Widespread Gene Delivery to the Nonhuman Primate Brain for the Treatment of Huntington’s Disease

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Huntington Disease: A significant unmet need in a large orphan population

• An autosomal dominant progressive neurodegenerative disease caused by triplet (CAG) expansion in the N-terminal region of the huntingtin gene.

• Mutant huntingtin confers a toxic gain-of-function leading to degeneration of medium spiny neurons and loss of cortical neurons.

• Death occurs within 15-20 years of disease onset; onset is inversely correlated to the number of CAG repeats.

• Estimated prevalence of manifest (diagnosed) HD patients: 4 to 10 per 100,000 in the western world

Sources: NORD; The Lancet Neurology, January 2011
Rationale for Htt Lowering Therapeutics for HD

• Preclinical data is robust and supportive of a transformative therapy
  – Therapeutic concept of “lowering mutant huntingtin” has been well validated
    • Antisense oligonucleotides (ASOs)
    • RNAi gene silencing
    • Genomic editing: zinc finger nucleases (ZFN’s), clustered regularly interspaced short palindromic repeats (CRISPR)
  – Preclinical testing of therapeutic concept in mouse models of HD showed correction of biochemical aberrations as well as significant improvement in motor function, and behavioral deficits
AAV-mediated RNAi for Huntington Disease

• Inhibitory RNAs can be designed to mimic primary miRNA stem-loops (artificial miRNAs), processed pre-miRNAs (short-hairpin RNAs) or mature miRNAs with perfect complementarity to their targets (small interfering RNAs).

• Adeno-associated viral vectors (AAV) can be used to achieve RNAi-mediated gene silencing in the brain.
rAAV as a Gene Delivery Vector for CNS Disorders

- Replication defective parvovirus
- Transduce non-dividing cells
- Nonpathogenic
- Vector production and purification methods have been established to produce large quantities of high quality vectors for clinical use
- A single intracranial administration AAV could provide long term suppression of Htt and long lasting therapeutic benefit
  - No complications due to repeat administration or devices
  - Intracranial procedures are routinely performed in the clinic
AAV1-miRNA-Htt Mediates Lowering of Htt and Correction of Phenotypic Deficits in YAC128 Mice

Mouse and Human Htt mRNA levels are reduced to equivalent levels

Inject at 2 months old
Sac at 5 months old
N=8/group
AAV1-miRNA-Htt Ameliorates Motor Deficits and Reduces Mutant Htt Aggregates in the Striatum of Aged YAC128 Mice

EM48 Immunostain

Rota Rod Test
Summary - Huntington

• Validated AAV-RNAi-mediated reduction of mHtt as a potential therapeutic strategy for Huntington's disease using the YAC128 mouse model

• Striatal injections of AAV2/1-miRNA-Htt into YAC128 mice resulted in:
  – significant reduction (~40%) of mutant and wild type huntingtin levels
  – partially corrected transcriptional dysregulation and reduced amounts of mHtt aggregates in the striatum, including aged YAC128 mice
  – functional improvement in behavioral and motor deficits in symptomatic YAC128 mice

• Sustained, partial reduction (~5 months) in Htt levels in YAC128 mice was not associated with overt toxicity; similar observations in non-human primates (McBride et al., 2011; Grondin et al., 2012)
Delivering Disease Modifying Therapeutics for HD

- Imaging studies show early and progressive loss of axons emanating from cortical neurons, including those projecting to the striatum, suggesting cortico-striatal connectivity dysfunction in HD.

- Reduction of mHTT from both striatal and cortical regions are required for optimal therapeutic benefit in BACHD mice compared to Htt reduction in either region alone.

- Therapies that only target the striatum or cortex may have limited effectiveness in patients.

