Secreted therapeutics: Monitoring durability of AAV5-miHTT gene therapy in Huntington disease

Marina Sogorb-González[1,2], Carlos Vendrell[1], Jolanda Snapper[1], Anouk Stam[1], Sonay Keskin[1], Elisabeth A. Spronck[1], Martin de Haan[1], Rienk Nieuwland[3], Sander J. van Deventer[1,2], Pavlina Konstantinova[1], Melvin M. Evers[1], Astrid Vallès[1]

[1]Department of Research & Development, uniQure biopharma B.V., Amsterdam, the Netherlands; [2]Department of Gastroenterology and Hepatology, Leiden University Medical Center, Leiden, the Netherlands; [3]Laboratory of Experimental Clinical Chemistry, Amsterdam UMC, The Netherlands

Poster No. 83

BACKGROUND

Huntington disease (HD) is a fatal neurodegenerative disorder caused by an autosomal dominant mutation in the huntingtin gene (HTT), which leads to mutant HTT (mHTT) protein aggregation, toxicity and neuronal cell death. The HTT-lowering therapy developed by uniQure is based on an engineered microRNA targeting HTT mRNA (miHTT) and delivered by adeno-associated viral vector serotype 5 (AAV5-miHTT).[1] AAV5-miHTT has demonstrated a long-term efficient HTT lowering *in vitro* and *in vivo* in the brains of different HD animal models after one-time infusion.[2-5]

The preclinical development of AAV5-miHTT is accompanied by translational challenges, and clinical biomarkers indicative of dosing and therapeutic efficacy in the central nervous system are very much needed.[6]

Recently, extracellular vesicles (EVs) have been identified as carriers of RNA species, including miRNAs, which are secreted into biological fluids directly from cells.[7] EV-associated miRNAs are becoming promising biomarkers for diagnosis and therapeutics in brain diseases.

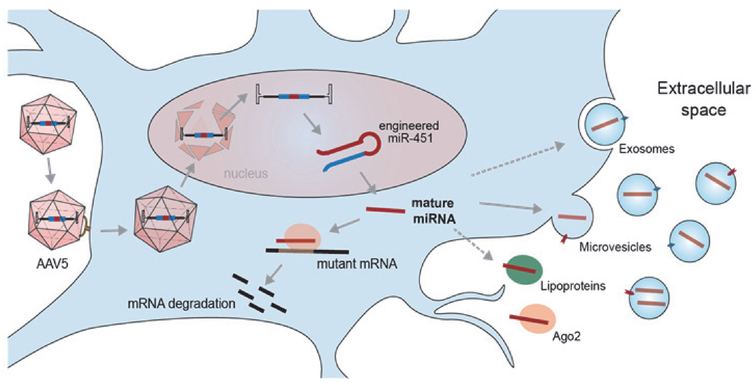

Figure 1. AAV-delivered miHTT molecules are released by neurons within extracellular vesicles.

OBJECTIVES

In this study, we investigated the potential use of EV-associated therapeutic miHTT molecules as suitable measurements to monitor the expression and durability of AAV-delivered therapeutic miRNAs in the brain.

- To study the **secretion** of therapeutic miHTT molecules from AAV5-miHTT-treated neurons.
- To **characterize** the association of therapeutic miHTT molecules with EVs and soluble proteins.
- To investigate the **longitudinal detection** of miHTT in cerebrospinal fluid (CSF) of non-human primates after one-time AAV5-miHTT brain infusion.

METHODS

Differentiation of HD patient iPSC-derived neurons

- Induced-pluripotent stem cells (iPSC) derived from an HD patient were induced and further differentiated into frontal brain-like neurons by dual inhibition of SMAD signaling (**Fig. 2**).[5]

