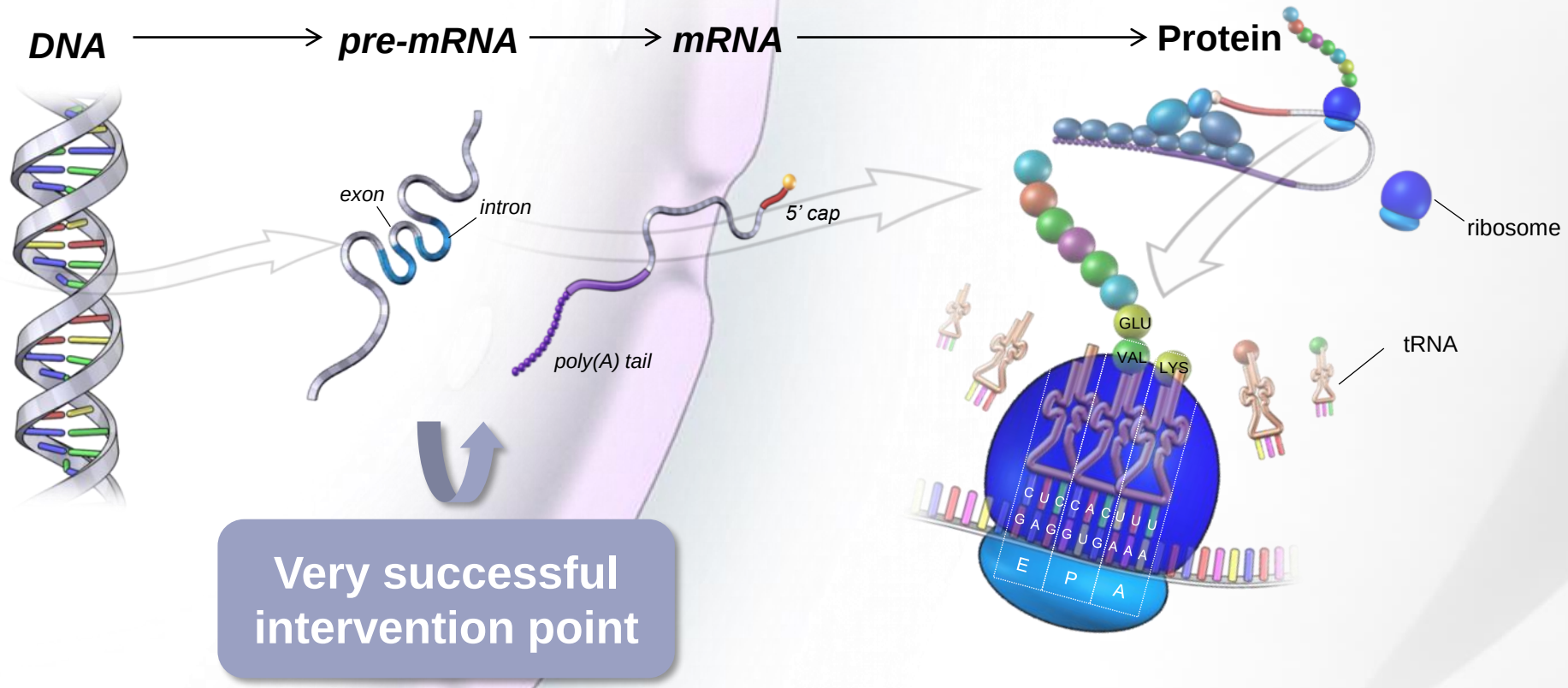
# Targeting Splicing to Discover and Develop Novel Small Molecule Therapeutics

Anu Bhattacharyya, PhD

# Post transcriptional control (PTC) focused company




**Very successful intervention point**

PTC uses small molecules to manipulate RNA processes for the purpose of treating challenging diseases

