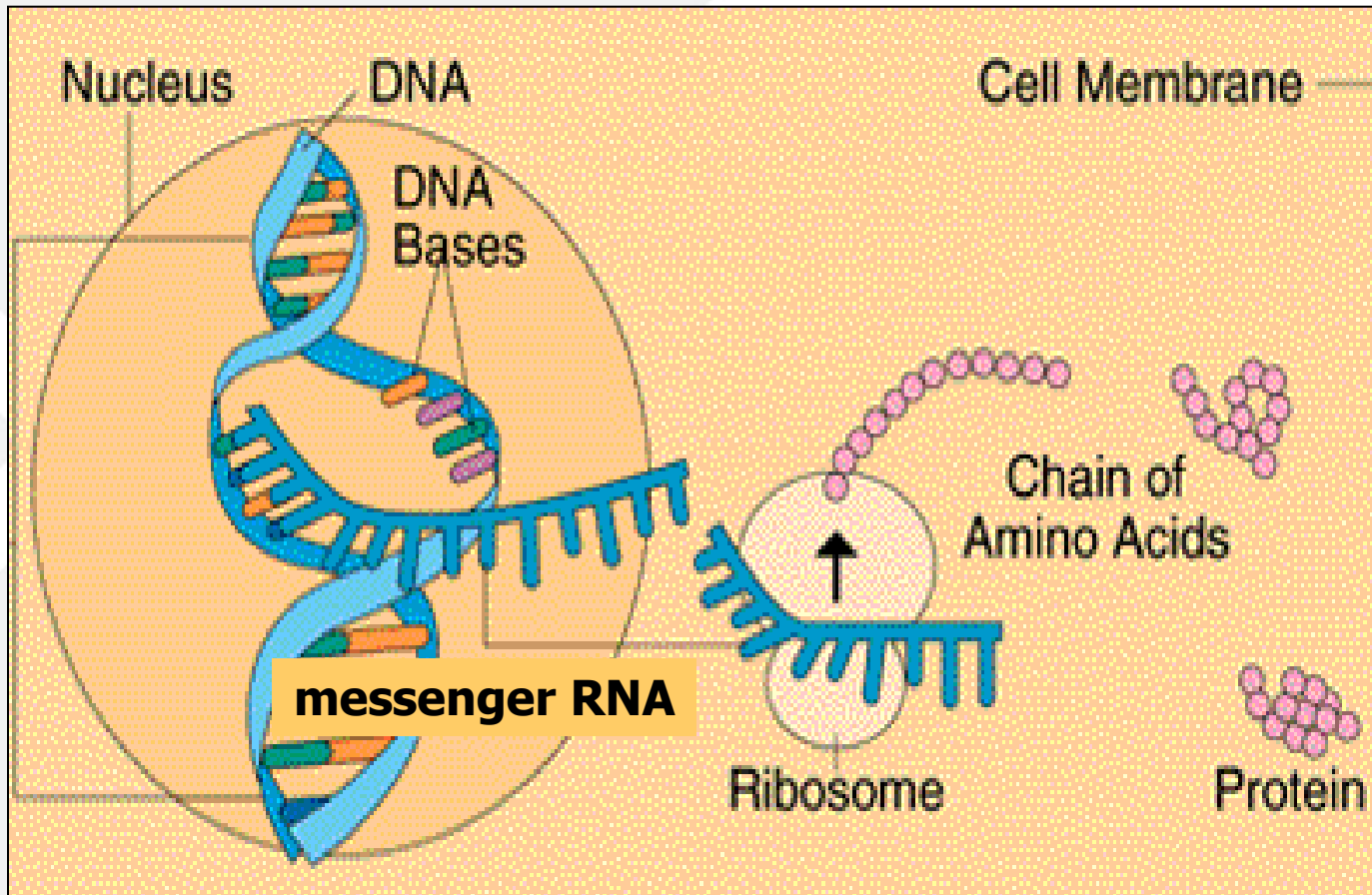
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Understanding the Disease-causing Protein...



DNA is copied to make RNA that is used for instructions on how to make Protein

a gene = a section of DNA



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

e.g. letters "CAG"
mean "add the
amino acid
'glutamine' "

(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



ORIGIN

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1 gctgccggga cgggtccaag atggacggcc gctcaggttc tgcttttacc tgcggccag
61 agccccattc attgccccgg tgctgagcgg cgccgcgagt cggcccagg cctccggga
121 ctgccgtgcc gggcgggaga ccgccatggc gaccctggaa aagctgatga aggcctcga
181 gtccctcaag tccttcagc agcagcagca gcagcagcag cagcagcagc agcagcagca
241 gcagcagcag cagcaacagc cgccaccgcc gccgccgccg ccgccgcctc ctcagcttcc
301 tcagccgccg ccgcaggcac agccgctgct gcctcagccg cagccgccc cgccgccgcc
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421 ttcagctacc aagaaagacc gtgtgaatca ttgtctgaca atatgtgaaa acatagtggc
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901 aaaggccttc atagcgaacc tgaagtcaag ctccccacc attcggcgga cagcggctgg
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1021 aaatgtgctc ttaggcttac tcgttctgt cgaggatgaa cactccactc tgctgattct
1081 taacatacta ctcacctaa gatattgat acccttacta caacacaga tcaaggacac
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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 ?