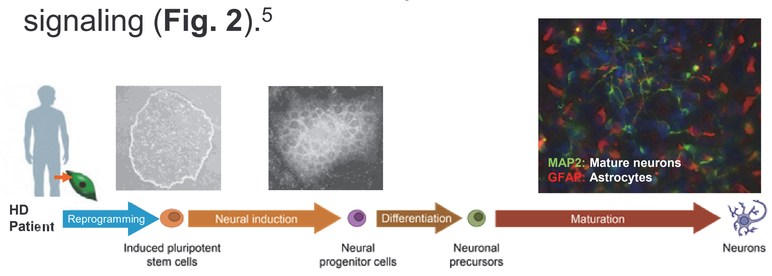

Figure 2. Differentiation of HD patient iPSCs into neuronal cells

Transduction of neurons and EV isolation

- The HD iPSC-derived neurons were transduced with AAV5-miHTT at different multiplicities of infection (MOI).
- 10 mL of cultured medium from transduced neuronal cells was collected and EVs were isolated by precipitation (ExoQuick-TC™), or size-exclusion chomatography (SEC).[8]

AAV5-miHTT treatment in non-human primates

Cynomolgus monkeys (*Macaca fascicularis*) were injected with formulation buffer or two doses of AAV5-miHTT locally in the caudate and putamen (100 µl/region, low dose $2x10^{12}$ gc/brain and high dose $2x10^{13}$ gc/brain) (n=6).

RESULTS

Successful AAV5-miHTT transduction results in HTT lowering and EV-associated secretion of miHTT molecules

- AAV5 dose-dependent levels of cellular miHTT expression (**Fig. 3A**) and mHTT protein lowering (**Fig. 3B**) were detected in iPSC-derived neurons.
- Therapeutic miHTT molecules were secreted in a dose-dependent manner from AAV5-treated neuronal cells at 5 and 12 days after transduction (**Fig. 3C**).

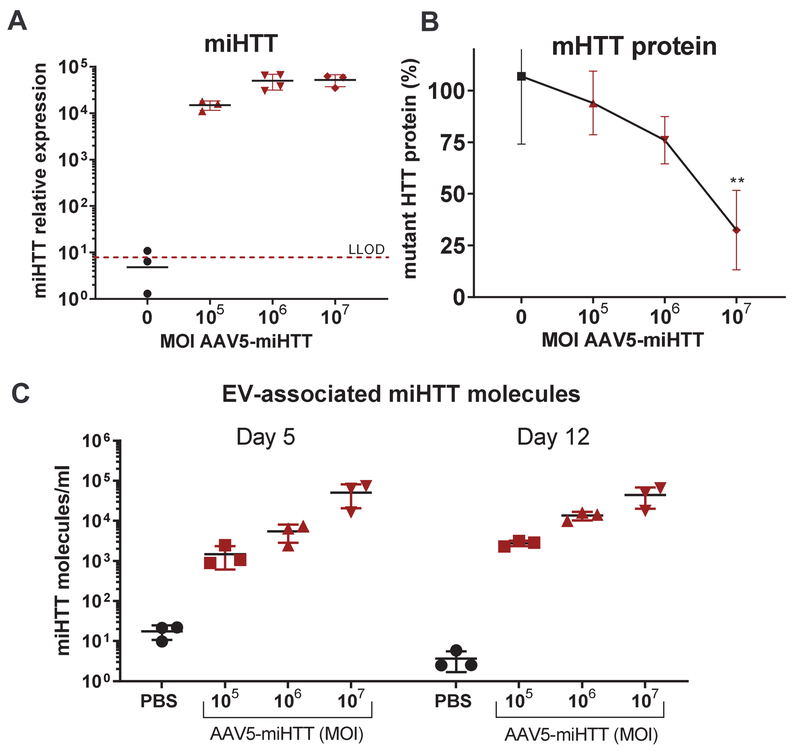

Figure 3. Dose-dependent (A) cellular miHTT expression, (B) mHTT protein lowering, and (C) miHTT molecules secreted within EVs from AAV5-miHTT-treated neuronal cells. *Mature miHTT molecules were quantified by standard curve-based TaqMan RT-qPCR.*

Characterization of EVs

- Vesicles precipitated from cultured medium were positive for EV and RNA-induced silencing complex (RISC-) protein markers by western blot (**Fig. 4A**) and visualized by transmission electron microscopy (**Fig. 4B**).