# Leaders in small molecule RNA-splicing technology

Development of SMA candidate as potential best-in-class treatment

13 years of discovering and developing drugs that target pre-mRNA splicing

Cutting-edge tech platform discovered and developed by PTC

**2nd Splicing Compound: A Development Candidate to treat Familial Dysautonomia**

Continue to exploit Splicing platform; addressing additional areas of unmet need

| Platform | Mechanism Targeted | Programs |
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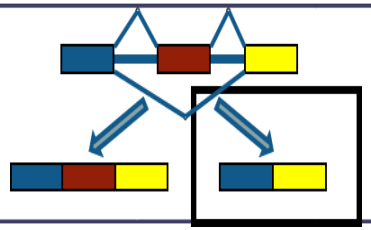
| Splicing |  Target-splicing events to restore or decrease protein levels | **SMA – SMN2**<br>**FD – IKBKAP**<br>**HD – HTT**<br>**Others** |

# The splicing technology is a proven platform to identify new therapeutics

Development of SMA candidate as potential best-in-class treatment

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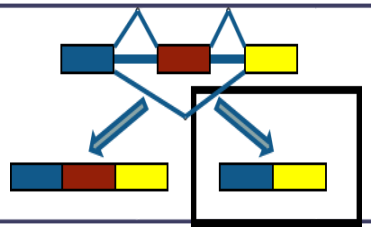
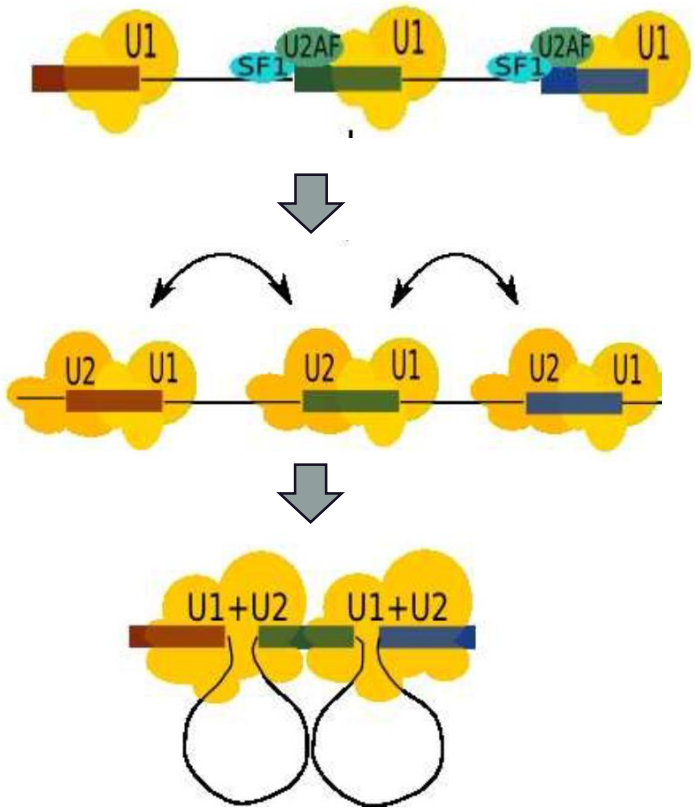
| Splicing |  Target-splicing events to restore or decrease protein levels | **SMA – SMN2**<br>**FD – IKBKAP**<br>**HD – HTT**<br>**Others** |

# Exon definition plays an important role in the regulation of alternative splicing




**U1** and **U2 snRNPs** recognize RNA sequence elements that are important for defining exons

**U1/U2 snRNPs** and associated splicing factors bind to the ends of an exon and a complex is built across it

Complexes on different exons join together to allow intron removal

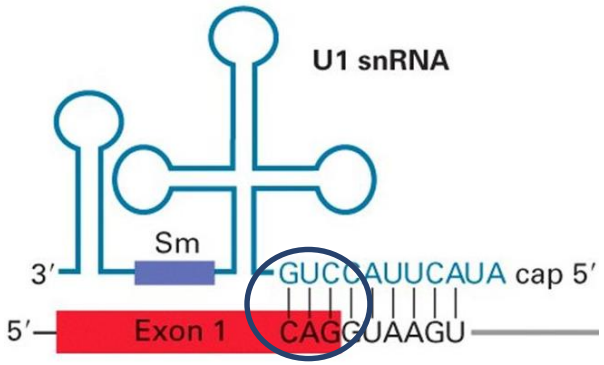
**Key intervention point:**

Much of splicing regulation (enhancers/repressors) and dysregulation (mutations) occur during this key step

