Targeting Splicing to Discover and Develop Novel Small Molecule Therapeutics

Anu Bhattacharyya, PhD
Post transcriptional control (PTC) focused company

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

Very successful intervention point
Leaders in small molecule RNA-splicing technology

<table>
<thead>
<tr>
<th>Platform</th>
<th>Mechanism Targeted</th>
<th>Programs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Splicing</td>
<td>Target-splicing events to restore or decrease protein levels</td>
<td>SMA – SMN2, FD – IKBKAP, HD – HTT, Others</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Platform</th>
<th>Mechanism Targeted</th>
<th>Programs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Splicing</td>
<td>Target-splicing events to restore or decrease protein levels</td>
<td>SMA – SMN2, FD – IKBKAP, HD – HTT, Others</td>
</tr>
</tbody>
</table>

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

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

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

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

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

**HD patient**

\[(CAG)_{>35}\]

\[\text{Ex 1} \quad \text{Ex X} \quad \text{Ex Y}\]

\[\text{Ex 1} \quad \text{Ex X} \quad \text{Ex Y}\]

**Favored mRNA**

Leads to toxic HTT protein

\[(CAG)_{>35}\]

\[\text{Ex 1} \quad \text{Ex X} \quad \text{Ex Y}\]

pseudo-exon

small molecule assisted exon definition
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

**HD patient**

\[(CAG)_{>35}\]

Ex 1 → Ex X → Ex Y

**Favored mRNA**

Ex 1 → Ex X → Ex Y

Leads to toxic HTT protein

\[(CAG)_{>35}\]

Ex 1 → Ex X → Ex Y

small molecule assisted exon definition

Degraded through translation-linked mRNA decay

\[\text{HTT mRNA lowering}\]

\[\text{HTT protein lowering}\]
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
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.