- Global delivery of AAV therapeutics to the entire human brain is not yet feasible however cortical and striatal targeting would likely provide significant therapeutic benefit.
Delivery of Therapeutic Agents to the Human Cortex is a Challenge

• Direct cortical infusions that would target a sufficient number of cortical neurons in affected regions of HD is unachievable in a human brain due to the architecture and volume of brain tissue

• Axonal and transsynaptic transport of vectors and vector-encoded genes at sites distal to injection have been observed with several AAV serotypes

• Can we achieve distribution of AAV vectors to the primate cortex following CED delivery of vectors into the striatum?
AAV Serotype Selection for Clinical Candidate

• Clinical Candidate Criteria
  – Maximal striatal transduction in a large brain
  – Predominantly neuronal tropism
  – Potential for cortical distribution

• Candidate Serotypes Evaluated
  – AAV1
    • Used in Proof of concept studies in Yac128 mice and Rhesus Monkey
    • Demonstrated neuronal and glial transduction in NHP brain
    • Safety in humans – 1st approved AAV gene therapy drug (Glybera) in the EU
  – AAV2
    • Demonstrated neuronal transduction in NHP and humans
    • Established safety record in multiple human clinical trials
AAV1-GFP Demonstrates Widespread Expression in the Mouse Brain Following Intra-striatal Delivery
The Scale-Up Problem: Human Brains are Big and Structurally More Complex

The Human brain is >1,000x larger than a mouse brain
Evaluating Brain Biodistribution of Intrastriatal Injections of AAV1-eGFP and AAV2-eGFP in NHPs for Serotype Selection

• Goals:

1. Distribution pattern of these vectors in the brain (striatal coverage, cortical coverage, others)?

2. Cellular tropism - cell types transduced in various brain regions by these vectors (neurons, microglia, astrocytes, others)?

3. Peripheral biodistribution – detection of GFP in tissue outside of the CNS following intrastriatal administration?
Evaluating Brain Biodistribution of Intrastriatal Injections of AAV1-eGFP and AAV2-eGFP in NHPs for Serotype Selection

Study Design
• Animals: Adult M/F Rhesus Monkeys pre-screened for neutralizing antibodies
• Study Duration: 30 Days
• Blood, serum, and CSF collected prior to surgery and at necropsy
• Peripheral tissues removed prior to PFA Perfusion
• Brain and Spinal cord perfused and collected into PFA

<table>
<thead>
<tr>
<th>Group</th>
<th>Production Method</th>
<th>Vector</th>
<th>Vector Concentration (vg/mL)</th>
<th>Vector Dose per hemisphere (vg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cohort 1 (TT)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AAV1-eGFP (n=3)</td>
<td>Triple Transfection (TT)</td>
<td>ssAAV2/1-CBA-eGFP</td>
<td>1.9x10^{12}</td>
<td>1.7x10^{11}</td>
</tr>
<tr>
<td>AAV2-eGFP (n=2)</td>
<td></td>
<td>ssAAV2/2-CBA-eGFP</td>
<td>1.9x10^{12}</td>
<td>1.7x10^{11}</td>
</tr>
<tr>
<td><strong>Cohort 2 (PCL)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AAV1-eGFP (n=2)</td>
<td>Producer Cell Line (PCL)</td>
<td>ssAAV2/1-CBA-eGFP</td>
<td>1.9x10^{12}</td>
<td>1.7x10^{11}</td>
</tr>
<tr>
<td>AAV2-eGFP (n=2)</td>
<td></td>
<td>ssAAV2/2-CBA-eGFP</td>
<td>1.4x10^{12}</td>
<td>1.3x10^{11}</td>
</tr>
</tbody>
</table>
Real Time MRI Guided Convection Enhanced Delivery To the NHP Caudate and Putamen

- CED uses bulk flow of agents through the extracellular space and allows delivery to a larger, more consistent treatment area than diffusion-based techniques.

- RCD employs interventional MRI to monitor distribution of therapeutic agents co-infused with gadolinium-related tracers.
AAV1-eGFP Produced Widespread Transduction in the Striatum and Cortex
AAV1-eGFP Neuronal Transduction in the Striatum

caudate x 20

GFP
NeuN
GFP/NeuN

putamen x 20

GFP
NeuN
GFP/NeuN
AAV1-eGFP Transduced Neurons and Astrocytes in the Cortex

Frontal Ctx

Cortical Layer V

Occipital Ctx
AAV2-eGFP Produced Widespread Transduction in the Striatum and Cortex
AAV2-eGFP Transduced Neurons in The Striatum and Cortex
AAV2-eGFP Neuronal Transduction in the Striatum

caudate x 20

GFP
NeuN
GFP/NeuN

putamen x 20

GFP
NeuN
GFP/NeuN
AAV2/1-eGFP (RM) Transduced a Greater Percentage of Neurons in the NHP Striatum Compared to AAV2/2-eGFP (RM)