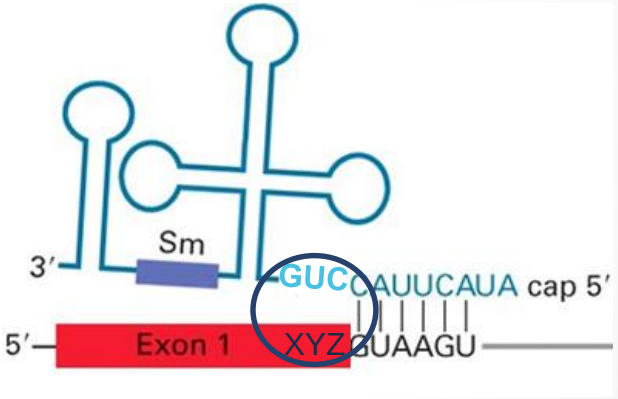
Exon definition involves interactions between the splice sites across the exon – a key step in mRNA splicing

# Alternative splicing is governed by interaction of U1 snRNP with canonical and non-canonical exons

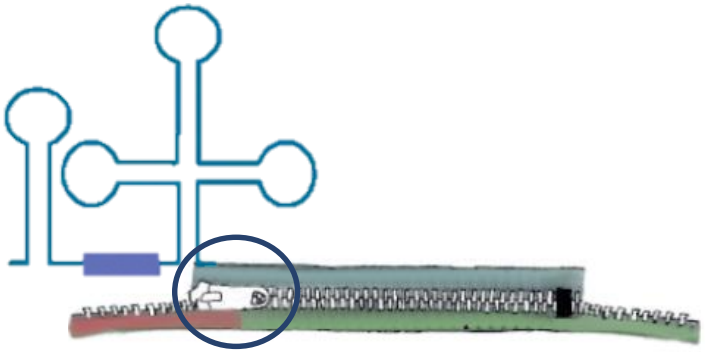
Mostly Canonical Exons




Fewer Non-Canonical Exons




Perfect or near-perfect base pairing to U1 snRNA



Weaker interaction requires help to invoke splicing

This represents an intervention point where small molecules can assist in modulating splicing

# The human genome has three types of non-canonical exons

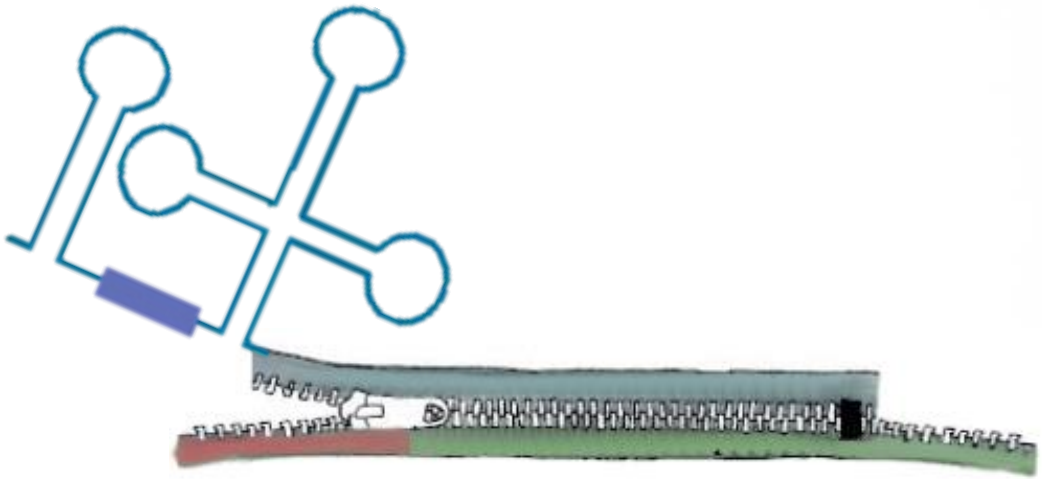
1. Endogenous non-canonical exons –10% of exons in the human genome
2. Mutation of canonical exons – DNA mutation that creates an exon with a non-canonical 5’ splice site
3. Pseudo-exons – Non-canonical exons that are not recognized and spliced

