# AAV-miQURE®-mediated targeting of hexanucleotide repeat expansion-containing transcripts in ALS C9orf72 mouse models

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## BACKGROUND

Amyotrophic lateral sclerosis (ALS) is an incurable and fatal neurodegenerative disease characterized by the progressive loss of cortical and spinal motor neurons leading to weakness, muscle atrophy, and, in a substantial number of patients, cognitive impairment [1]. The most common genetic cause of familial ALS (30-50%) is an expanded hexanucleotide repeat ($G_4C_2$) in the first intron of chromosome 9 open reading frame 72 (*C9ORF72*) gene. The repeat expansion undergoes bidirectional transcription and causes cellular toxicity due to RNA foci and dipeptide repeat proteins production [1]. Therapeutic options are limited and there are no current treatment options that substantially change the course of C9orf72 ALS [2]. Therefore, there is a need to develop new gene therapy strategies to effectively target pathological transcripts of the *C9ORF72* gene.

## OBJECTIVE

To develop an AAV gene therapy (**Fig. 1A**) that selectively lowers mutant intronic C9orf72 transcripts (**Fig. 1B**).

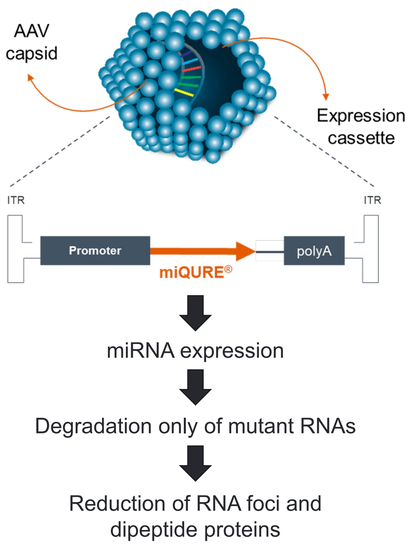


***Fig. 1A Features of the miQURE® technology***

- ✓ *AAV-miC9O (targeting sense mutant C9orf72) transduces motor neurons*
- ✓ *Vector uncoats in the nucleus and mediates transgene expression*
- ✓ *The hairpin structured precursor is transported to the cytoplasm and further processed to mature guide miRNA. No passenger strand is formed from miQURE®, strongly limiting the risk of off-target activity*
- ✓ *Mature miRNA selectively binds mutant C9orf72 mRNA, leading to degradation of the toxic target transcripts*

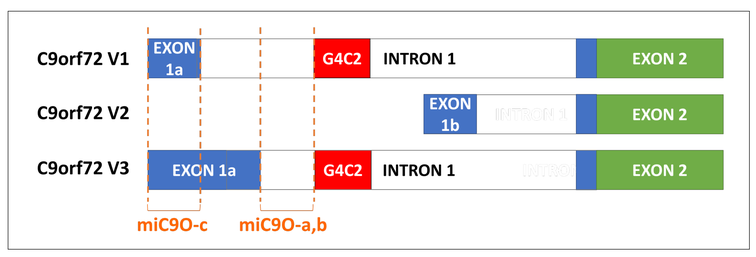


***Fig. 1B Schematic representation of human C9orf72 transcript variants.*** miQURE® targets only variant 1 (V1) and variant 3 (V3) C9orf72 mRNAs. miC9O target sequences are marked in orange.

## METHODS

### *In vivo* studies in ALS mouse models

To provide proof-of-concept in mice, two C9orf72 models were used:

- **AAV9-$(G_4C_2)_{149R}$ mouse model**. AAV9 was used to deliver 149 repeats of the hexanucleotide $G_4C_2$ motif, along with the 5' (119 bp) and 3' (100 bp) flanking regions of the C9orf72 gene. AAV9 was injected into the lateral ventricles of wild-type pups on postnatal day 0 [3]. Eight weeks post AAV9 injection, AAV-miC9O vectors were administered bilaterally in the striatum at mid and high dose and the mice were sacrificed 8 weeks post AAV-injection.

- **Transgenic BAC-C9-112 mice.** The Tg(C9orf72_3) line 112, uses a bacterial artificial chromosome (BAC) to deliver the full-length C9orf72 sequence with the disease-associated expansion [4]. Mice were selected to express ~500 repeats. At 8 weeks of age mice were administered bilaterally in the striatum at mid dose with AAV-miC9O vectors and sacrificed 12 weeks post AAV-injection.