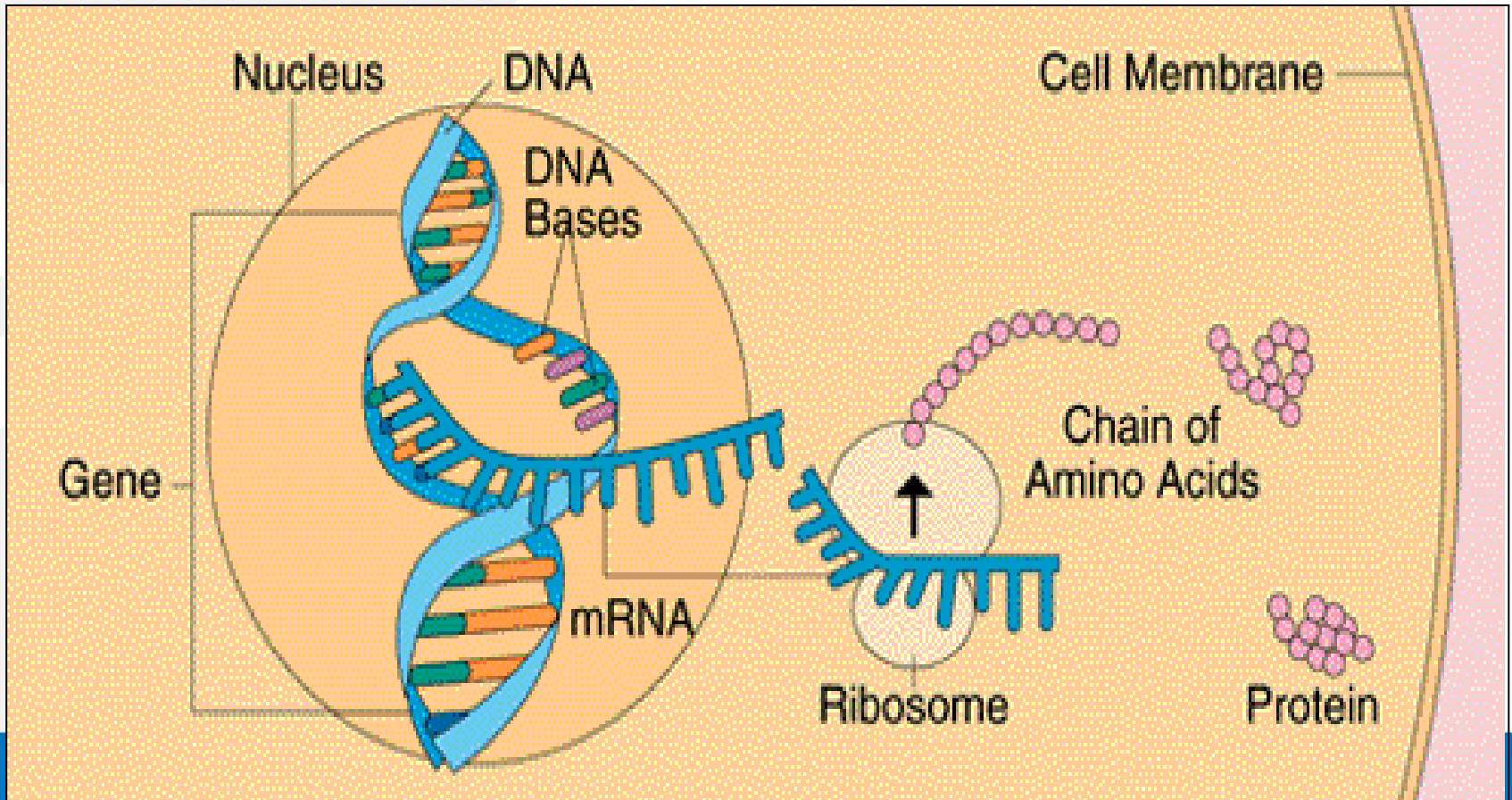
- Mice
 - Mutant mice expressing half the amount of huntingtin are normal
 - 1995 – Mabel P. Duyao, Massachusetts General Hospital
 - 1995 – Scott Zeitlin, Columbia University
- Human
 - An individual with half the amount of huntingtin is normal
 - 1994 – Christine M. Ambrose, Massachusetts General Hospital
 - Suggest that lowering huntingtin is safe

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

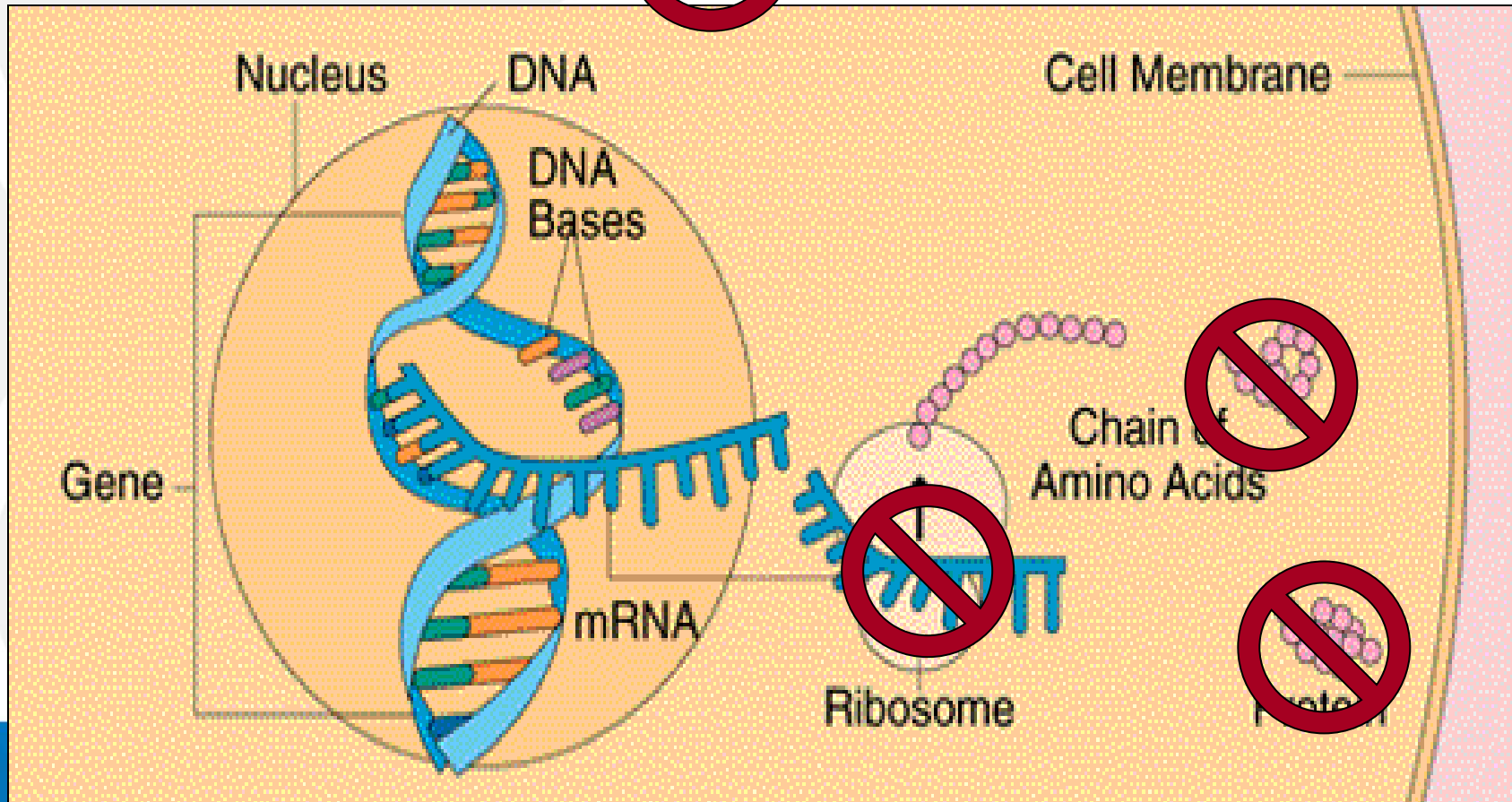
- Normally, DNA → mRNA → protein **Natural Way To Reduce Production Of Protein**