# PTC developed multiple programs that target the recognition step of splicing

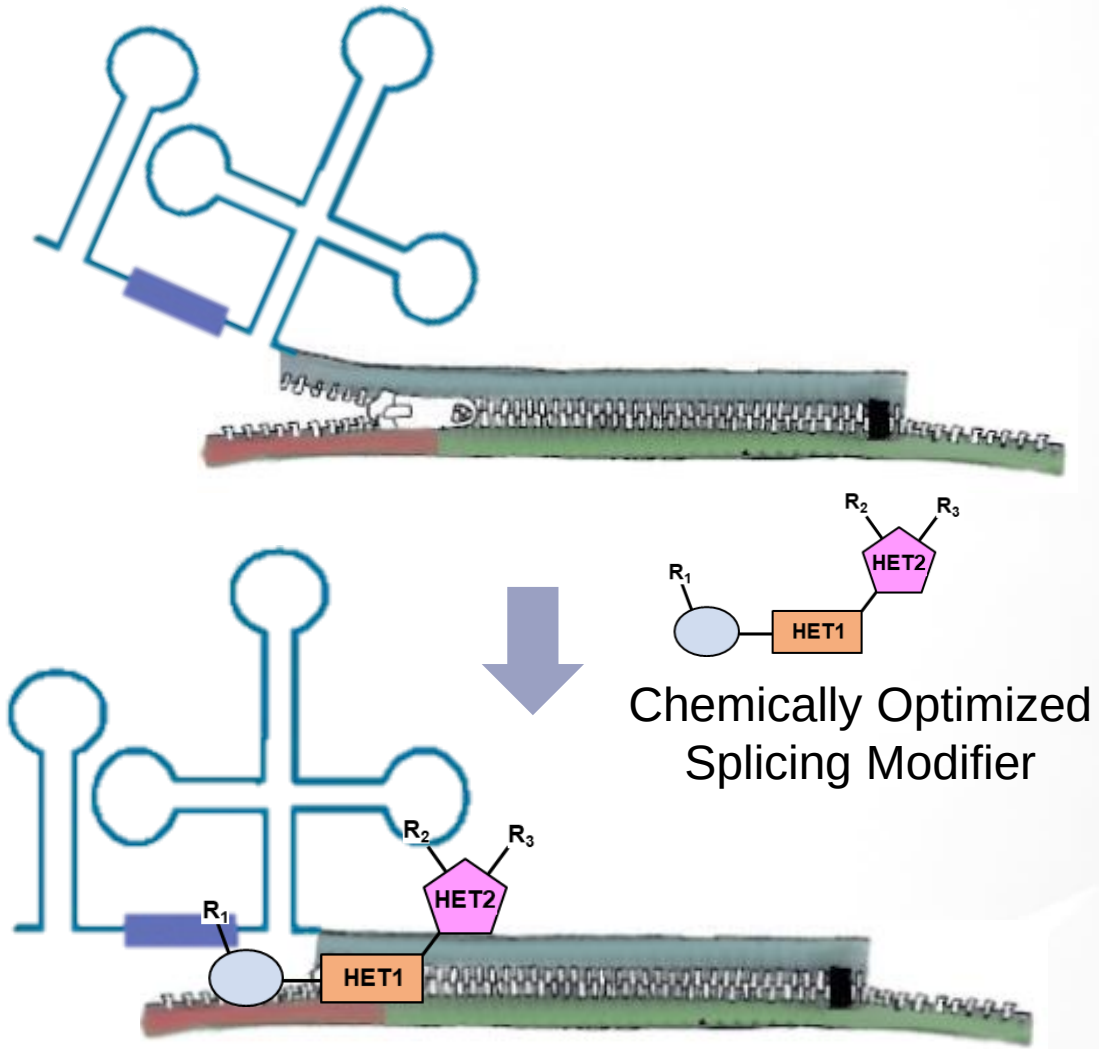
1. Endogenous non-canonical exons –10% of exons in the human genome
    - SMN2 exon 7 Inclusion
2. Mutation of canonical exons – DNA mutation that creates an exon with a non-canonical 5’ splice site
    - Familial dysautonomia: IKBKAP Exon 20 Inclusion
3. Pseudo-exons – Non-canonical exons that are not recognized and spliced
    - Htt pseudo-exon

