vMiX™, an innovative AAV-based RNA interference platform: from *in vitro* development to *in vivo* validation by targeting genes involved in ALS

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AVIADOBIO

vMiX™

P0944

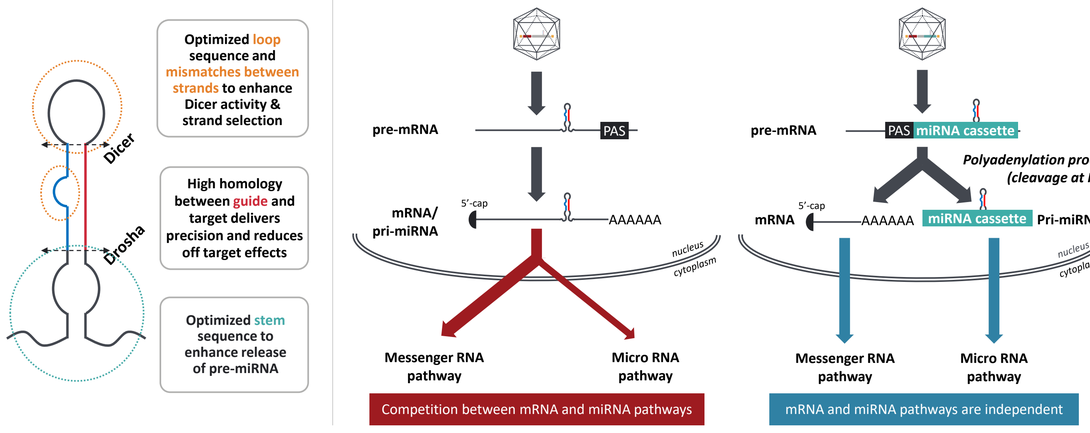
OBJECTIVE

To design and test a novel adeno-associated viral (AAV) vector gene silencing platform that could be applied to a wide range of genes implicated in neurodegenerative diseases, including those that cause amyotrophic lateral sclerosis (ALS).

INTRODUCTION

- vMiX™ is a novel AAV vector gene silencing platform capable of expressing micro-RNAs (miRNA) to target any transcript in any species (see **Poster P0089**).
- The regular design of the miRNA vector based on a messenger-RNA (mRNA) expression cassette with an RNA Pol II promoter results in a miRNA hairpin included in a mRNA sequence, which can then be exported into the cytoplasm before being cleaved by Drosha, causing competition between the two pathways (**Figure 1B**).
- The vMiX™ vector design allows separation of mRNA and miRNA sequences during pre-mRNA cleavage at PAS (polyadenylation process), leading to two independent pathways (**Figure 1C**). The mRNA part of vMiX™ can also be used to express another therapeutic gene.
- Gain-of-function mutation in genes including ataxin-2 (*ATXN2*), *FUS*, *C9ORF72* and superoxide dismutase 1 (*SOD1*) have been identified as causative of ALS, a neurodegenerative disorder resulting in progressive loss of voluntary muscle control, paralysis and fatal respiratory failure.
- We sought to demonstrate proof-of-concept of the vMiX™ platform's ability to target *ATXN2* and *SOD1* to support continued development towards clinical application.

