Leveraging the vMiX™ platform to target alpha-synuclein for the treatment of Parkinson's disease and other synucleinopathies

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AVIADOBIO

vMiX™

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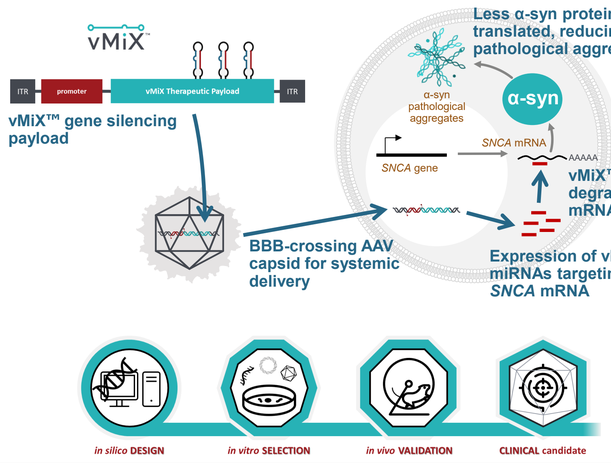
OBJECTIVE

- **To design vMiX™ miRNAs targeting *SNCA* and evaluate their mRNA knockdown and guide processing performance *in vitro.***
- **To characterise the top vMiX-*SNCA* miRNAs vectorised into a BBB-crossing AAV capsid *in vitro,* and evaluate the lead miRNA *in vivo*.**

INTRODUCTION

- α-synuclein (α-syn) is a small protein (14 kDa) encoded by *SNCA* that plays a key role in neuronal synaptic activity[1].
- α-syn aggregation is the central pathological hallmark of Parkinson's disease (PD), Lewy body disease (LBD) and multiple system atrophy (MSA) – progressive conditions for which no disease-modifying treatments are currently available[2].
- *SNCA* gene silencing is supported by human disease evidence and has shown promise in preclinical models, where lowering *SNCA* mRNA led to reductions in intracellular α-syn aggregates in the brain and the prevention and reversal of pathology *in vivo*[3-7].
- vMiX-*SNCA* combines a therapeutic payload based on our proprietary miRNA expression platform (vMiX™) with a BBB-crossing AAV capsid that can achieve broad CNS biodistribution. It is being developed as a single-dose, intravenously delivered *SNCA*-lowering gene therapy for PD and other synucleinopathies (**Figure 1**).

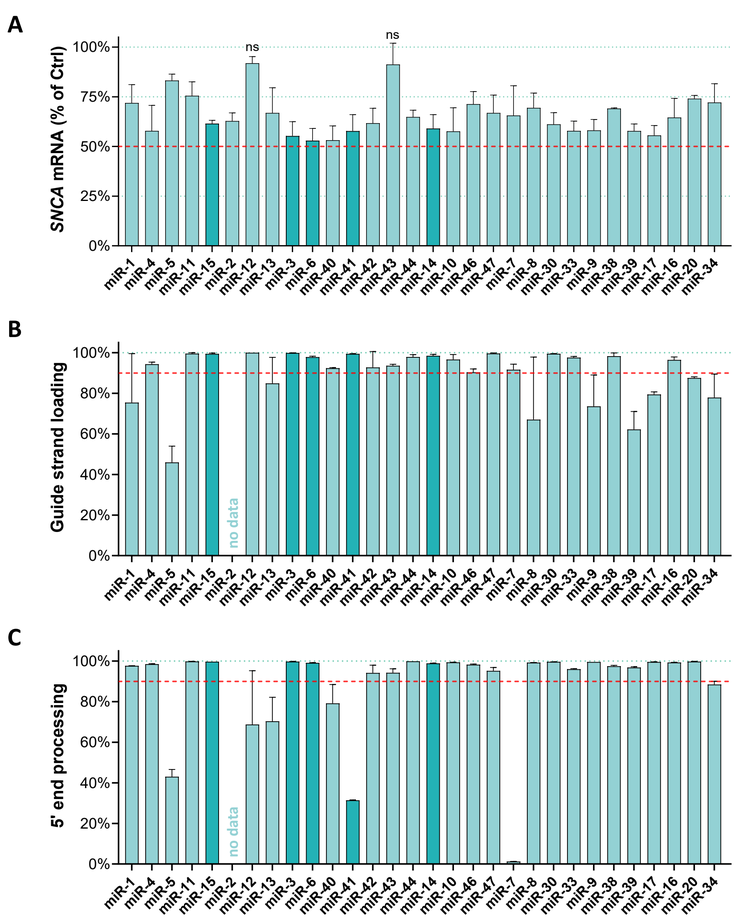
Figure 1: Therapeutic strategy and development process of vMiX-*SNCA*



