Assessment of miQURE™ efficacy and safety in Spinocerebellar Ataxia Type 3 neurons

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BACKGROUND

Spinocerebellar ataxia type 3 (SCA3) is a progressive and fatal neurodegenerative disorder. Clinical manifestations include progressive gait ataxia with the involvement of cranial nerves. An expansion of a CAG trinucleotide repeat in the ATXN3 gene causes the accumulation of aberrant, toxic ataxin-3 protein in brain regions located in the posterior fossa. An ataxin-3 silencing approach was investigated using the next-generation miQURE™ technology (Miniarikova J, et al., 2016). Artificial microRNAs were engineered to downregulate the ATXN3 gene and packaged into adeno-associated viral vectors (AAV-miATXN3) suitable for in vivo administration (Martier R, et al., 2019)

Mechanism-of-action of AAV-miATXN3

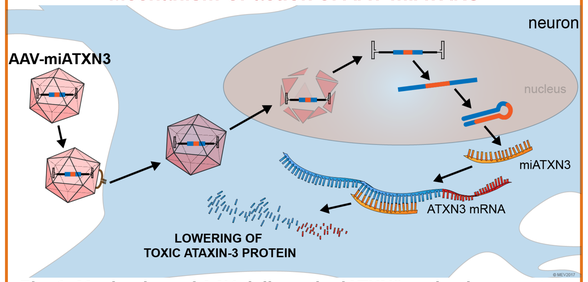

Fig. 1. Mechanism of AAV delivered miATXN3 and subsequent inhibition of ATXN3 expression

- *AAV-miATXN3 binds to neuronal cell-surface receptors and is internalized.*
- *Transport to the nucleus and uncoating of the miATXN3 transgene which remains mostly episomal.*
- *Expression and processing of the miATXN3 transgene by the endogenous RNA interference machinery.*
- *Hairpin structured precursor is transported to the cytoplasm and further processed to mature guide miATXN3. No passenger strand is formed, strongly limiting the risk of off-target activity.*
- *Mature miATXN3 is loaded in the RNA-induced silencing complex and binds ATXN3 mRNA.*
- *ATXN3 mRNA is cleaved and degraded, resulting in lowering of ataxin-3 protein translation.*

STUDY AIMS

- Establish knockdown efficiency of AAV-miATXN3 in neurons and astrocytes
- Determine safety and off-target effects of AAV-miATXN3 through (small) RNA sequencing

METHODS

Culturing and transduction of human iPSC derived neurons and astrocytes

- Human iPSCs obtained from 2 SCA3 patients and 2 healthy controls were differentiated into frontal brain like neurons (**Fig. 2)** as described by (Chambers *et al.,* 2009)
- Control astrocytes were generated using the STEMdiff™ kit from Stem Cell technologies as previously described (Keskin S, et al., 2019)
- Mature astrocytes and neurons were transduced with AAVs at multiplicity of infection ranging from $1x10^6$ to $1x10^7$

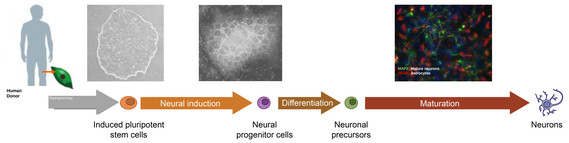

Fig. 2. Human iPSCs were differentiated into frontal brain-like neurons by dual inhibition of SMAD signaling (Chambers *et al.* 2009)

RNA sequencing and analysis of gene expression

- Astrocytes were harvested 6 days and neurons 10 days after transduction
- RNA sequencing was performed by Baseclear, Leiden
- Gene expression analysis was performed by Fios Genomics, Edinburgh, UK

RESULTS

AAV-miATXN3 is highly effective in human iPSC neurons and astrocytes

- AAV-miATXN3 was able to efficiently transduce human iPSC neurons and astrocytes
- Dose-dependent miATXN3 guide strand expression was observed in transduced cells (**Fig 3**)

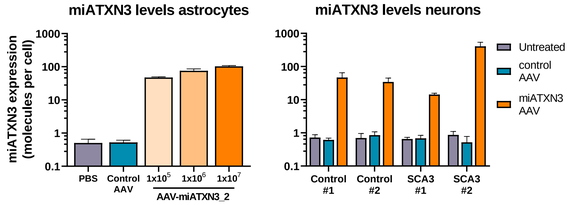

Fig. 3. miATXN3 is highly expressed in neurons and astrocytes. *Control astrocytes and control- or SCA3 neurons were transduced with AAV-miATXN3. After 6 days (astrocytes) or 10 days (neurons) RNA was isolated and expression of mature miATXN3 was determined by Taqman qPCR.*

