Effective *SOD1* targeting with vMiX™, an innovative AAV-based RNA interference platform

L. Reilly,[1] Z. Walker,[1] D. Ojutalayo,[1] C. Andreassi,[1] N. Sheikh,[1] E. Dellow,[1] N. Ali Mohammadi Nafchi,[2] O. Brock,[2] A. Abhilash,[1] L. Ireland,[1] R. Elsayed,[1] A. Ule,[1] P.M. Coan,[1] C.J. Miranda,[1] J. Isaac,[1] C.E. Shaw,[1,2] D.Y. Lee,[1] Y.B. Lee,[1,2] R. Joubert[1]

[1]AviadoBio Ltd, London, UK; [2]Maurice Wohl Clinical Neuroscience Institute, King's College London, London, UK.

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vMiX™ TST-24

OBJECTIVE

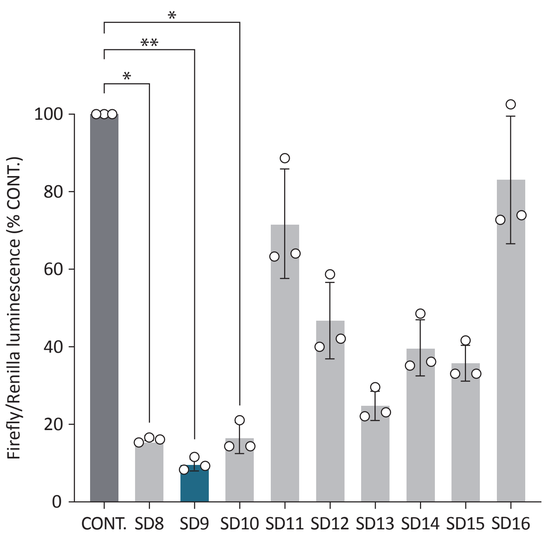
To design, screen, and select effective microRNA (miRNA) candidates using the vMiX™ platform for efficient silencing of *SOD1,* a causative gene in amyotrophic lateral sclerosis (ALS).

INTRODUCTION

- ALS is a severe neurodegenerative disease characterized by progressive motor neuron loss, leading to muscle atrophy, paralysis, and respiratory failure. *SOD1*, the first gene linked to ALS, accounts for 12–20% of familial ALS and 1–3% of sporadic ALS cases. Toxic gain-of-function *SOD1* mutations cause protein misfolding and aggregation.
- Recent approval of Qalsody® (tofersen) validates gene silencing as a therapeutic treatment approach for SOD1-ALS. However, the need for frequent, chronic antisense oligonucleotide injections creates significant treatment burden for patients and caregivers, and also non-trivial associated healthcare resource utilization. Consequently, there is interest in long-term, potentially one-time treatments that could meaningfully reduce treatment burden over time.
- Using vMiX™, a novel adeno-associated virus (AAV)-based RNA interference platform utilizing miRNA for gene silencing,[1] we aimed to design, screen, and select effective miRNA candidates for efficient *SOD1* knockdown, evaluating their performance *in vitro* and *in vivo*.

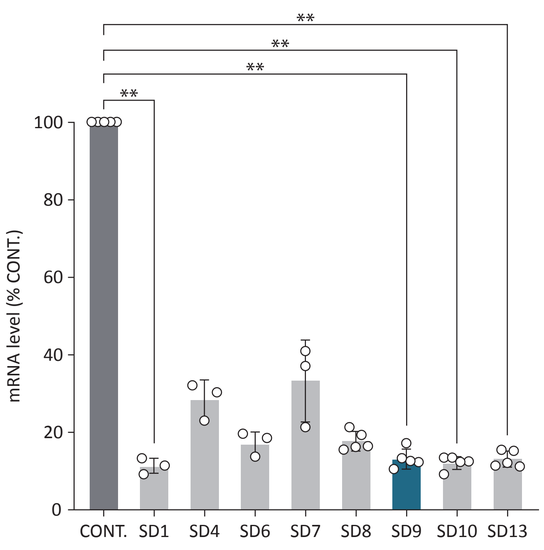
In vitro screening of *SOD1* miRNA candidates

Figure 1: *In vitro* development of miRNA targeting *SOD1* using a luciferase assay



