QRX-704, a novel antisense oligonucleotide therapy designed to prevent HD pathology while maintaining HTT function

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Introduction

QRX-704 is a novel antisense oligonucleotide-based therapeutic approach, aiming to mitigate mutant Huntingtin (mHTT) toxicity, while maintaining physiological HTT function. Proteolytic cleavage of mHTT generates toxic N-terminal fragments that are hypothesized to be the main contributor to the pathogenesis of Huntington disease (HD). These fragments are formed from a cascade of proteolytic cleavages, initiated by caspase-6 cleavage at position D586. Importantly, HTT586 cleavage is required for HD-like phenotypes in the YAC128 mouse model[1]. Moreover, mHTT cleavage in turn increases caspase-6 activity, driving pathology in a toxic forward-feedback loop[2] (**Figure 1A**). While toxicity stemming from mHTT cleavage is the primary pathogenic mechanism, data indicates that HTT loss-of-function exacerbates pathology[3]. In addition, maintaining wild type HTT function may be critical for safety of HTT-targeted therapeutics when treating patients for a long period of time.

Figure 1A. Caspase-6 cleavage of mHTT drives generation of toxic small N-terminal fragments in forward feedback loop

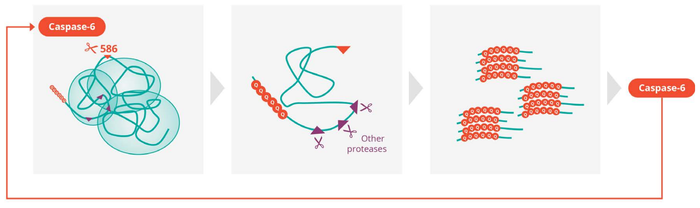
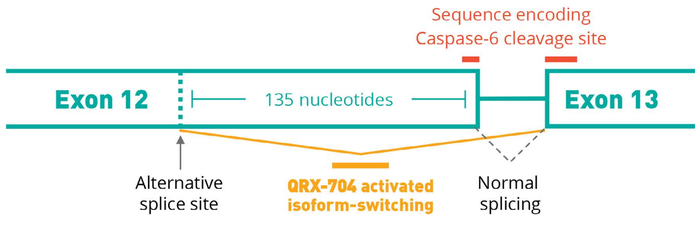


Figure 1B. QRX-704 activates alternative exon 12 splice isoform, removing HTT586 caspase-6 site



Objectives

QRX-704 functions at the pre-mRNA level by activating an alternative HTT splice-isoform (HTT Δ12), removing the critical HTT586 caspase-6 cleavage site, thus preventing formation of toxic N-terminal fragments[4] (Figure 1B). The aim of the study is to pharmacologically characterize QRX-704 for preclinical development, and describe the novel HTT Δ12 isoform.

Material and Methods

QRX-704 splice-switching activity was tested and optimized in cultured **HD fibroblasts** (GM04857) and **HD iPSC-derived mature neural cultures** (ND42223), both from Coriell Institute, assessed by droplet digital PCR and western blotting. Biophysical and biochemical characterization of HTT Δ12 was performed using purified protein, with **caspase-6 cleavage assays**, **CD-spectroscopy**, and **post-translational modification mapping**. Intracerebroventricular (ICV) bolus administration of QRX-704 in in wild type and **YAC128 mice**, was used to study **pharmacodynamic activity**, quantified by droplet digital PCR, **biodistribution** assessed by fluorescent in situ hybridization, **reactive gliosis** was studied by immunohistochemistry. To better quantify HTT Δ12 protein, **exon 12 isoform-specific antibodies** were developed and used to detect HTT Δ12 in YAC128 brain tissue.

Results

QRX-704 sequence optimization in HD fibroblasts and iPSC-neurons

Oligonucleotide lead candidate sequences screened and optimized in HD fibroblasts, assessed by droplet digital PCR and western blot (**Figure 2A**). Results were verified in HD iPSC neural culture (**Figure 2B**).