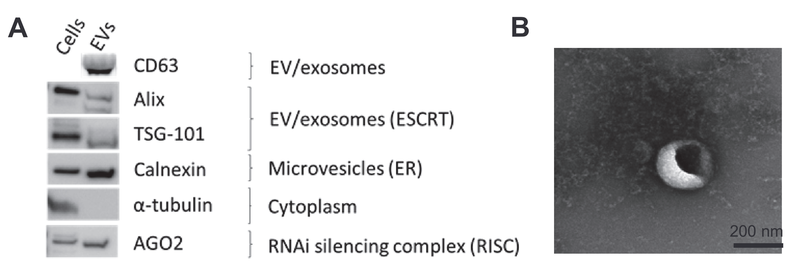

Figure 4. Characterization of EV precipitated from cultured medium of neuronal cells by (A) western blot and (B) transmission electron microscopy (TEM). *Cell lysates were used as positive controls.*

Therapeutic miHTT molecules are associated with EVs and soluble proteins

- Therapeutic miHTT molecules were detected in association with EVs and soluble proteins, efficiently separated by size-exclusion chromatography (SEC) (**Fig. 5**).

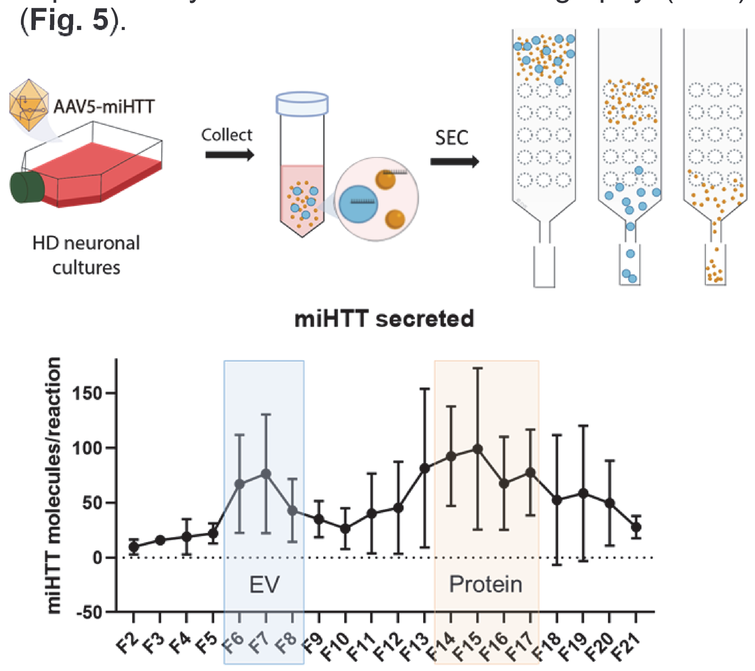

Figure 5. Secreted miHTT molecules were detected in association with EVs, as well as soluble proteins separated by SEC. *Levels of miHTT molecules in different SEC fractions corresponding to EV (blue) and protein (orange) containing fractions complexes.*

Intrastriatal injection of AAV5-miHTT results in widespread distribution of miHTT in the brain of non-human primates

- Non-human primates received a one-time intrastriatal infusion of AAV5-miHTT and CSF was collected at different time points (**Fig. 6A**).
- Widespread distribution of therapeutic miHTT molecules were detected across main brain areas (**Fig. 6B**).