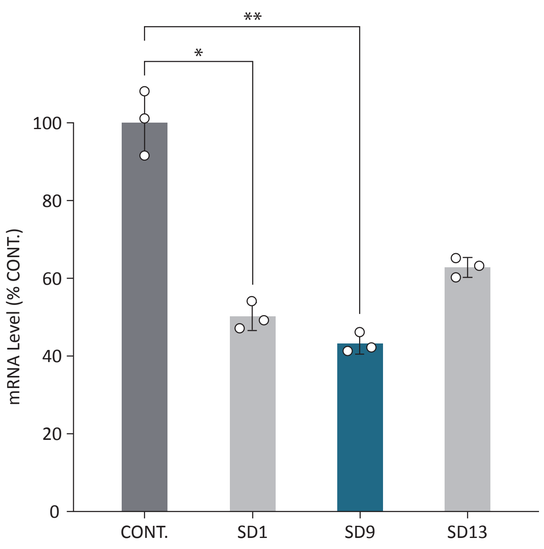
- miRNAs targeting across the coding sequence of *SOD1* were designed and cloned into a reporter plasmid and co-transfected in HEK293 cells.
- 24 hours post-transfection, a luminescence assay was performed.
- Certain miRNA sequences reduced detected levels of luminescence by up to 90% (**Figure 1**; **SD9**; Kruskal-Wallis with Dunn's multiple comparisons, *p<0.05,**p<0.01).

Figure 2: *In vitro* analysis of *SOD1* knockdown following transfection with the vMiX™ vector



- Eight miRNAs which most effectively reduced luminescence were cloned into the vMiX™ vector to assess knockdown of endogenous *SOD1*.
- HEK293 cells were transfected with vMiX™ vectors and cultured for 48 hours before harvesting and RNA extraction.
- All candidate miRNA successfully induced knockdown of *SOD1*, varying from 65% to 90% reduction in expression compared to a non-targeting miRNA control (**Figure 2**; **SD9**; Kruskal-Wallis with Dunn's multiple comparisons, **p<0.01).

Figure 3: *In vitro* analysis of *SOD1* knockdown following transduction with the vMiX™ vector

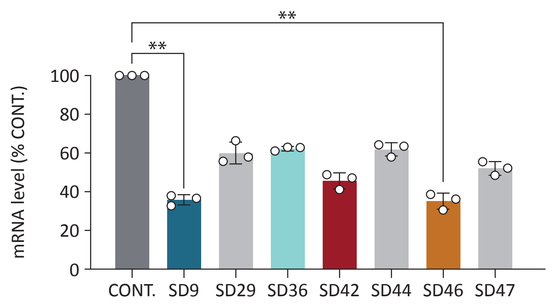


- AAV9 vectors were produced for the three miRNAs that most effectively reduced *SOD1* expression.
- HEK293 cells were transduced with vMiX™ vectors and cultured for 48 hours before harvesting and RNA extraction.
- Candidate miRNAs reduced expression of *SOD1* by up to 57% (**Figure 3**; **SD9**; Kruskal-Wallis with Dunn's multiple comparisons, *p<0.05,**p<0.01).

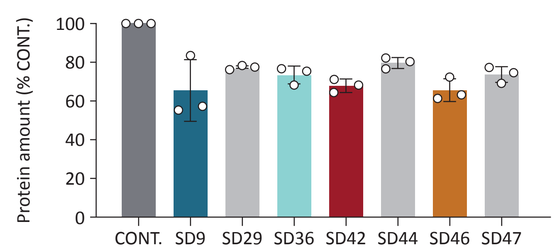
Lead candidate selection screening of *SOD1* miRNA candidates

Figure 4: *In vitro* screening of candidate miRNAs targeting *SOD1*

A) *SOD1* mRNA level



B) *SOD1* protein level

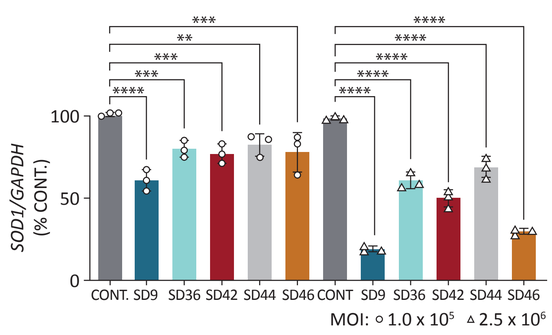


A) miRNAs targeting *SOD1* in human and additional species were designed and cloned into the vMiX™ vector. HEK293 cells were transfected, and RNA harvested as before. All candidates successfully induced knockdown of *SOD1*, varying from 38% to 65% reduction in expression compared to a non-targeting miRNA control (**Figure 4A**; one-way ANOVA with Dunn's multiple comparisons, **p<0.01).

