Partial lowering of total huntingtin levels to treat adults with Huntington’s disease (HD): potential benefits and theoretical risks from human studies and animal models

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Summary
HD is caused by the toxic form of a protein called huntingtin (HTT), and reducing the amount of this protein improves the health of animals engineered to develop HD. A new drug in development for HD, RG6042 (R07234292), partially lowers the amount of both the toxic form of HTT (mHTT) and the non-toxic form (wtHTT). A search of scientific literature identifies no safety risks from partially lowering wtHTT in adulthood.

Methods
• Peer-reviewed journal articles covering the following topics were included in this review:
  • studies in humans relevant to wtHTT lowering
  • partial wtHTT lowering therapies in normal rodents and non-human primates
  • transgenic animal models of HD
  • partial wtHTT lowering therapies in transgenic rodent models of HD
  • genetic inactivation of wtHTT in otherwise normal rodents
  • genetic manipulation of wtHTT in transgenic rodent models of HD
  • Primary literature on in vitro studies was excluded, but recent reviews article on in vitro findings were included.

Results

Studies in humans
• In a Phase 1/2a study (NCT02219036), wtHTT and mHTT lowering with RG6042 in adults with HD was generally safe and well tolerated up to four monthly doses.1
• In case studies of people with rare genetic variations, loss of one normal allele does not cause HD, and people with homozygous CAG expansions develop normally with a similar age of HD onset to heterozygotes.24 (Table 1)

Table 1: Effect of wtHTT lowering in humans

<table>
<thead>
<tr>
<th>Study type</th>
<th>Genotype</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>RG6042 Phase 1/2a1</td>
<td>wtHTT and mHTT lowering with four monthly doses of RG6042</td>
<td>Generally safe and well tolerated</td>
</tr>
<tr>
<td>Case study2</td>
<td>Woman with one normal and one disrupted HTT allele</td>
<td>No detectable abnormal phenotypes at 46 years of age</td>
</tr>
<tr>
<td>Case series4</td>
<td>Homozygous CAG-expansion mutations</td>
<td>Normal development up to onset of HD. No effect on onset of illness and pre-onset</td>
</tr>
<tr>
<td>Epidemiology study5</td>
<td>Transcription-lowering variants of either mHTT or wtHTT alleles</td>
<td>Lowering of mHTT delayed HD onset by a mean of 9.3 years. Lowering of wtHTT hastened HD onset by a mean of 3.3 years</td>
</tr>
</tbody>
</table>

Partial wtHTT lowering in adult rodents
• Partial lowering of wtHTT in normal adult rodents was generally safe and well tolerated over 2–6 months with no reports of alternation in motor performance or activity (Table 2)12
• Partial lowering of wtHTT and mHTT in animal models of HD had beneficial effects (Table 3)13,14,15,16

Table 2: Studies on partial lowering of endogenous wtHTT in normal adult rodents

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Magnitude of wtHTT reduction (%)</th>
<th>Follow-up duration</th>
<th>Mouse strain</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reversible lowering</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Miha6526</td>
<td>75 (mRNA)</td>
<td>3–4 mo</td>
<td>FBV/N</td>
<td>No alterations in motor coordination or activity</td>
</tr>
<tr>
<td>Non-reversible lowering</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV-shHTT</td>
<td>~55 (mRNA)</td>
<td>9 mo</td>
<td>C57BL/6</td>
<td>Altered striatal gene expression</td>
</tr>
<tr>
<td>AAV2/1-miRNA-mHTT</td>
<td>45 (mRNA); 55 (protein)</td>
<td>2–5 mo</td>
<td>FBV/N</td>
<td>No alterations in motor performance or activity</td>
</tr>
<tr>
<td>AAV-mi2-AFP</td>
<td>~70 (mRNA)</td>
<td>4 mo</td>
<td>NR</td>
<td>No neuronal loss or neurotoxicity in histological analyses</td>
</tr>
<tr>
<td>AAV-mi24-AFP</td>
<td>~65 (mRNA)</td>
<td>4 mo</td>
<td>C57BL/6</td>
<td>Minimal striatal toxicity in histological analysis</td>
</tr>
</tbody>
</table>

Partial wtHTT lowering in normal adult non-human primates
• No safety criteria were identified in any studies describing partial lowering of wtHTT in normal non-human primates, including up to 9 months of treatment with a 6-month recovery period in INON/Leavitt chronic toxicology studies (Table 4)16,17

Table 3: Studies on partial lowering of wtHTT and mHTT in animal models of HD

<table>
<thead>
<tr>
<th>Treatment</th>
<th>wtHTT reduction*</th>
<th>Model</th>
<th>Benefits</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reversible lowering</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Miha6526</td>
<td>wtHTT and mHTT (~75% maximum, normalization after 4 mo; mRNA)</td>
<td>BACHD mouse (Hdh&lt;sup&gt;129S5B/ScJ&lt;/sup&gt;)</td>
<td>Improved motor function; Tendency towards longer lifespan</td>
<td>No atonement of beneficial functional effects vs mHTT-specific ASO</td>
</tr>
<tr>
<td>AAV-mi2-AFP</td>
<td>mHTT maximum protein reduction: 89% (mHTT); 71% (mHTT); 76% (mHTT)</td>
<td>Hu/h178</td>
<td>Increased cognition and behavioral phenotype across all studied ASOs</td>
<td>Potency of ASO on mHTT lowering appears to predict more improvement across experiments between the three studied ASOs</td>
</tr>
<tr>
<td>Non-reversible lowering</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AAV2-shHTT</td>
<td>wtHTT and mHTT (60% at 4 wks, 75% at 4 mo; mRNA)</td>
<td>HD-N171- 820 mouse (Hdh(+/-))</td>
<td>None</td>
<td>No difference in GABA-ergic neuronal survival and inclusion load ratios versus mHTT reduction</td>
</tr>
<tr>
<td>LV-shHTT</td>
<td>wtHTT only (56–79%; mRNA)</td>
<td>Non-transgenic rat (Hdh&lt;sup&gt;+/+&lt;/sup&gt;)</td>
<td>None</td>
<td>No signs of toxicity</td>
</tr>
<tr>
<td>AAV2/1-miRNA- mHTT</td>
<td>wtHTT and mHTT (75% at 12 wks; mRNA and protein)</td>
<td>VPC128 mouse (Hdh&lt;sup&gt;+/+&lt;/sup&gt;)</td>
<td>None</td>
<td>No overt striatal toxicity or neuroinflammation in histopathological specimens</td>
</tr>
</tbody>
</table>

Conclusions
• Lowering wtHTT showed little/no effects in humans or adult animals and non-allele specific HTT lowering is beneficial in HD animal models.
• Genetic ablation showed inconsistent effects and is not relevant to HTT lowering with ASOs, which is partial, transient, reversible and titratable.
• Non-allele specific lowering of HTT with RG6042 remains a promising HD therapeutic strategy and this is being further investigated in an open-label extension to the Phase 1/2a study.

Abbreviations
AAt, adenosine-associated virus; ASO, antisense oligonucleotide; GABA, y-aminobutyric acid; HD, Huntington’s disease; HTT, mouse Huntington’s disease homolog; Hdh, huntingtin; LV, lentivirus; mHTT, mutant HTT; mRNA, messenger RNA; miRNA, micro RNA; mo, month; NR, not reported; shRNA, short hairpin RNA; wks, weeks; wtHTT, wild type HTT.

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References
Full reference list available on request.

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