Sustained mutant huntingtin lowering in the brain and cerebrospinal fluid of Huntington disease minipigs mediated by AAV5-miHTT gene therapy

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BACKGROUND
HTT-lowering therapies hold great promise to slow down or halt neurodegeneration in Huntington disease (HD). We have developed an engineered microRNA targeting human huntingtin (HTT), delivered via adeno-associated viral vector serotype 5 (AAV5-miHTT), leading to efficient HTT-lowering in vitro and in vivo in rodent models. Transgenic HD (tgHD) minipigs, ubiquitously expressing human mutant HTT (548-amino acid N-terminal human fragment with 124 repeats), are suitable to study proof-of-concept of HTT-lowering in a large brain. In this ongoing study, we aim to assess the therapeutic window of our approach in a large animal model. We observed sustained target engagement and strong efficacy up to 1 year after one-time intrastrtrial injection of AAV5-miHTT in tgHD minipigs.

Mechanism-of-action (MoA) of AAV5-miHTT

1. Upon parenchymal injection AAV5-miHTT binds to neuronal cell-surface receptor and is internalized.
2. Transport to the nucleus and uncoating of the miHTT transgene which remains mostly epimorphic.
3. Expression and processing of the miHTT transcript by the endogenous RNA interference machinery.
4. HaRpin structured precursor is transported to the cytoplasm and further processed to mature guide miHTT. No passenger strand is formed, allowing limitation of off-target activity.
5. Mature miHTT is loaded in the RNA-induced silencing complex and binds HTT mRNA.
6. HTT mRNA is cleaved and degraded, resulting in lowering of huntingtin protein translation.

RESULTS

OBJECTIVES

- Surgical target acquisition: evaluate feasibility of MRI-guided convection-enhanced delivery (CED) of AAV5-miHTT in tgdH minipigs
- Biodistribution: assess long-term distribution of vector DNA in different brain areas
- Long-term efficacy: measure miHTT and human HTT mRNA and protein expression in several brain regions
- Biomarkers: assess expression of mutant HTT protein in cerebrospinal fluid (CSF)

METHODS

Animals
- Young adult tgdH Göttingen minipigs (males and females, age 4-8 months), origin Libechov (n=30)
- Treatment groups:
  - Control (untreated)
  - AAV5-miHTT treated (1.2 x 1011 gc/animal)
- Per treatment group, interim sacrifices:
  - 6 months (n=3/group)
  - 12 months (n=4/group)
  - >24 months (n=8/group) [still in-life period]

AAV5-miHTT administration

- MRI-guided CED (Renishaw pre-clinical drug delivery system), bilateral into caudate and putamen (100 µL per structure) at maximum rate 3 µL/min (Fig. 2).
- CSF was collected pre- and post-injection (every 3 months)
- Brains were cut in 4mm slices, and 4mm punch taken:

6 months sacrific: n=27 punches/hemisphere
12 months sacrific: n=65 punches/hemisphere

- Brain punches were divided for bioanalysis:
  - DNA isolation → vector DNA (Q-PCR)
  - RNA isolation → miHTT (RT-qPCR)
  - Tissue lysate → miHTT protein (2ST-M1H Single assay)

- The extensive pumping scheme throughout the whole cerebral cortex at 12 months post-injection, revealed a widespread vector DNA distribution, with detectable levels in all cortical regions analyzed (Fig. 5).
- Mature miHTT molecules were detected in virtually all brain areas from animals treated with AAV5-miHTT, in accordance to vector DNA levels (Fig. 6).

CONCLUSION
Here, we show strong, widespread and sustained (up to 12 months) target engagement and efficacy of one-time intrastrtrial administration of AAV5-miHTT in tgdH minipigs. The current results of this ongoing study on mutant HTT protein lowering in several brain regions, support the continuation of our program into the clinic.

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