Figure 2A. RNA modulation in HD fibroblasts

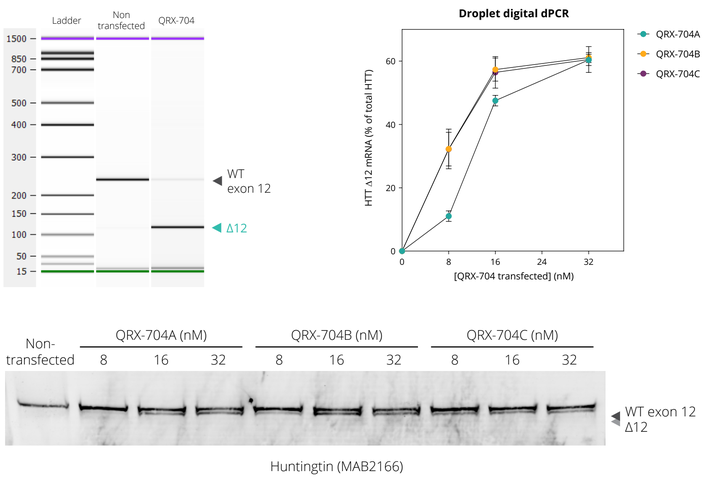
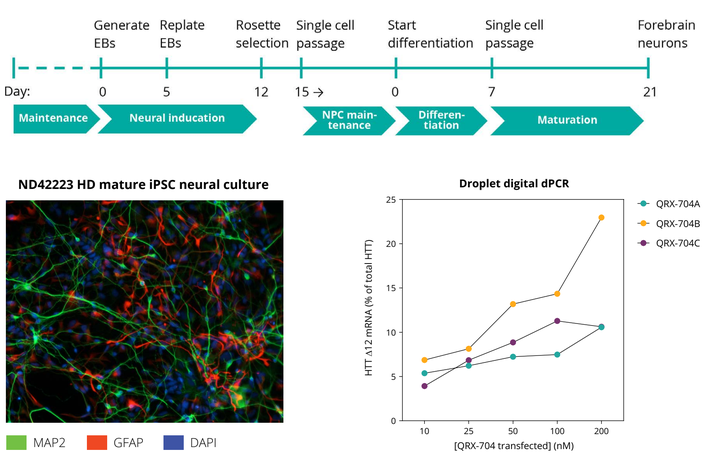


Figure 2B. RNA modulation in HD iPSC neurons



Results (continued)

HTT Δ12 is resistant to HTT586 caspase-6 cleavage

Caspase-6 cleavage of HTT Δ12 isoform assessed in lysates from COS7 cells expressing cDNA (**Figure 3A**), or HD fibroblasts transfected with QRX-704 (**Figure 3B**), followed by incubation with recombinant active caspase-6.

Figure 3A. COS7 cells

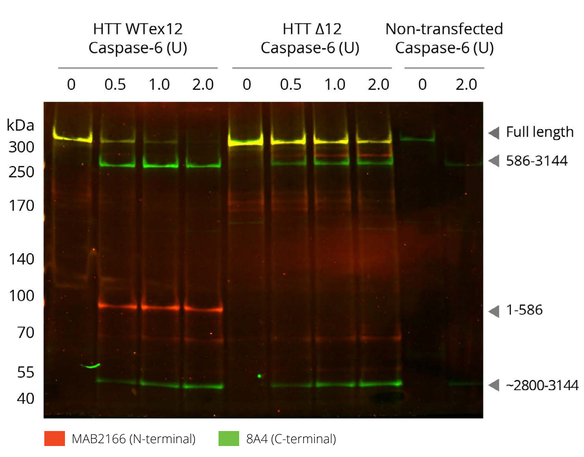
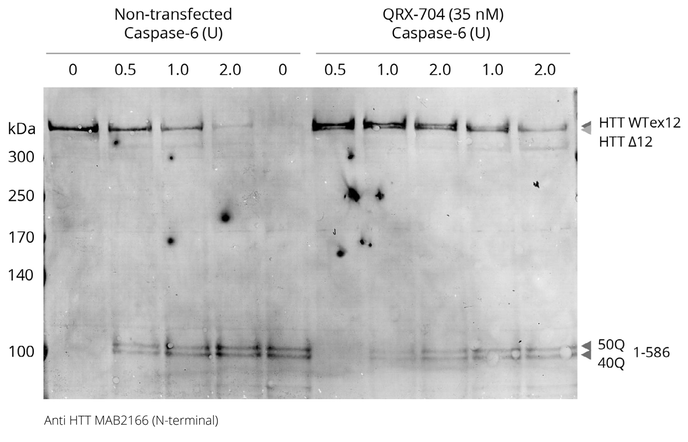


Figure 3B. HD Fibroblasts (CAG40/50)



HTT Δ12 maintains overall folding and biochemical characteristics of canonical HTT

Biochemical analysis of purified 23Q or 78Q WT exon 12 or Δ12 HTT isoforms derived in Sf9 insect cells, assessed by SDS- and native PAGE (**Figure 4A**), CD spectroscopy (**Figure 4B**) and PTM analysis (**Figure 4C**).