Figure 1: Design of the vMiX™ vector

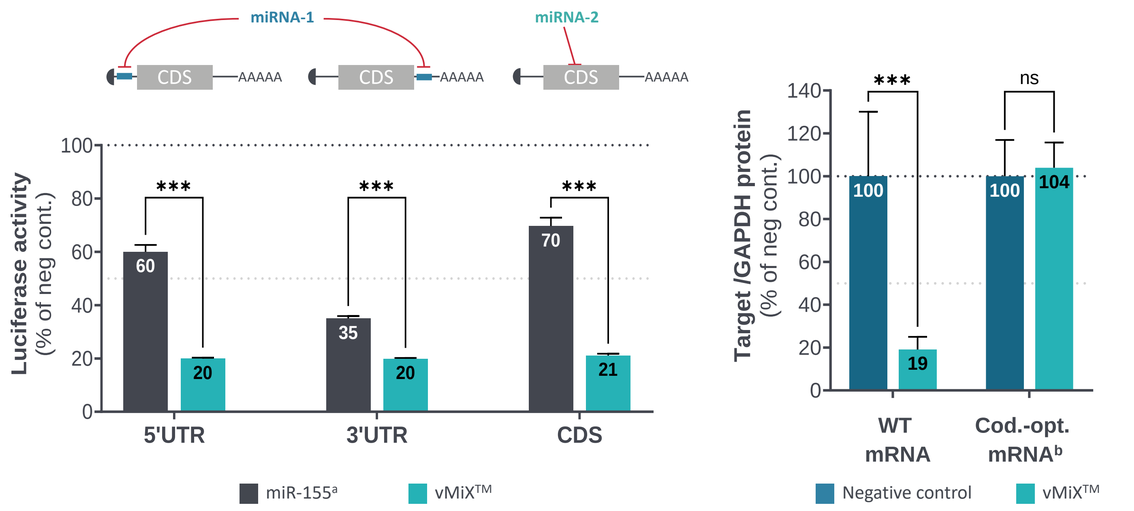


In vitro development of vMiX™ vector

A) Using luciferase reporter assays to assess mRNA knockdown levels, the vMiX™ vector efficiency was compared with that of commercially-available miR-155 miRNA constructs with identical guides.

B) vMiX™ vector specificity was assessed by comparing knockdown levels against wild-type and codon-optimized mRNA coding for the same protein (three mutations outside the seed sequence).

Figure 2: *In vitro* development of vMiX™ vector



A) miR-155: vector using the regular miR-155 hairpin scaffold in the regular mRNA-based expression cassette. **B)** Codon-optimized mRNA sequence with three different nucleotides outside seed sequence. Mean ± SD; two-way ANOVA (*** p<0.001).

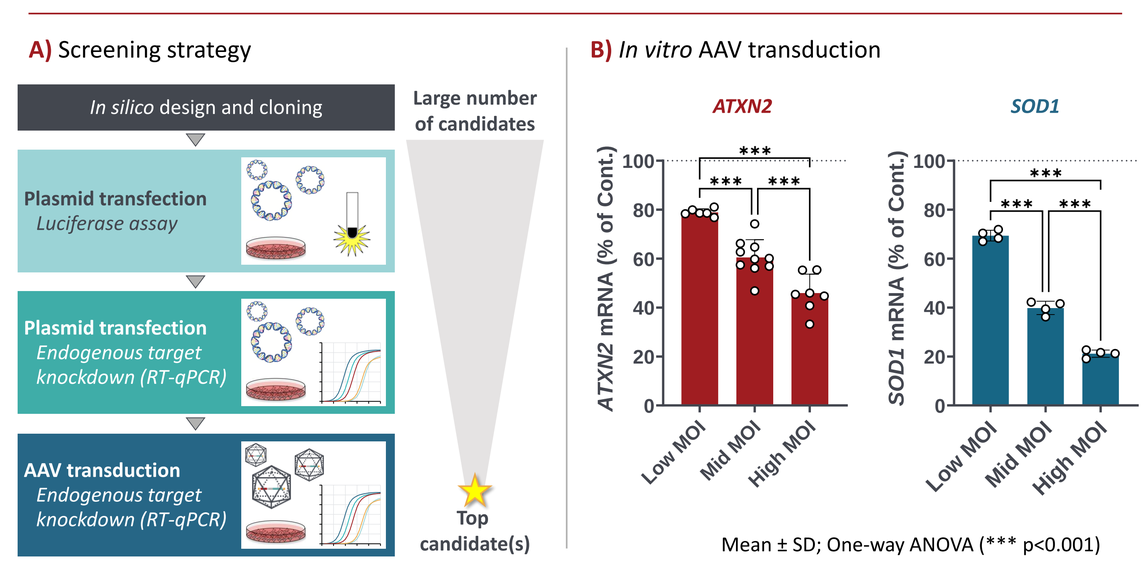
- The vMiX™ vector achieved greater levels of luciferase knockdown compared with miR-155 miRNA constructs: 80% versus 30–75%, respectively (**Figure 2A**).
- Protein analysis revealed the vMiX™ vector achieved an 81% knockdown of a wild-type target and had no activity against the codon-optimized mRNA coding for the same protein (**Figure 2B**).

In vitro application of vMiX™ to target two ALS genes, *ATXN2* and *SOD1*

A) A schematic of the screening strategy of miRNA candidates from *in silico* design to *in vitro* transduction is shown in **Figure 3A**.

B) vMiX™ AAVs expressing the best candidates were transduced into mammalian cells and their activity against the target's endogenous mRNA was assessed.