B) HEK293 cells were transfected and cultured for 72 hours before harvesting and protein extraction. All candidates induced knockdown of *SOD1*, from 21% to 35% reduction in protein levels compared to a non-targeting miRNA control (**Figure 4B**).

Figure 5: *In vitro* transduction with candidate miRNA guides targeting *SOD1*

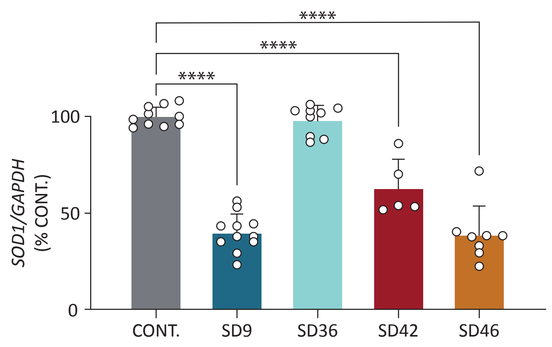
SOD1 mRNA level



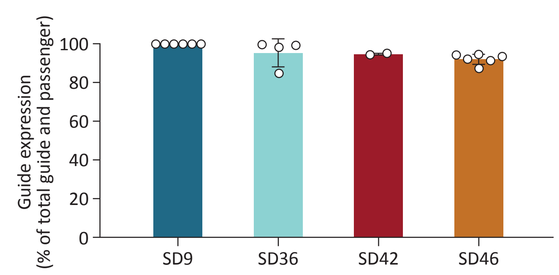
- AAV9 vectors were produced for five miRNAs that effectively reduced *SOD1* expression. HeLa cells were transduced with vMiX™ vectors and cultured for 72 hours before harvesting and RNA extraction.
- Candidate miRNAs reduced expression of *SOD1* by up to 70% (**SD46**) or 81% (**SD9**; **Figure 5**; two-way ANOVA with Dunnett's multiple comparisons, **p<0.01; ***p<0.001; ****p<0.0001).

Figure 6: *In vivo* confirmation of *SOD1* knockdown with vMiX™

A) *SOD1* mRNA level



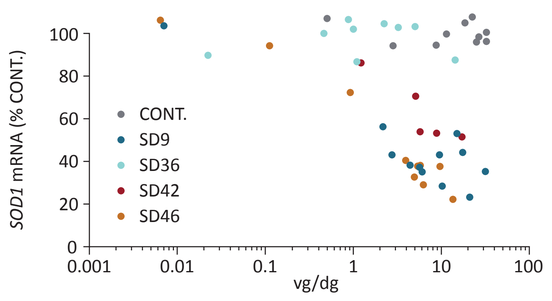
B) miRNA guide/passenger expression



A) Neonatal mice expressing the human mutated G93A *SOD1* received intracerebroventricular injections of a vMiX™ AAV9-miR-SOD1. After six weeks, analysis of cortical samples demonstrated an efficient knockdown of *SOD1* mRNA (**Figure 6A**; one-way ANOVA with Dunnett's multiple comparisons, ****p<0.0001).

B) Small RNA sequencing miRNA processing analysis revealed candidate guides produced a similar average amount of guide species and low amounts of passenger. SD46 displayed the most accurate guide processing at the expected 21nt length (data not shown).

C) *SOD1* knockdown vs vg/dg



C) Analysis of cortical samples demonstrated an efficient knockdown of *SOD1* mRNA across a broad range of vector genome (vg)/ diploid genome (dg) quantifications. SD9 and SD46 display correlations between the amount of guide expressed and vg/cell quantifications (data not shown).

CONCLUSIONS

- **We have developed and demonstrated that the novel RNA interference platform vMiX™ has the capacity to efficiently silence a gene associated with both familial and sporadic ALS.**
- **This platform shows broad adaptability for an AAV-based RNA interference approach to target a range of diseases.**

REFERENCES: [1]Joubert R, *et al*. Presented at ESGCT Annual Congress 2024. Poster P0944.

ABBREVIATIONS: **AAV:** adeno-associated virus; **ALS:** amyotrophic lateral sclerosis; **ANOVA:** analysis of variance; **miRNA:** microRNA; **MOI:** multiplicity of infection; **mRNA:** messenger RNA; ***SOD1*:** superoxide dismutase 1; **vg/dg:** vector genome/diploid genome

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