RNA Interference

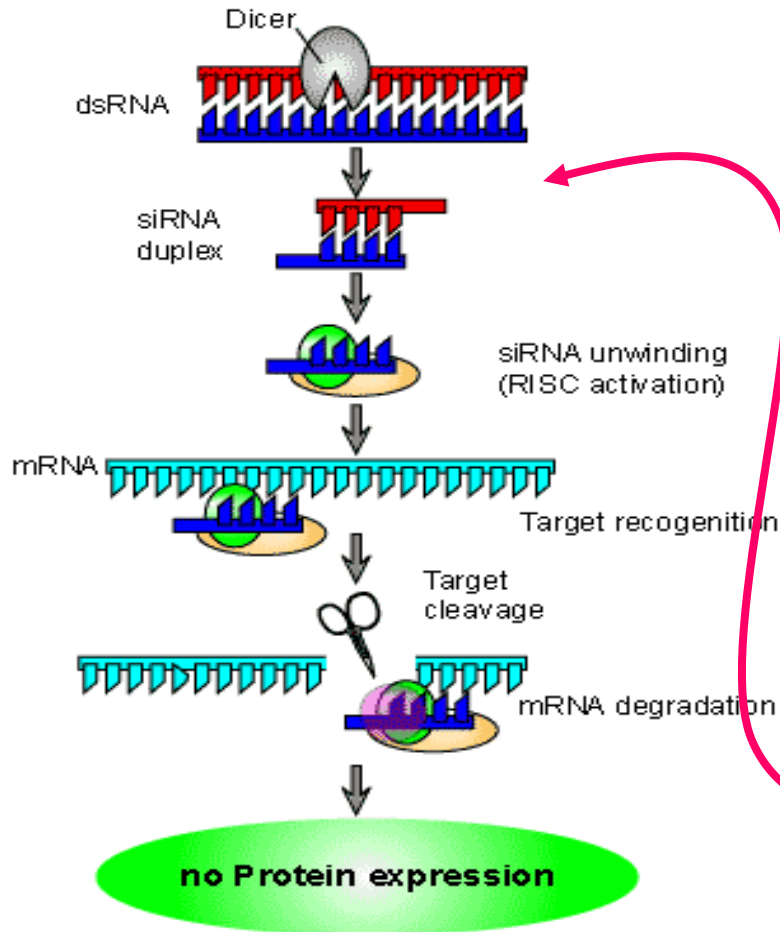
- Normally, DNA → mRNA → Protein

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)

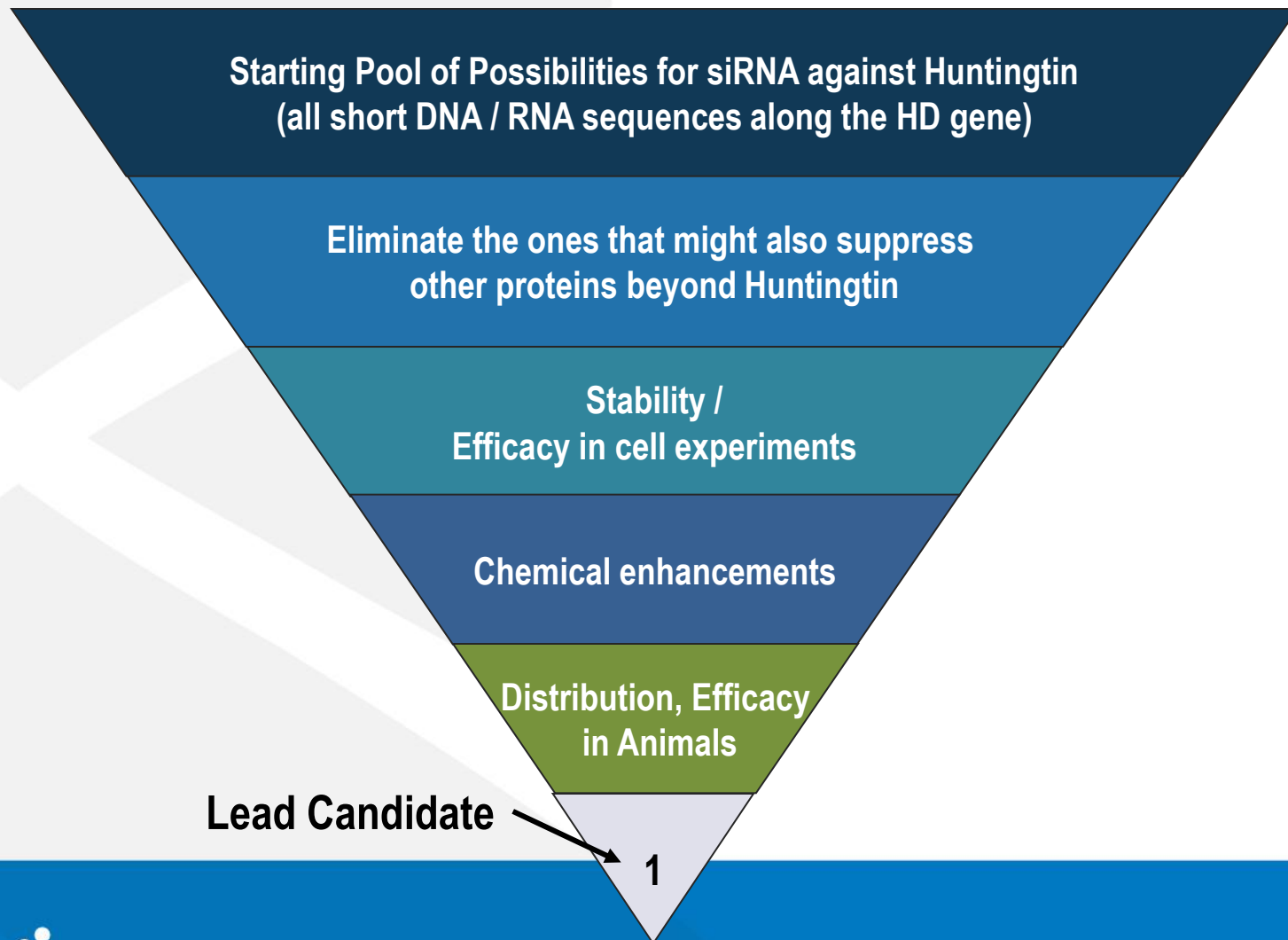
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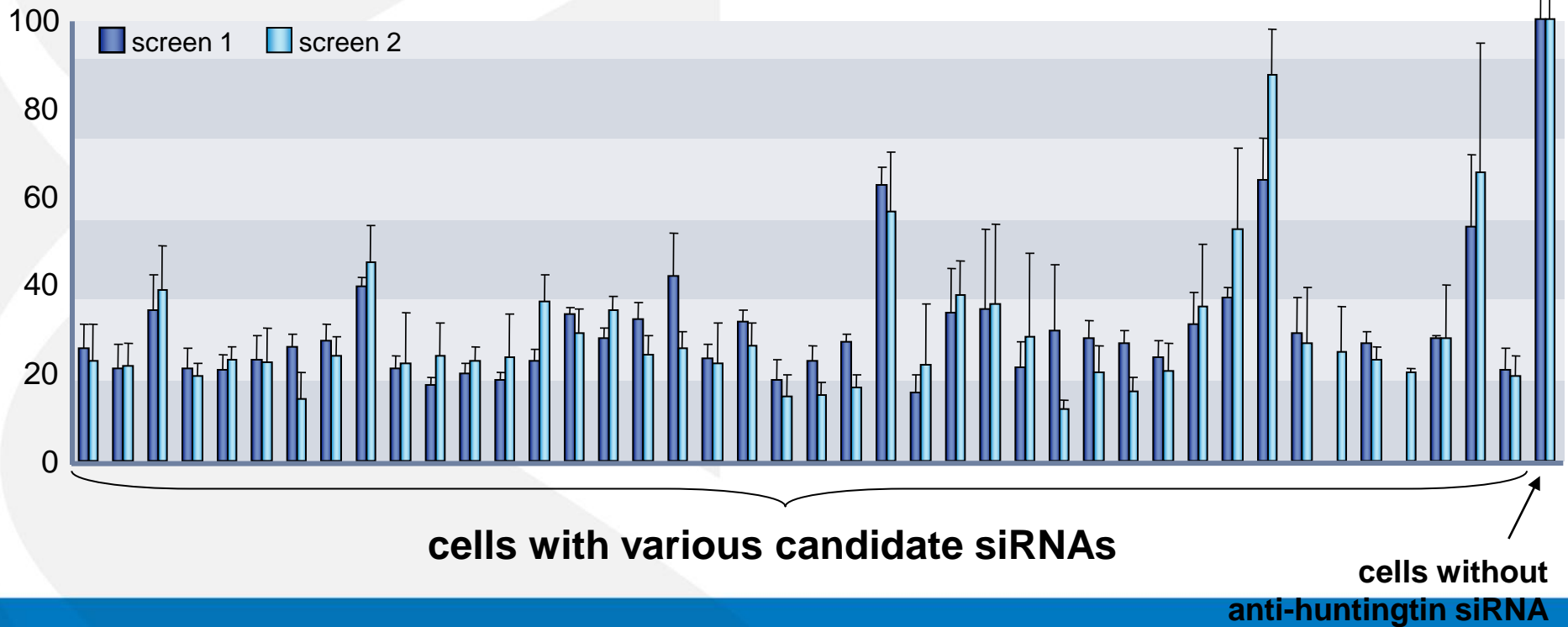
Finding The Drug