Figure 4A. PAGE of purified proteins

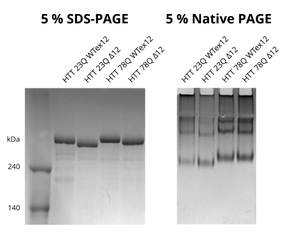


Figure 4B. CD-spectroscopy

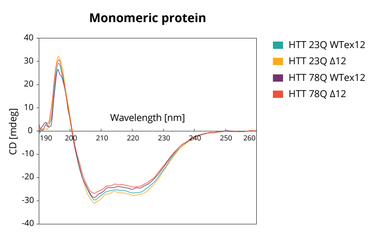
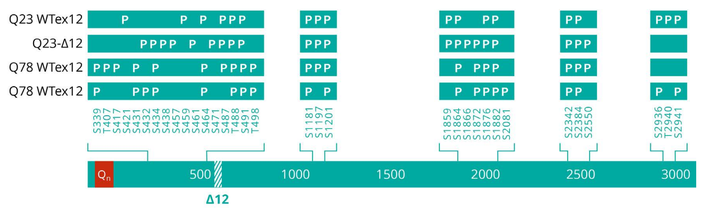


Figure 4C. Post translational modification mapped by mass spectrometry Phosphorylated sites with Mod Score >19 displayed



HTT WT exon 12 or HTT Δ12 overexpressed in SK-N-SH or COS7 cells indicates normal intracellular localization (**Figure 5A**), and unfolding/digestion pattern in pulse proteolysis (**Figure 5B**).

Figure 5A. Intracellular localization - SK-N-SH cells overexpressing HTT isoforms

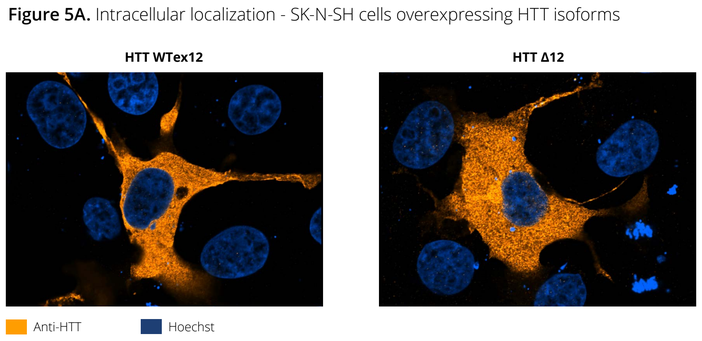
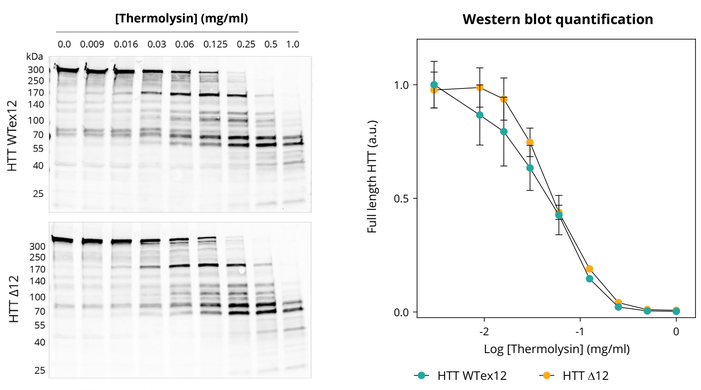


Figure 5B. Pulse proteolysis



In vivo evaluation of QRX-704 activity

Dose-response evaluation of QRX-704 activity in YAC128 HD model administered by intracerebroventricular injection, assessed by droplet digital PCR (**Figure 6A**). Biodistribution and astrocytosis was assessed in CD1 WT mice by fluorescent in situ hybridization (**Figure 6B**) and immunohistochemical staining for GFAP (**Figure 6C**).