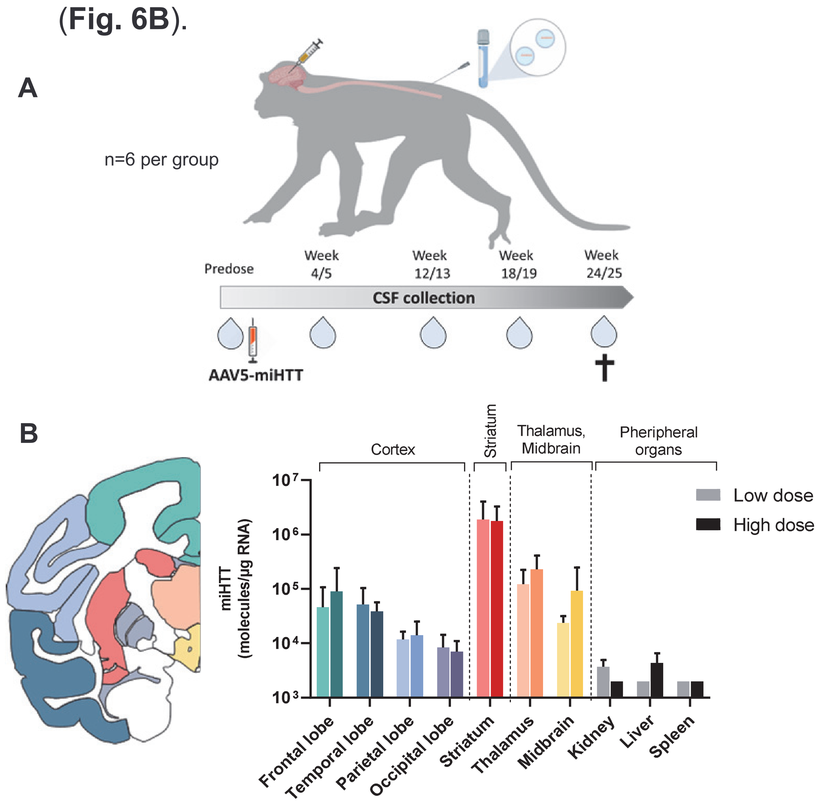

Figure 6. Intrastriatal injection and miHTT expression in non-human primates. *(A) injection and (B) biodistribution of miHTT (molecules/µg input RNA) in different brain areas. Scheme on the left indicates color-coded brain regions, corresponding to the colors on the right graph.*

Detection of therapeutic miHTT molecules in CSF confirms secretion and durability of one-time AAV5-miHTT therapy

- Dose-dependent mature miHTT molecules were detected in CSF EVs up to six months after intrastriatal injection in non-human primates (**Fig. 7**).

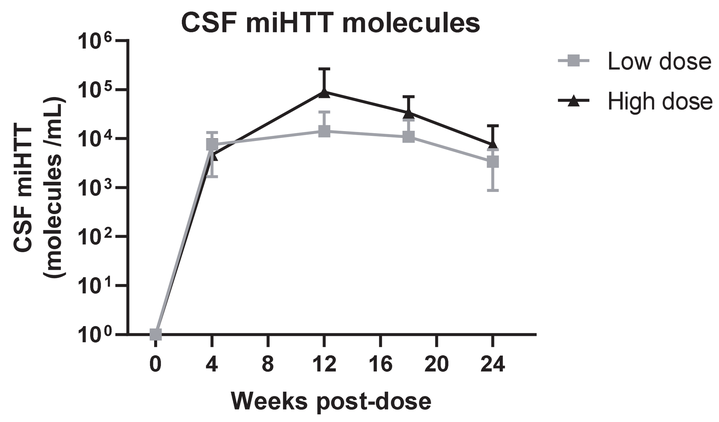

Figure 7. Quantification of EV-associated miHTT molecules in CSF. *Molecules per mL of CSF detected by TaqMan qPCR at different time points after striatal treatment with two doses of AAV5-miHTT.*

CONCLUSIONS

- AAV5-miHTT efficiently transduces HD patient iPSC-derived neurons, and results in mHTT protein lowering.
- Therapeutic miHTT molecules are secreted in a dose-dependent manner from AAV5-treated neuronal cells in association with EVs, as well as soluble proteins.
- Therapeutic miHTT molecules are detected in CSF up to six months after one-time intrastriatal injection of AAV5-miHTT in non-human primates.

The detection of EV-associated miHTT molecules in CSF suggests this is a promising translational marker to monitor long-term expression of AAV5-miHTT therapy in the brain

REFERENCES

1. Miniarikova J, *et al.* (2016) Mol Ther Nucleic Acids 22(5):e297.
2. Miniarikova J, *et al.* (2017) Gene Ther. 24(10):630-639.
3. Evers MM, *et al.* (2018) Mol Ther. 26(9):2163-2177.47
4. Spronk E, *et al.* (2019) Mol Ther Methods Clin Dev. 13: 34-343.
5. Keskin S, *et al.* (2019). Mol Ther Methods Clin Dev. 15:275-284.
6. Valadi H, *et al.* (2007) Nature Cell Biology. 9(6):654-9.
7. Miniarikova J, *et al.* (2018) Mol Ther. 4;26(4):947-962.
8. Boing AN, *et al.* (2014) J Extracellular Vesicles. 3:23430.

Presented at CHDI 2020 the 15th Annual Huntington's Disease Therapeutics Conference: A Forum for Drug Discovery & Development, February 24-27, Palm Springs