Alnylam's Screening Program



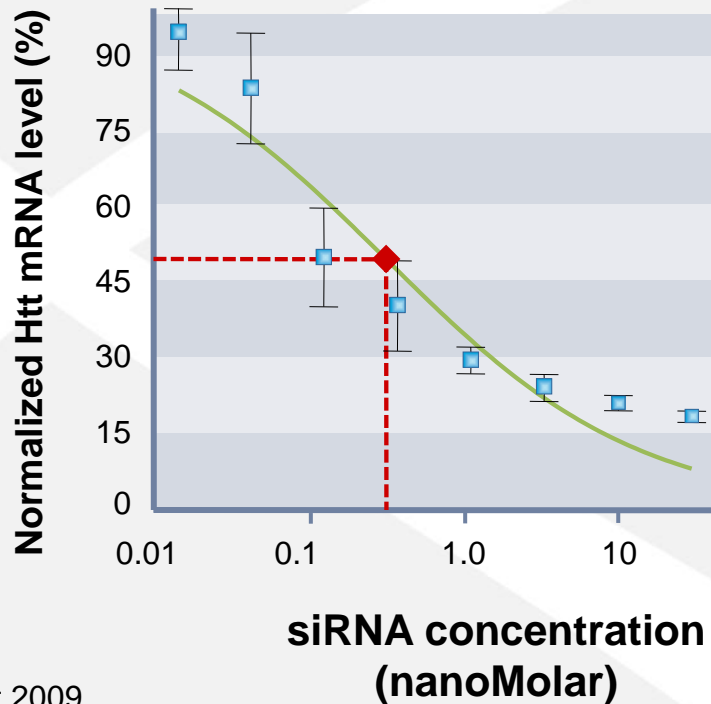
Candidate siRNAs Screening in Cell Experiments

Normalized Huntingtin mRNA level (%)

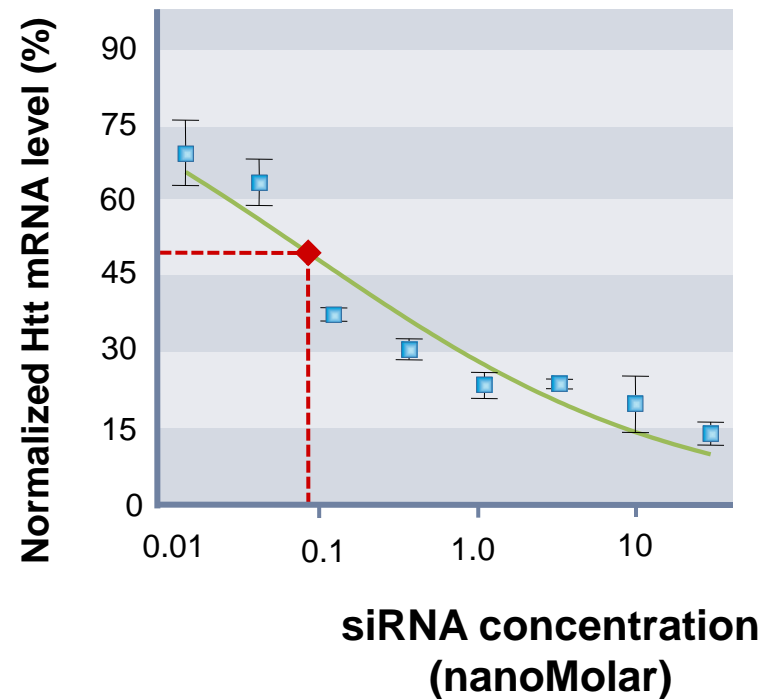


Finding the Most Potent siRNA In Cell Experiments: How Much Suppression, With How Much siRNA?

