AAV5-miHTT gene therapy demonstrates broad vector distribution and strong mutant huntingtin lowering in a Huntington’s disease minipig model

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Huntington’s disease (HD) is an autosomal dominant neurodegenerative disorder caused by a CAG trinucleotide repeat expansion in the first exon of the HTT gene. This CAG repeat expansion results in an expanded polyglutamine repeat in the huntingtin protein, causing toxic gain-of-function and affecting numerous cellular processes. Lowering the translation of mutant huntingtin and thereby reducing downstream toxic effects should be therapeutically beneficial.

The gene lowering therapy developed by uniQure is based on an engineered microRNA targeting huntingtin mRNA (miHTT). The DNA expression cassette encoding the miHTT is delivered to the cell using adeno-associated viral vector serotype 5 (AAV5-miHTT).

Here, we have investigated distribution, efficacy and tolerability of AAV5-miHTT treatment in the currently largest existing animal model of HD, the transgenic HD (tgHD) minipig (Baxa et al., 2013). The tgHD minipig expresses a human HTT fragment of 12 exons with 145 CAG/CAA repeats next to the human HTT promoter.

Widespread AAV5-GFP distribution upon striatal and thalamic injection in tgHD minipigs

Convection-enhanced delivery of 1x10^13 and 3x10^13 total genome copies (gc) of AAV5-miHTT, 1x10^13 gc AAV5-GFP and PBS + 5% sucrose into the striatum and thalamus of tgHD minipigs and followed for 3 months.

Cerebrospinal fluid (CSF) was taken pre- and post-injection for cytokine and mutant huntingtin protein measurements. Brain biopsies (*) were taken for distribution and pathology analysis.

Widespread dose-dependent AAV5-miHTT vector distribution and miHTT expression

Quantitative PCR showed widespread dose-dependent distribution of the AAV5-miHTT vector.

Strong reduction of mutant HTT mRNA by AAV5-miHTT

Mutant HTT-specific TaqMan assay showed a strong reduction of mutant HTT mRNA in all transduced regions upon AAV5-miHTT treatment.

Strong reduction of mutant huntingtin protein by AAV5-miHTT

Single-molecule counting immunoassay showed a dose-dependent reduction of mutant huntingtin protein in biopsies taken from tgHD minipig brain regions.

Trend towards reduced mutant huntingtin protein in CSF of tgHD minipigs after AAV5-miHTT treatment

Longitudinal trend towards reduced mutant huntingtin protein levels in the CSF of tgHD minipigs upon single intraparenchymal AAV5-miHTT injection.

The combination of widespread vector distribution, extensive huntingtin lowering, long-term expression and tolerability observed with AAV5-miHTT, support further investigation of efficacy and safety in this tgHD minipig model and continued preclinical development of HTT-lowering gene therapy for HD.