Each category has many potential druggable targets

# How do splicing modifiers target the recognition step of splicing?

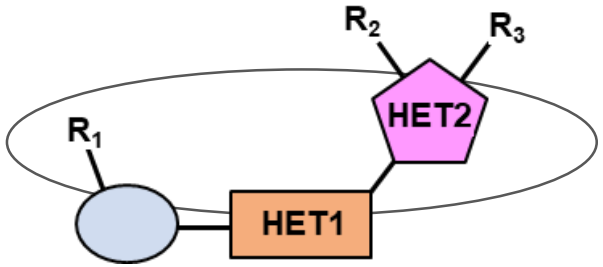


# Binding of splicing modifiers to the non-canonical U1-pre-mRNA structure induces splicing



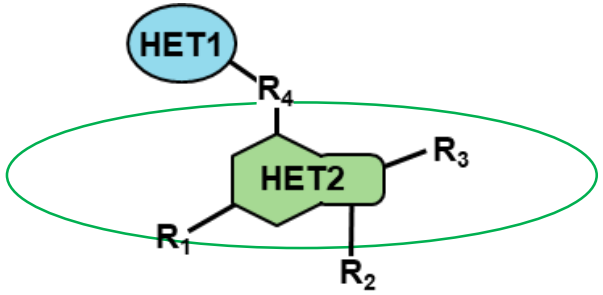
# Defining the universe of small molecule splicing modifiers