siRNA #1
IC50 ~ 0.3 nM

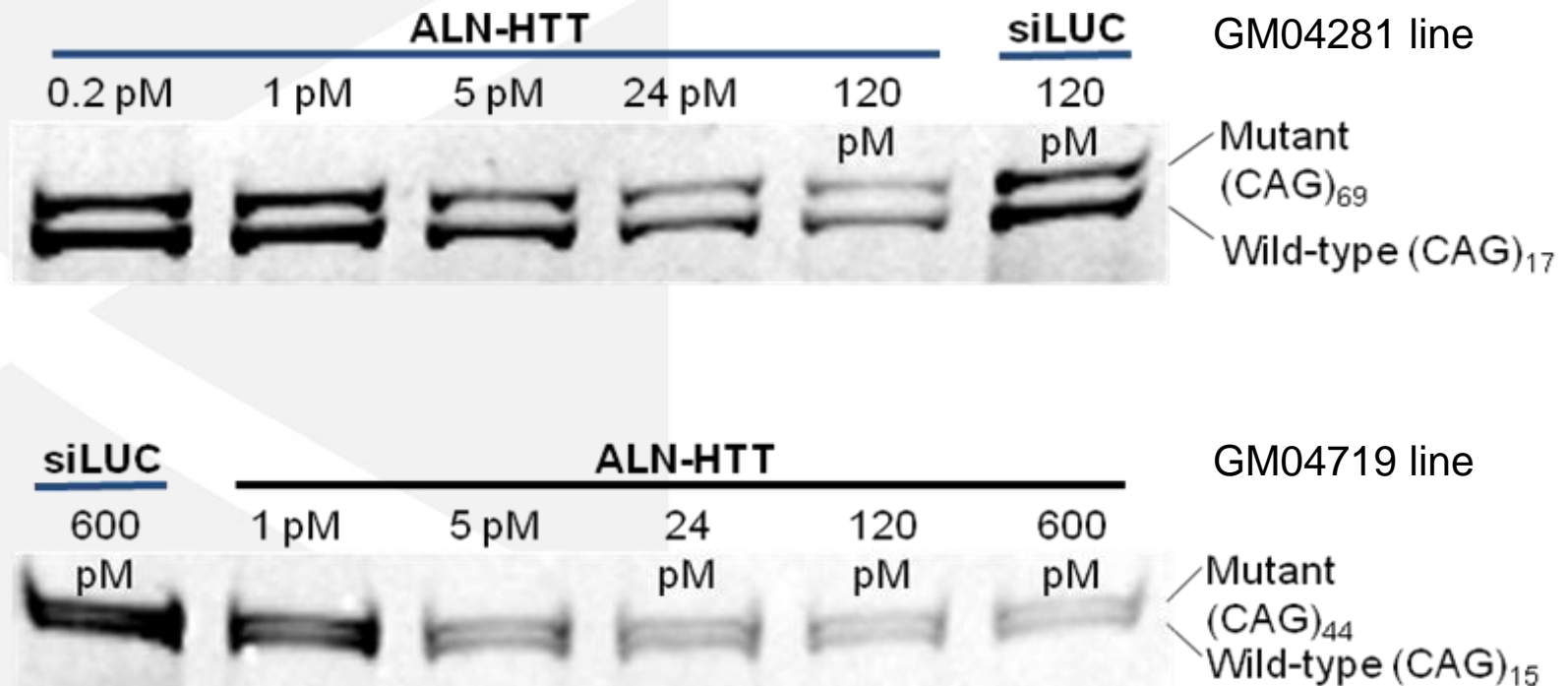


siRNA #2
IC50 ~ 0.08 nM

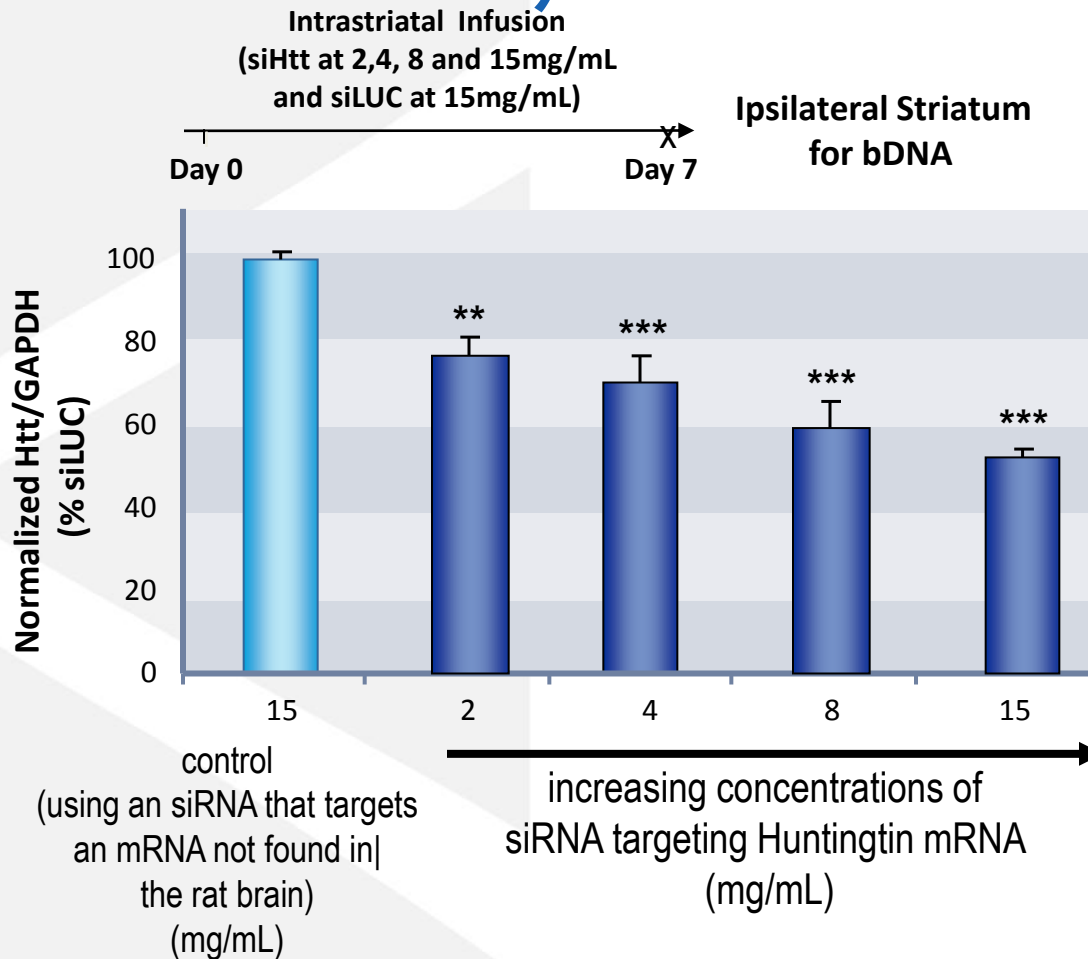


SFN, Oct 2009

siRNA Reduce Amount of Expanded Protein (As Well As Normal Length Protein) In Cells From HD Patients



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



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

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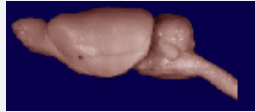
SFN, Oct 2009