Figure 6A. FVB-YAC128, 2 weeks after single ICV administration

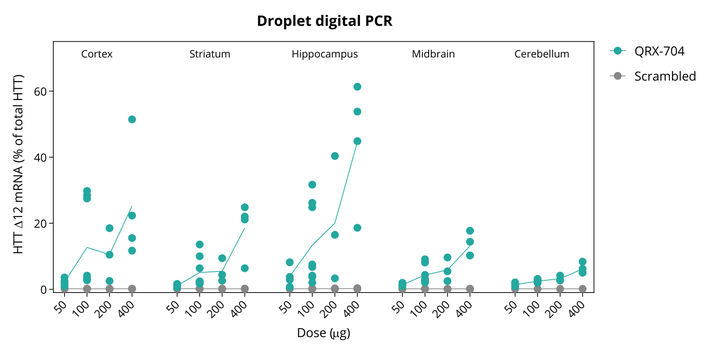


Figure 6B. CD1 mouse 14 days after ICV-administration of QRX-704 (400 µg)

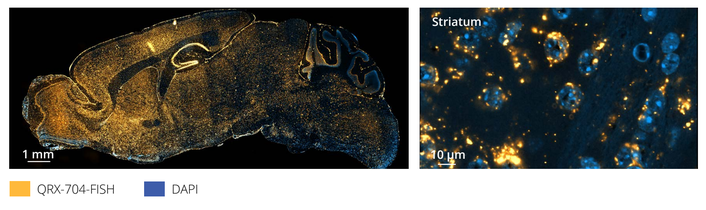
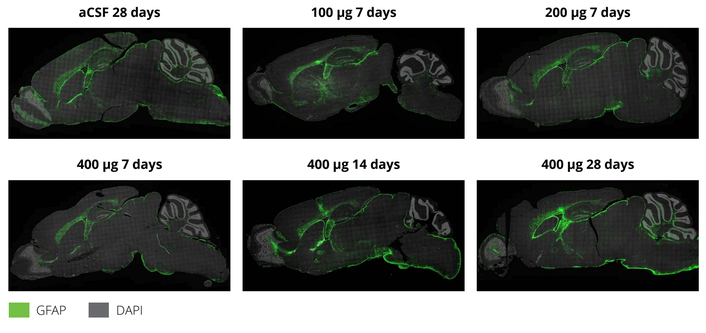


Figure 6C. GFAP immunohistochemistry



Generation of exon 12 isoform-specific monoclonal and polyclonal antibodies, directed against peptides for wt exon 12 or truncated Δ12 (**Figure 7A**), tested by western blot analysis (**Figure 7B**).

Figure 7A. Isoform specific antibodies

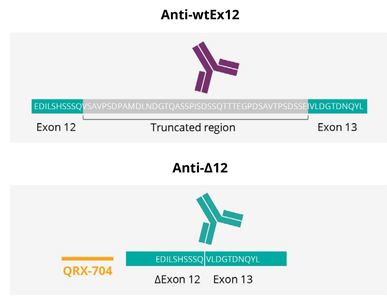
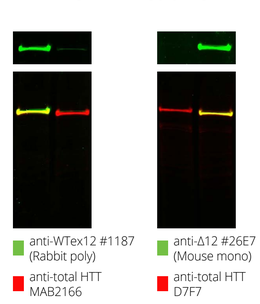
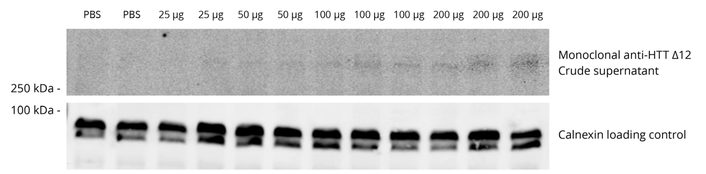


Figure 7B. COS7 cells overexpressing HTT WTex12/Δ12 isoforms



QRX-704 was administered to FVB-YAC128 mice and sacrificed after 14 days for protein analysis, using crude antibody supernatant, which allowed detecting HTT Δ12 protein (**Figure 8**).

Figure 8. Western blot, cortex



Conclusion

QRX-704 constitutes a novel therapeutic approach to HD, potentially preventing toxicity of mHTT while maintaining HTT function.

- **QRX-704 activates a Caspase-6 resistant splice isoform: HTTΔ12**
- **Initial biochemical analyses indicate no major change to global protein folding and biochemistry after exon 12 truncation.**
- **HTTΔ12 RNA and protein was detected in YAC128 brains after ICV administration of QRX-704.**
- **Administration did not cause overt astrogliosis up to 28 days.**

Literature

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