SMA



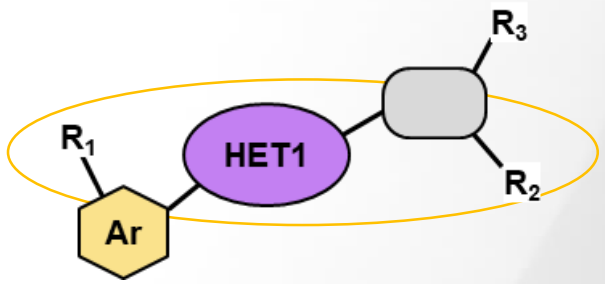

*SMN2*

Familial Dysautonomia




*IKBKAP*

Huntington's Disease

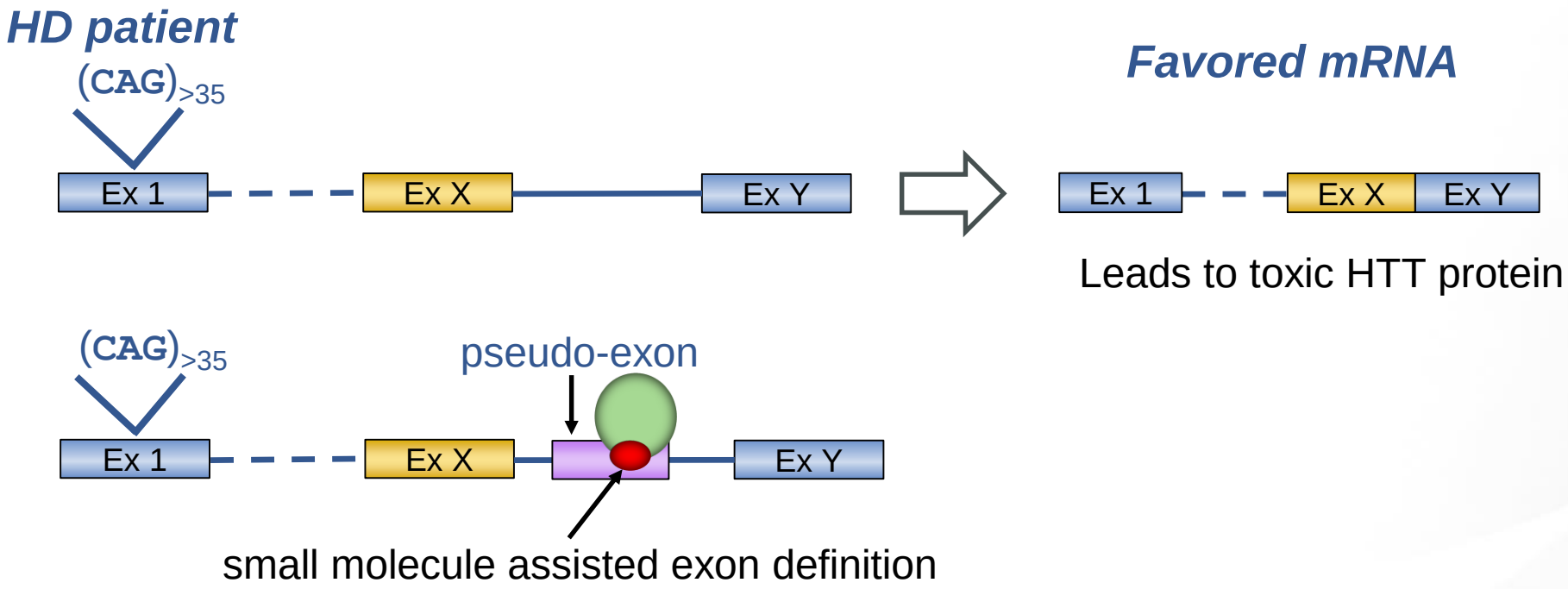



*HTT*

Pre-mRNA splicing provides a rich set of targets to discover and develop new small molecule therapeutics

# Applying splicing technology to treat Huntington's Disease

- Program objective
  - Identify small-molecules that lower HTT levels by targeting gene expression through alternative splicing
  - Optimize orally delivered molecules that lower HTT in key areas of the brain and other HD-targeted peripheral organs