AAV-miATXN3 leads to only minor gene expression changes in neurons, suggesting a favorable safety profile

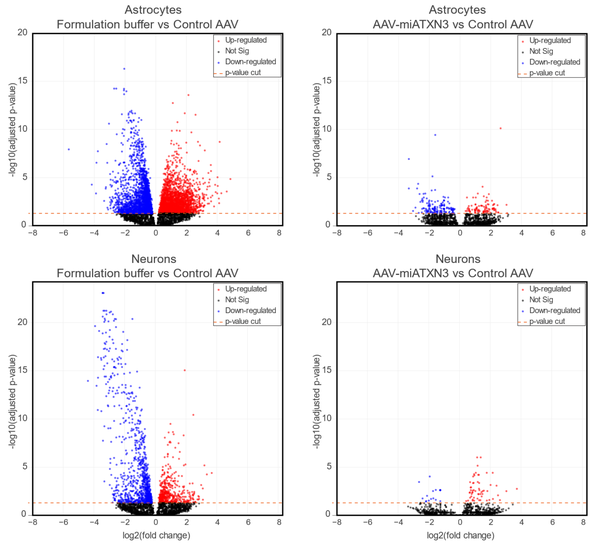

Fig. 4. Differential gene expression in astrocytes and neurons transduced with AAV-miATXN3. *When comparing a (non expressing) control AAV capsid versus formulation buffer, many differentially expressed genes are observed (left panels). However, comparing AAV-miATXN3 to the control AAV revealed only minor changes in gene expression (right panels).*

miATXN3 is processed into exclusively guide strands in human iPSC-neurons, minimizing off-target effects

- Small RNAseq confirmed efficient processing of miATXN3 without passenger strand formation (**Fig 5**)

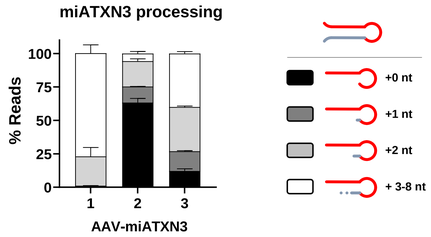

Fig. 5. Small RNA sequencing analysis of miATXN3 processing in human iPSC neurons. *The sequence distribution of the different guide strand lengths mapping to miATXN3_1, miATXN3_2 and miATXN3_3 pre-microRNA sequences were calculated in % reads. The secondary miATXN3 structure based on miRBase prediction are shown on the right panel, including their predicted 22 nucleotide (nt) guide strands shown in red and the untrimmed nucleotides in grey.*

No saturation of RNAi machinery by miATXN3 indicates good cellular tolerability

- The total pool of microRNAs expressed was investigated in transduced neurons by small RNA seq
- Of all microRNA counts, 0.003% to 5.7% were aligned to the mature miATXN3 candidate sequences **(Fig 6)**
- Despite high expression levels of miATXN3, no potentially harmful dysregulation of RNAi machinery or differential miRNA expression was observed
- These results mirror previous off-target analyses for Huntington miQURE™ (Keskin S, et al., 2019)

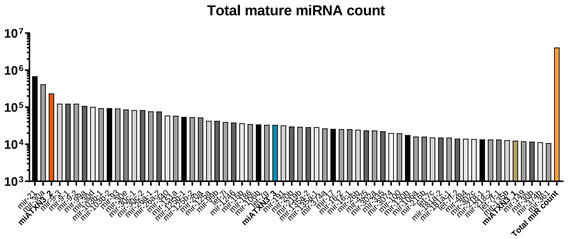

Fig. 6. Small RNA sequence analysis of AAV-miATXN3 treated human iPSC neurons. *Small RNA sequencing was performed 2 weeks post transduction. The total amount of small RNA reads corresponding to the three miATXN3 candidates are shown in colors. Top 60 of most abundant small transcripts are shown in grey.*

CONCLUSIONS

The miQURE™ based AAV-miATXN3 was investigated for efficacy and safety in human iPSC derived neurons and astrocytes.

- AAV-miATXN3 transduces astrocytes and SCA3 neurons with high efficiency
- miATXN3 is processed exclusively into guide strands, minimizing risk of off-target effects
- Expression of miATXN3 causes no saturation of RNAi machinery
- miATXN3 induces very limited gene expression differences in neurons and astrocytes

REFERENCES

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2. Martier R, et al, (2019) Mol Ther Methods Clin Dev. 28;15:343-358.
3. Chambers SM, et al., (2009) Nat Biotechnol. 27(3):275-80.
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