### *In vivo* processing and abundancy of therapeutic miRNAs

Mature miRNA processing and abundancy were investigated in mice bilaterally injected in the striatum with high dose of AAV-miC9O-a and miC9O-b. Mice were sacrificed 4 weeks post injection and total RNA from striatum was used for small RNA sequencing.

## RESULTS

### Dose-dependent knockdown of mutant intronic C9orf72 mRNA in AAV9-$(G_4C_2)_{149R}$-induced mice

- Mature miRNA expression was confirmed in the striatum (**Fig. 2**)

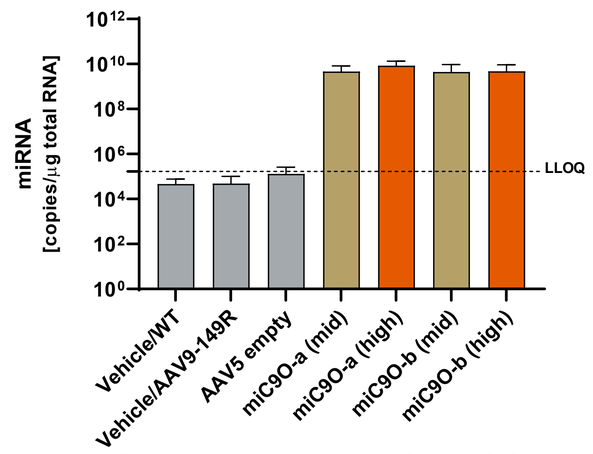


***Fig. 2 Mature miRNA expression in striatum of AAV9-$(G_4C_2)_{149R}$ - induced mice.*** miC9O-a and miC9O-b were quantified by specific RT-QPCR assays on striatum tissues from AAV-treated and untreated groups (n=6, except for miC9O-b high n=5).

- Strong, dose-dependent human mutant intronic C9orf72 mRNA knockdown (≤89%) was achieved in the striatum of AAV-miC9O-a and AAV-miC9O-b injected mice (**Fig. 3**).

- A trend in human mutant intronic C9orf72 mRNA lowering (up to ~50%) was observed in rostral cortex (graph not shown).

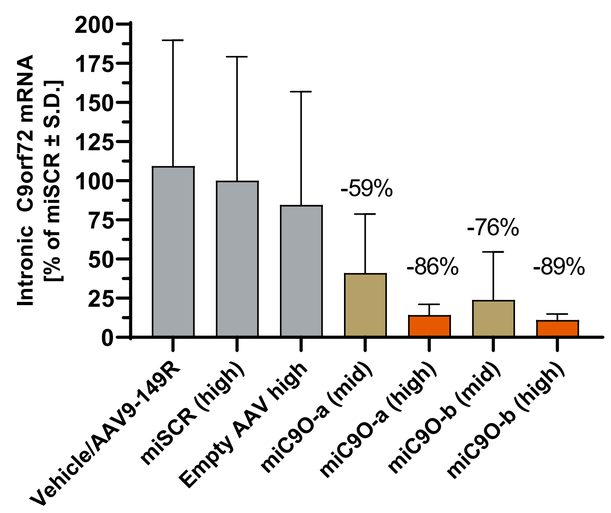


***Fig. 3 Intronic C9orf72 mRNA expression in striatum of AAV9-$(G_4C_2)_{149R}$ - induced mice.*** RT-QPCR was performed was performed in AAV-treated and untreated groups (n=6, except for miC9O-b high n=5).

### AAV-miC9O-a drives efficient mutant intronic C9orf72 mRNA lowering in BAC-C9-112 mice

- Significant human intronic C9orf72 mRNA knockdown (up to 78%) was reached in the striatum of AAV-miC9O-a injected mice (**Fig. 4**)

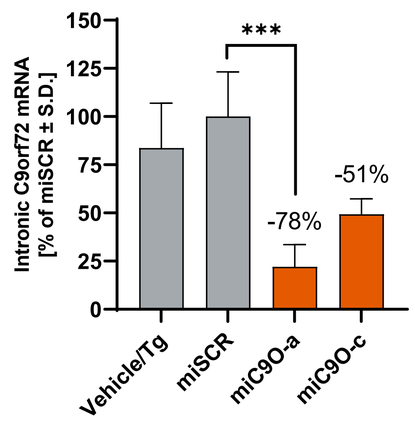


***Fig. 4 Intronic C9orf72 mRNA expression in striatum of BC-C9-112 mice.*** RT-QPCR was performed in AAV treated (n=3, except for miC9O-c n=2) and untreated groups (n=5). Statistics: one-way ANOVA with Dunnett's post-hoc test.