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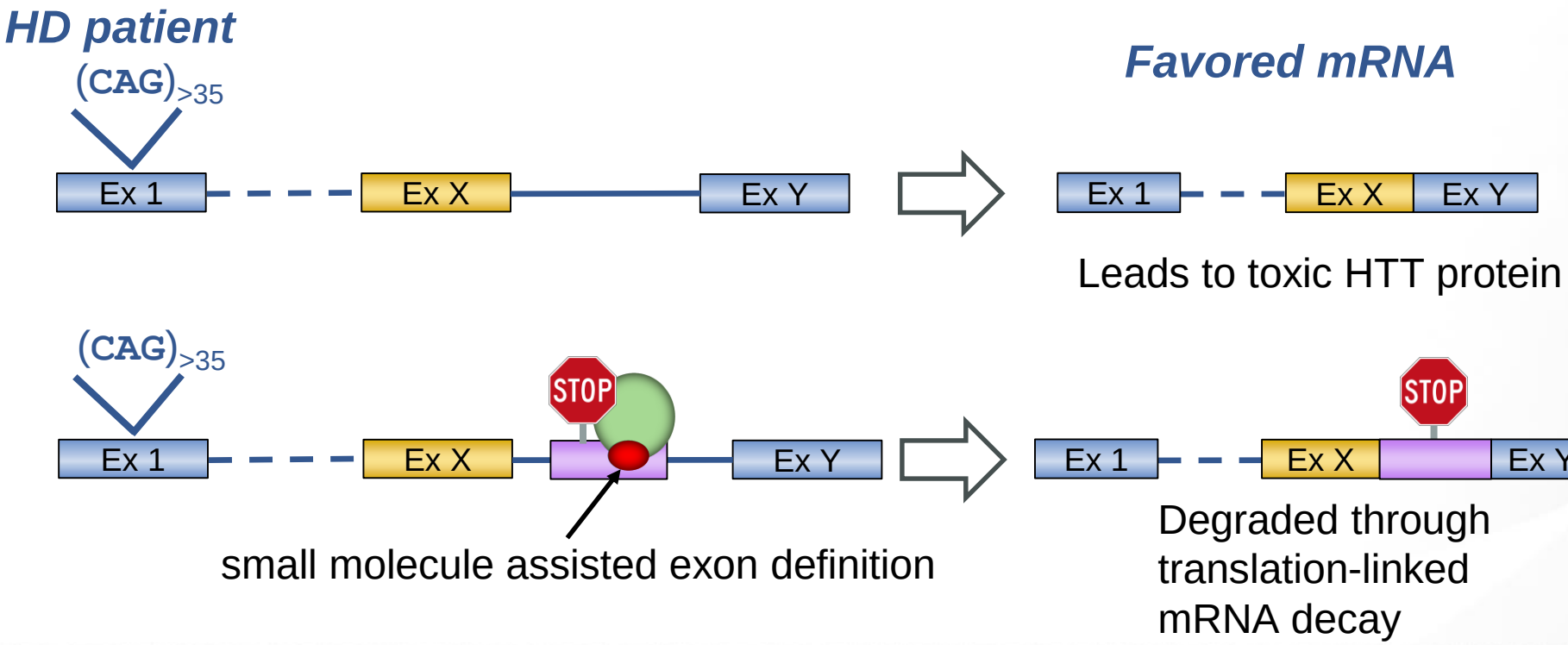
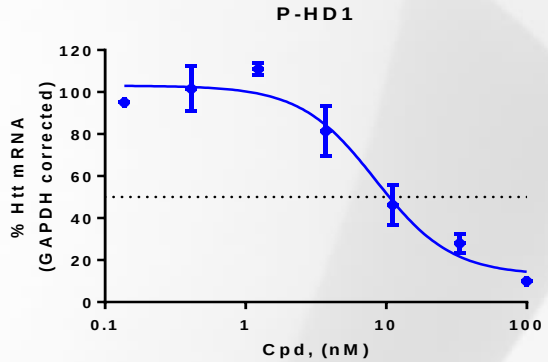
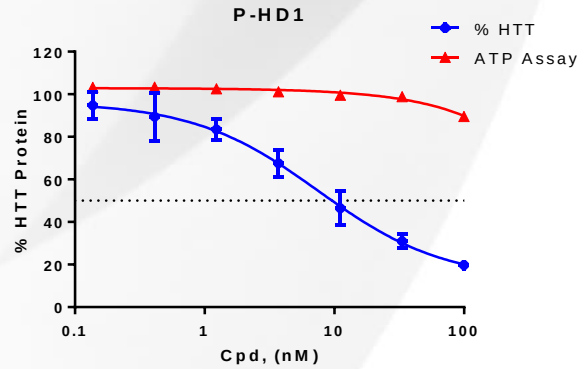