Figure 3: *In vivo* confirmation of vMiX™ knockdown activity with *ATXN2* and *SOD1*



Mean ± SD; One-way ANOVA (*** p<0.001)

- The best performing candidate yielded dose-dependent knockdown of *ATXN2* and *SOD1 in vitro*, with a maximum knockdown of 54% and 79%, respectively (**Figure 3B**).

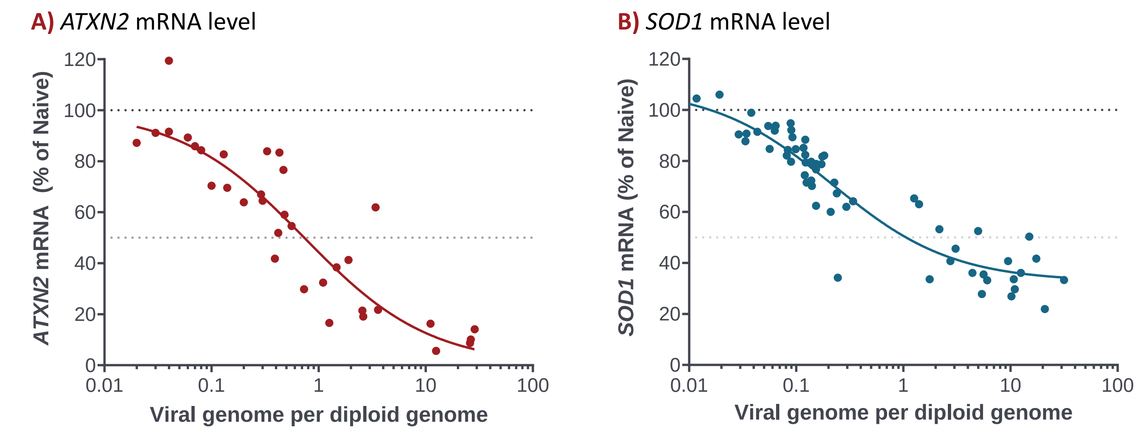
In vivo confirmation of vMiX™ knockdown activity

ATXN2 and *SOD1* mRNA knockdown was assessed 6–7 weeks after injection in two studies:

A) Neonatal mice expressing the human *ATXN2* transgene (hBACQ72) received intracerebroventricular injections of a vMiX™ AAV9-miR-ATXN2 in a dose-range study.

B) Neonatal mice expressing the human mutated G93A *SOD1* received intracerebroventricular injections of a vMiX™ AAV9-miR-SOD1 in a pilot and longitudinal cohort study.

Figure 4: *In vivo* confirmation of vMiX™ knockdown activity with *ATXN2* and *SOD1*



- AAV9-miR-ATXN2 demonstrated a dose-dependent response in human *ATXN2* mRNA knockdown mice (**Figure 4A**), up to a maximum knockdown of 89.1% with 10–30 viral genome/diploid genome (vg/dg).
- AAV9-miR-SOD1 demonstrated a dose-dependent response in human *SOD1* mRNA knockdown mice, with the decrease dependent on the level of AAV transduction across the cortex and spinal cord (**Figure 4B**).
- **The two vMiX™ vectors performed the same way, with an EC_{50} of approx. 1 vg/dg.**

CONCLUSIONS

- **For the first time, we show an RNA of interest (an miRNA here) can be expressed outside the canonical promoter-transgene-termination-signal expression cassette.**
- **The design of the vMiX™ vector allowed early decoupling of mRNA and miRNA sequences to avoid competition between the two pathways.**
- **The hybrid hairpin improved the essential step of miRNA processing, allowing a high fidelity of the predicted guide sequence and a good guide-to-passenger ratio (see Poster P0089).**
- **The vMiX™ vector's activity was confirmed *in vitro* and *in vivo* against two different targets (*ATXN2* and *SOD1*) involved in ALS and showed potential for treating ALS (see Posters P0167 and P0280).**

ABBREVIATIONS: **AAV:** adeno-associated viral; **ALS:** amyotrophic lateral sclerosis; **ANOVA:** analysis of variance; ***ATXN2:*** ataxin-2; **CDS:** coding sequence; **EC_{50}:** half maximal effective concentration; **mRNA:** messenger RNA; **miRNA:** microRNA; **MOI:** multiplicity of infection; **ns:** non-significant; **PAS:** polyadenylation process; **RT-qPCR:** reverse transcription-quantitative polymerase chain reaction; **SD:** standard deviation; ***SOD1*:** superoxide dismutase 1; **UTR:** untranslated region; **vg/dg:** viral genome/diploid genome; **WT:** wild type.

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