- Healthy (V2) human C9orf72 mRNA expression was not affected by AAV-miC9O-a and AAV-miC9O-c treatment, confirming selective silencing of mutant transcripts only (**Fig. 5**).

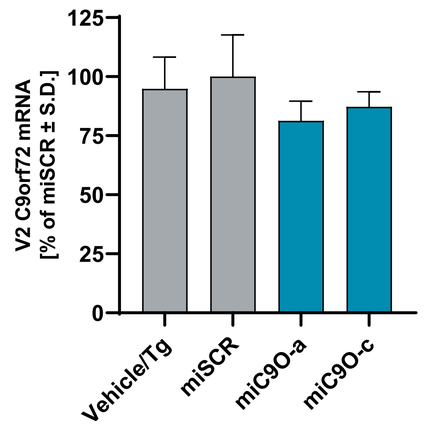


***Fig. 5 V2 C9orf72 mRNA expression in striatum of BC-C9-112 mice.*** RT-QPCR was performed in AAV treated (n=3, except for miC9O-c n=2) and untreated groups (n=5).

- Total human C9orf72 mRNA expression was not lowered by AAV-miC9O-c. A trend for lowering was observed in AAV-miC9O-a injected mice as consequence of the strong lowering of mutant C9orf72 mRNAs, impacting total mRNA levels (**Fig. 6**).

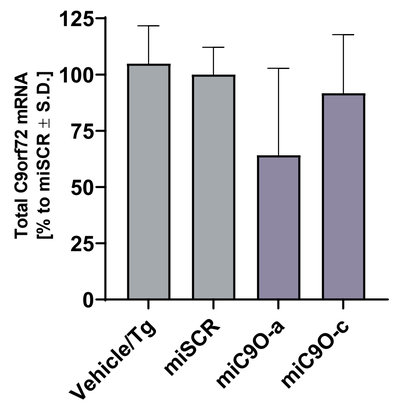


***Fig. 6 Total C9orf72 mRNA expression in striatum of BAC-C9-112 mice.*** RT-QPCR was performed in AAV treated (n=3, except for miC9O-c n=2) and untreated groups (n=5).

### miC9O-a and miC9O-b expression in the brain does not exceed the level of endogenous miRNAs

- Sequencing results revealed that the expression of mature miC9O-a and miC9O-b is within the levels of endogenous miRNAs, and hence there is a low risk of interference with endogenous miRNA pathways. In **Fig. 7** the processing of miC9O-a and miC9O-b is shown.

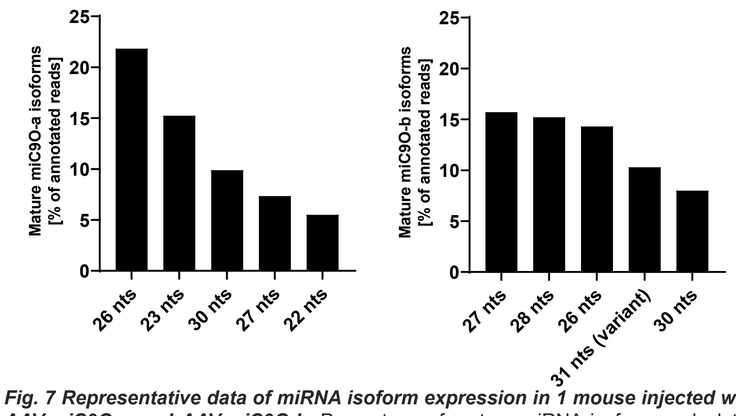


***Fig. 7 Representative data of miRNA isoform expression in 1 mouse injected with AAV-miC9O-a and AAV-miC9O-b.*** Percentage of mature miRNA isoforms calculated on the total of annotated reads.

## CONCLUSIONS

- We showed *in vivo* PoM of AAV-miQUREs in selectively silencing of the mutant C9orf72 transcripts
- Small RNA analyses revealed that the level of mature miC9Os is not overcoming the one of endogenous miRNAs, and therefore there is a low risk of interference with endogenous miRNA pathways
- Current results support further investigation of distribution, efficacy, tolerability, and safety of AAV-miC9Os in small and larger animals

## *REFERENCES*

1. Smeyers et al. (2021). Front. Cell. Neurosci. 15:661447
2. Hautbergue et al. (2021). Curr Opin Neurol. 34(5):748-755
3. Chew et al. (2019). Mol Neurodegener. 14(1):9.
4. O'Rourke et al. Neuron. 88(5):892-901.

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