Our Mission

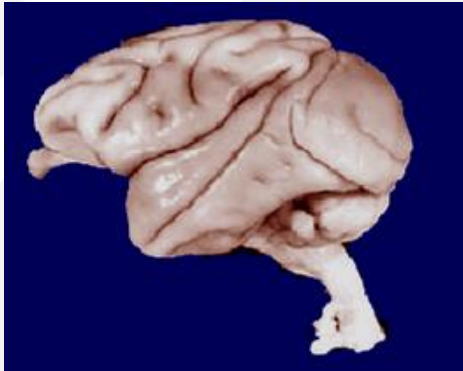
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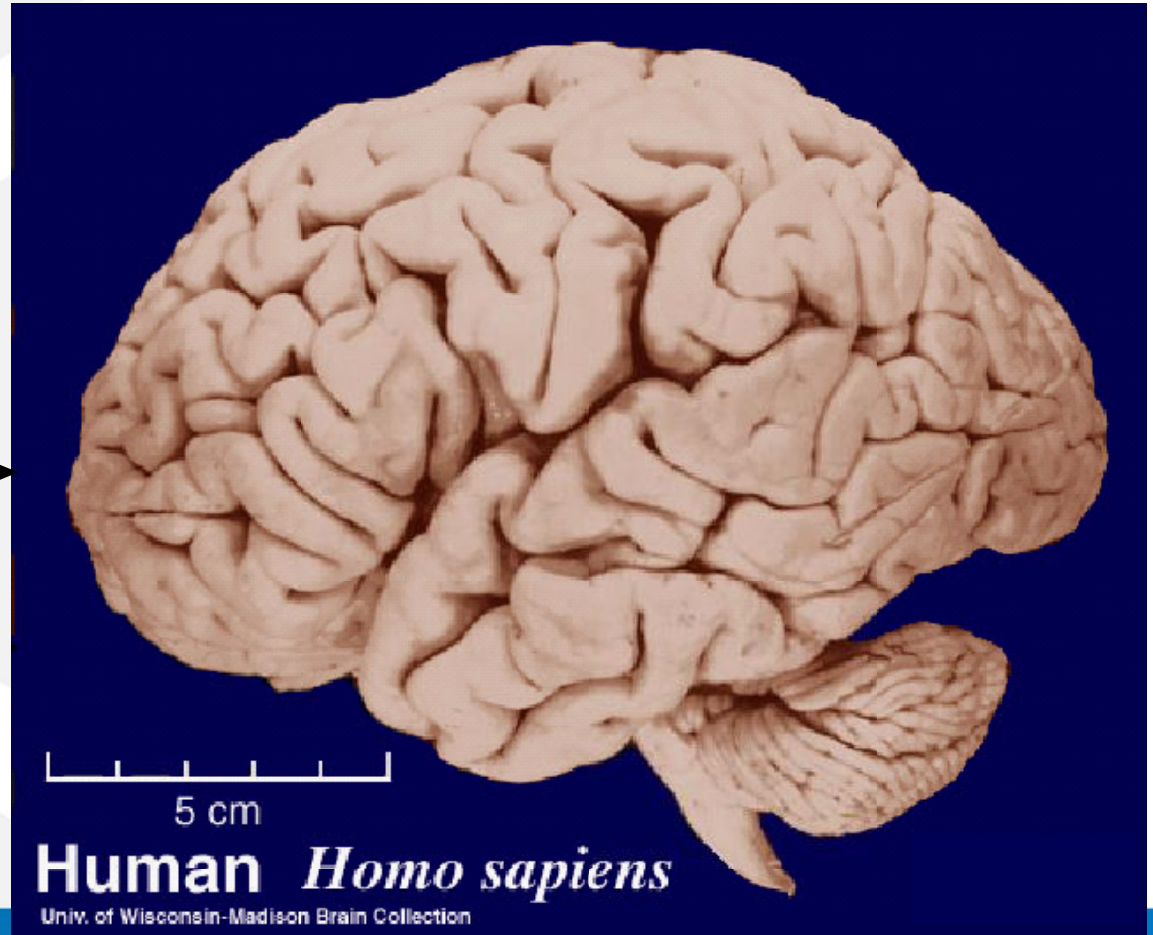
How Does siRNA Get Into Rat (Or Other) Brain? ... We Put It There, Directly.



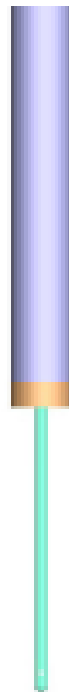
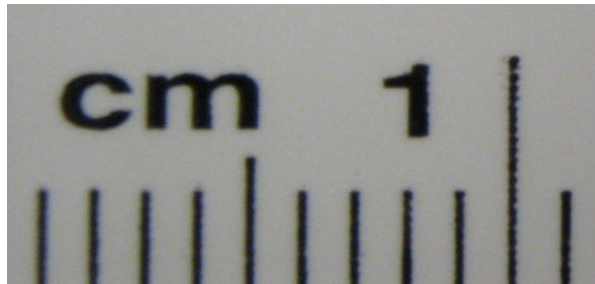
Rat brain



Rhesus monkey brain

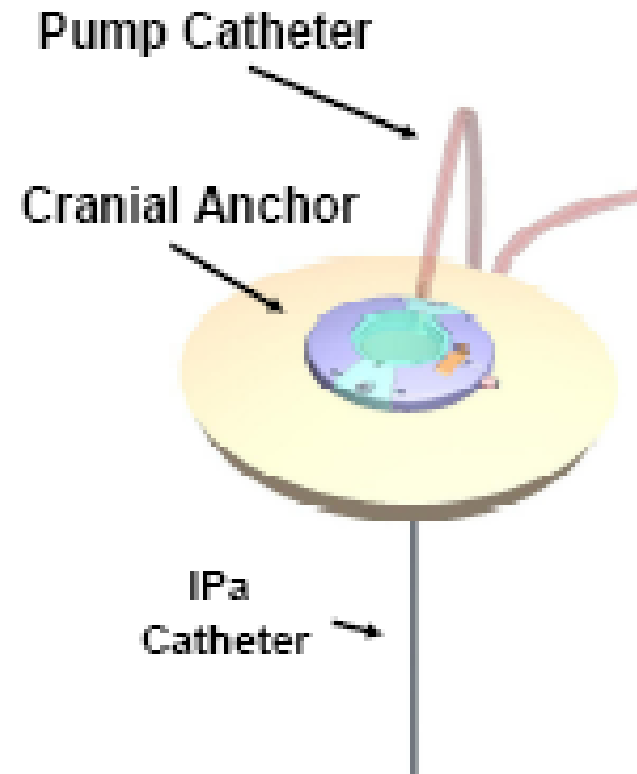


Direct Infusion Into Brain Using Catheter (Tube)



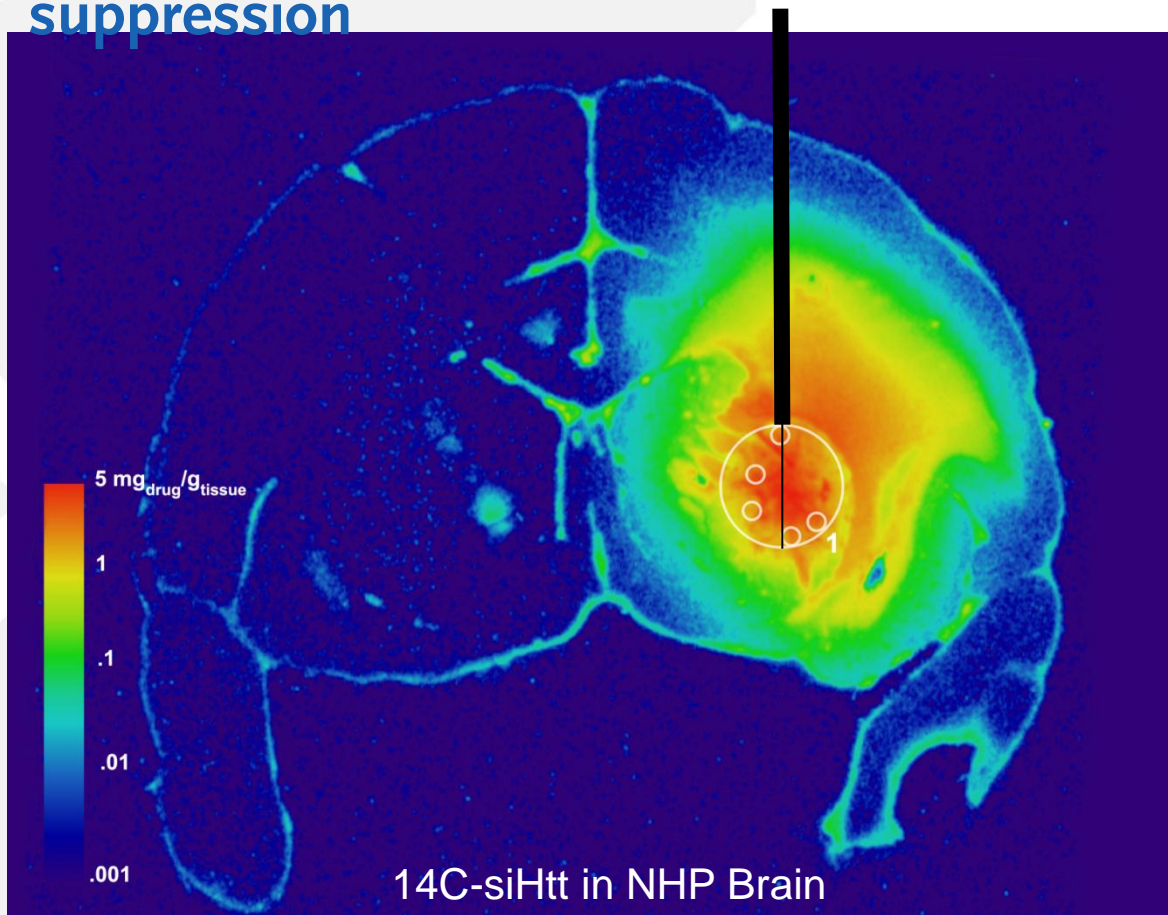
IPa Catheter
(Elastomeric polymer)