Efficiency of neuronal transduction by ssAAV2/1-CBA-EGFP and ssAAV2/2-CBA-EGFP (RM) vectors within the targeted/transduced brain structures of the NHP brain

<table>
<thead>
<tr>
<th>Targeted region</th>
<th>Cyrus MMU39553 ssAAV2/1-CBA-EGFP (RM)</th>
<th>Quest MMU40167 ssAAV2/1-CBA-EGFP (RM)</th>
<th>Kier MMU39808 ssAAV2/2-CBA-EGFP (RM)</th>
<th>Basil MMU39417 ssAAV2/2-CBA-EGFP (RM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left putamen</td>
<td>58.6 ± 7.61 %</td>
<td>57.1 ± 10.5%</td>
<td>53.1 ± 12.7%</td>
<td>52.4 ± 11.8%</td>
</tr>
<tr>
<td>Right putamen</td>
<td>50.5 ± 9.78 %</td>
<td>73.2 ± 9.88%</td>
<td>52.1 ± 10.6 %</td>
<td>50.1 ± 4.5 %</td>
</tr>
<tr>
<td>Left caudate</td>
<td>57.0 ± 7.13 %</td>
<td>51.8 ± 9.42 %</td>
<td>43.0 ± 14.5 %</td>
<td>43.4 ± 8.5 %</td>
</tr>
<tr>
<td>Right caudate</td>
<td>59.1 ± 9.11 %</td>
<td>70.5 ± 8.38 %</td>
<td>52.1 ± 7.22 %</td>
<td>61.2 ± 12.9 %</td>
</tr>
</tbody>
</table>

Average across monkeys and sites
- AAV1: 59.7%
- AAV2: 50.9%
Both AAV2/1-eGFP and AAV2/2-eGFP efficiently transduce neurons within the striatum

Green region depicts the primary area of GFP transduction (robust signal with densely distributed GFP+ neurons (results in panel A))

GFP+ neurons were also detected in regions outside the primary areas of GFP transduction (results in panel B)
AAV1-eGFP or AAV2-eGFP did not transduce microglia

AAV2/2-eGFP (putamen x 20)

AAV2/2-eGFP (putamen x 40)
3D reconstruction of AAV1-eGFP injected NHP: GFP Signal Mapped onto MRI Scan

- Red = Cortex
- Green = GFP in the cortex
- Blue = GFP in Putamen
- Violet = STN
- Light blue = Substantia Nigra

BRAINLAB iPlan
• **Red** = Cortex
• Red = Cortex
• **Red** = Cortex
• **Blue** = GFP in Putamen

AAV1-GFP
>90% cortical coverage observed in this NHP

- **Red** = Cortex
- **Blue** = GFP in Putamen
- **Green** = GFP in the cortex
- **Violet** = STN
- **Light blue** = Substantia Nigra
Cortico-striatal Connections Provide Avenues for AAV Transport

All AAV-eGFP Vectors Were Well Tolerated Up to 30 Days Post Injection

• No Adverse Events
• Blood and CSF clinical chemistry all normal
• Anti-AAV antibody titers in serum and CSF at necropsy all within normal limits
• Body weights prior to surgery and at necropsy were not significantly different between groups
No eGFP mRNA Expression was detected in Peripheral Tissues for all AAV-eGFP (RM) Vectors

QPCR on samples 1 month following injection of AAV-eGFP (RM) Vectors into the NHP striatum
Summary

• All vectors were well tolerated and no AAV-related adverse events were observed up to 30 days post injection

• AAV1 achieved widespread striatal and cortical transduction of neurons and astrocytes following CED delivery to the caudate/putamen

• AAV2 achieved widespread striatal and cortical transduction of mostly neurons following CED delivery to the caudate/putamen

• No detectable GFP was observed in any peripheral tissues analyzed
Conclusion

Transduction patterns of both AAV1 and AAV2 following CED delivery to the caudate and putamen suggest these vector serotypes would provide suitable distribution profiles for delivery of HD therapeutics.
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