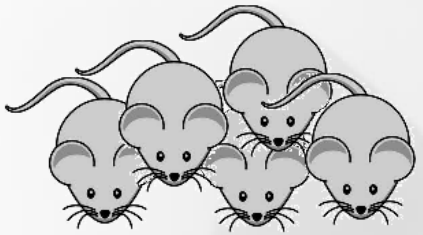

HTT mRNA lowering

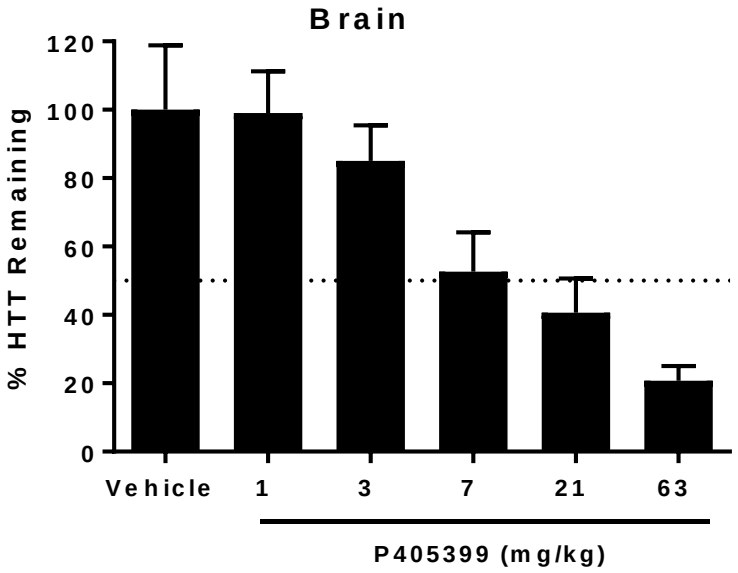


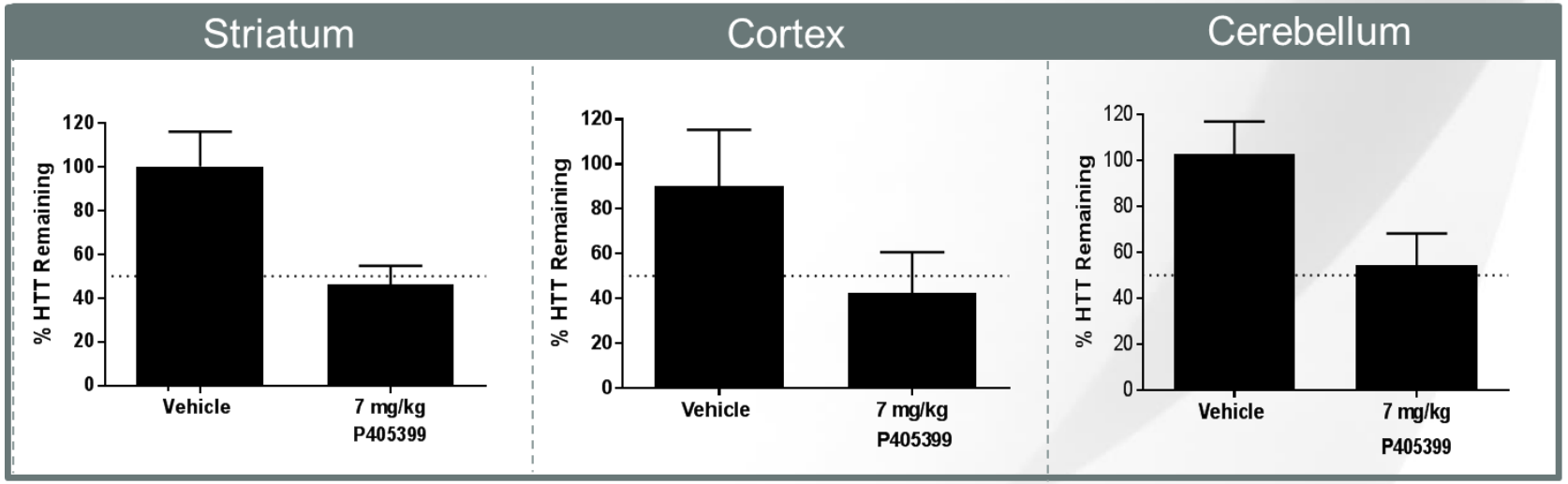

HTT protein lowering

# Splicing modifiers reduce levels of human HTT in HD mouse brain

- Transgenic mice carrying human mutant *HTT* gene
- Orally administered for 21 days





# PTC's vision of targeting splicing regulation

- Developed a deep understanding of the druggable interactions in the splicing of pre-mRNA
- Identified and advanced small molecules that directly interact with RNA-RNA complexes of the spliceosome
- Targeted the diversity of unique RNA structures generated at the recognition step in splicing
- Moving forward
  - Developing knowledge of the structural diversity within the multiple steps of splicing beyond the recognition step
  - Using that knowledge to develop small molecules for each targeted splicing event
    - Developing small-molecule splicing modifiers to target a broad range of genetic disorders

# *measured by moments*

Everyone has a different definition of progress. For the last 20 years, we've measured our progress researching rare disease in moments. Smiling ones and crying ones. Moments spent with our boys' families and ones with their friends. We know that every step forward comes after several steps backward, because we've lived it—whether spending time with families in their homes or with our scientists researching in our labs.

It can be easy to lose yourself as you progress further. Although we've grown, our heart remains in the same place, because we've never measured ourselves like larger companies do. Our biggest accomplishment has always been the time we can give to all of our families. Whether it's hours, days, months, or years, every small moment is a big win.

PTC THERAPEUTICS