Length = 5mm for Rhesus



Direct Infusion of siRNA into Brains of Monkeys

Distribution of siRNA and correlation with Htt mRNA suppression

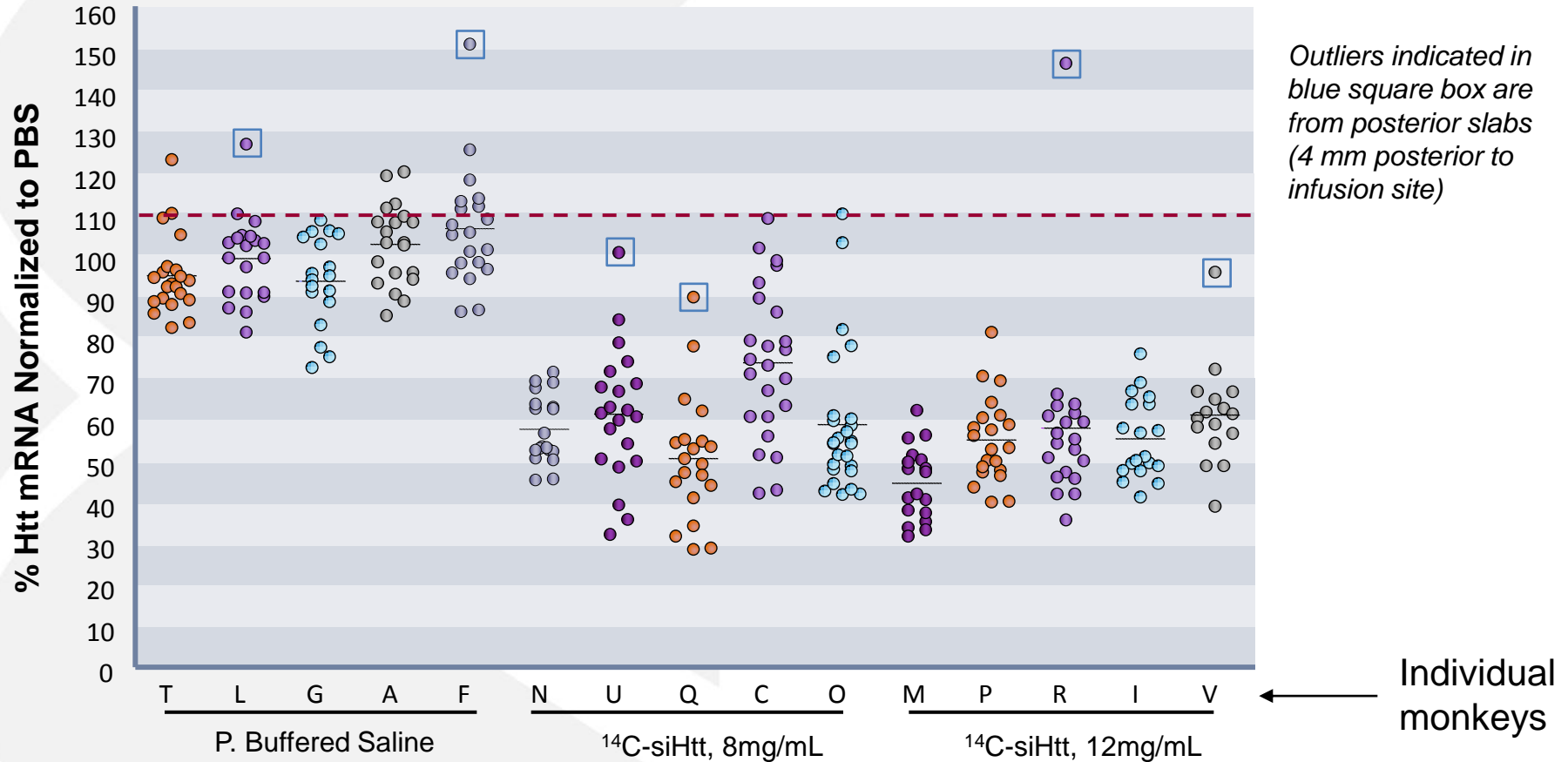


Tissue location	% Suppression of Htt mRNA
1	39%
2	28%
3	47%
4	51%
5	40%

Keystone: RNA Silencing., Jan 2010

Huntingtin mRNA Suppression and siRNA Distribution in Rhesus Monkey Brain

Consistent Htt mRNA Suppression Across Punches and Animals



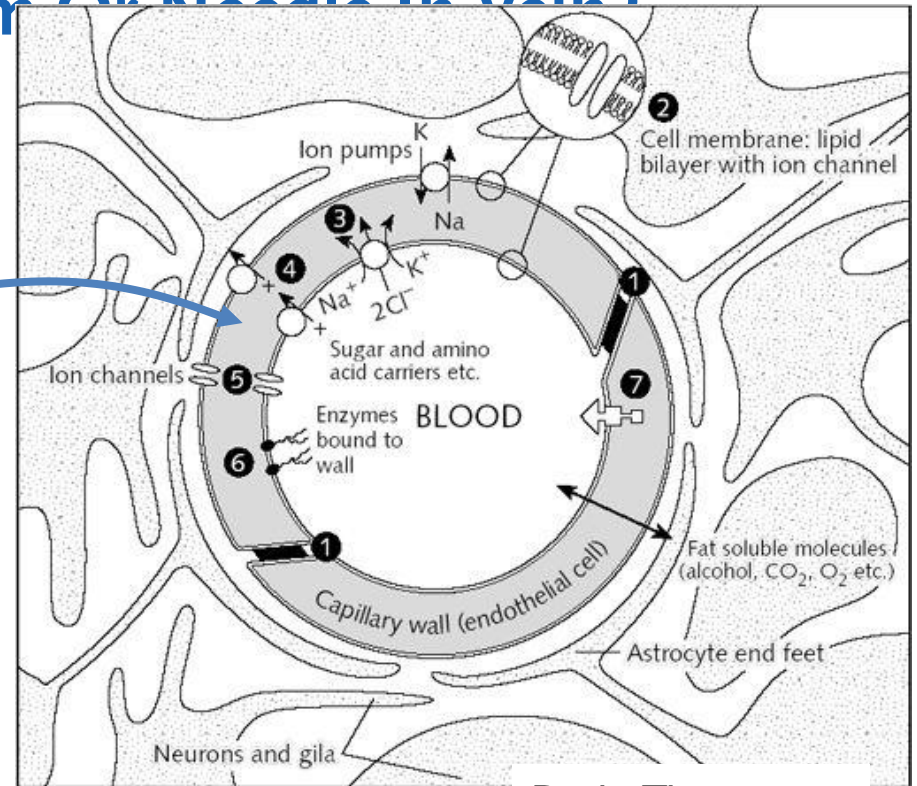
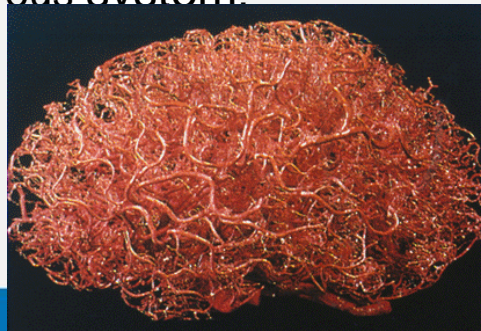
Keystone: RNA Silencing., Jan 2010

Why Can't siRNA be a Pill,

Or Given By Shot In Arm Or Needle In Vein?

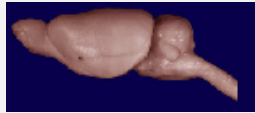
- siRNA cannot readily survive digestive tract
- Enzymes in serum can degrade siRNA in blood very quickly (although chemical stabilization methods exist)
- Main reason:
 - Blood-brain barrier excludes large molecules such as siRNA from entering the central nervous system.

Plastic cast of all the blood vessels in the brain

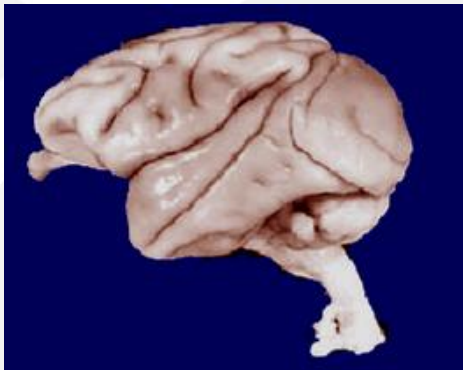


Magnified view (diagram) of a blood vessel in brain tissue.

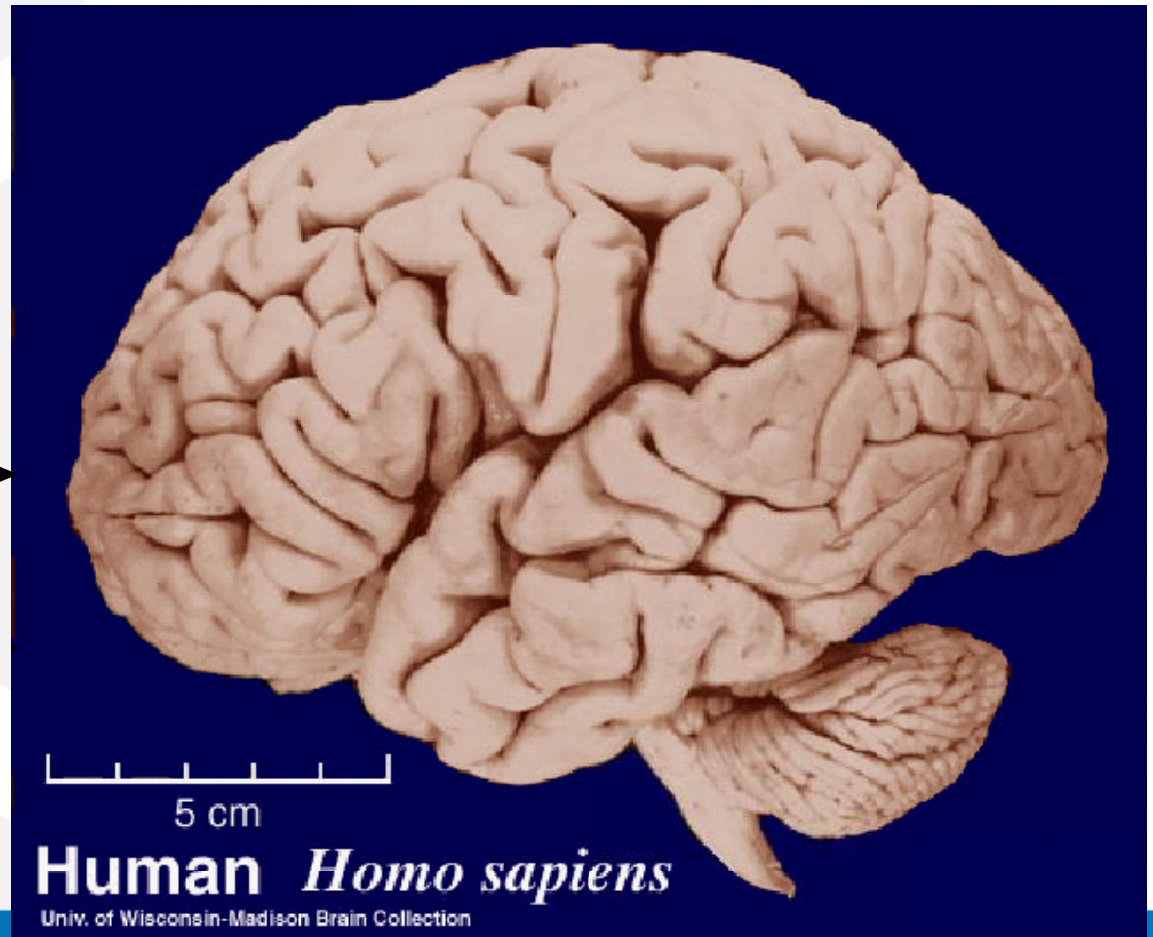
How Will People Receive Long-term Treatment?



Rat brain



Rhesus monkey brain

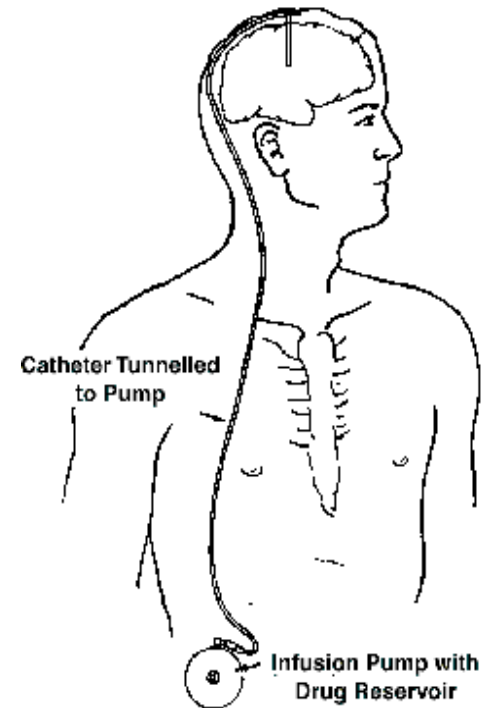


Develop a treatment for Huntington's Disease that uses a pump to deliver, directly into the person's brain tissue, a drug that reduces the amount of the disease-causing protein in the brain.

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.