In vitro screening of *SNCA* miRNAs

- 30 miRNAs targeting *SNCA* mRNA were designed and cloned into vMiX in a single hairpin format.
- *SNCA* mRNA knockdown activity and processing fidelity of the miRNAs were evaluated in a human neuroblastoma cell line (KELLY) (**Figure 2**).
- The miRNAs were ranked based on *SNCA* mRNA knockdown and guide processing performance, as well as *in silico* target engagement across human, mouse and NHP *SNCA* mRNA orthologues.
- This selection process identified miR-3, miR-6, miR-14, miR-15 and miR-41 as the top five miRNAs for further development.

Figure 2: *SNCA* mRNA knockdown and guide processing of 30 *SNCA* miRNAs in a vMiX single hairpin configuration

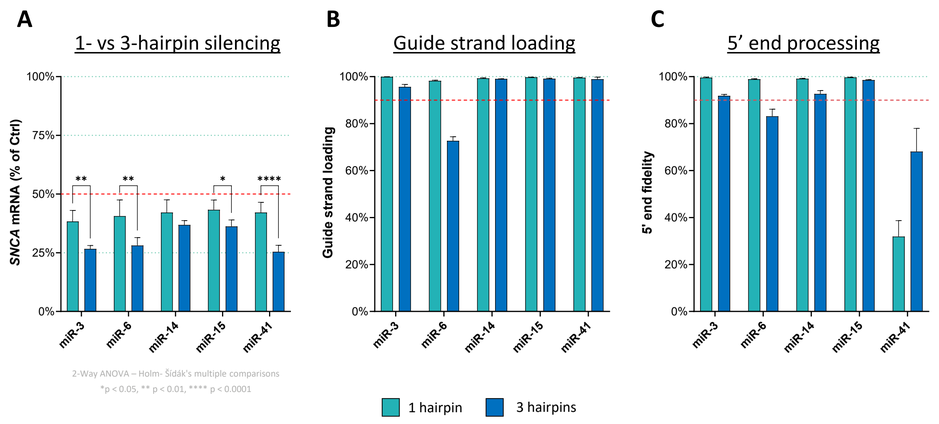


RNA was harvested from KELLY cells 48 h after plasmid transfection and analysed for **(A)** *SNCA* mRNA knockdown, expressed as % of a non-targeting miRNA control (n=3; ANOVA with Holm-Šídák's multiple comparisons test, P <0.05; ns= non-significant relative to the non-targeting miRNA control). Small RNA-seq (Illumina) was used to measure **(B)** Guide strand loading and **(C)** 5' end processing fidelity of the miRNAs (n=2). Threshold for ideal guide processing is ≥ 90%. The top five miRNAs are shown in jade. Data represented as mean + SD.

vMiX multiplexing improves *SNCA* mRNA silencing

- vMiX triple hairpin payloads were generated for the top five *SNCA* miRNAs.
- *SNCA* mRNA knockdown and guide processing performance of the triple hairpin payloads were assessed against their single hairpin equivalents in KELLY cells (**Figure 3**).
- Based on *SNCA* mRNA knockdown and guide processing performance in the final vMiX triple hairpin configuration, miR-3 and miR-15 were identified as the top two miRNAs to further develop.

Figure 3: *SNCA* mRNA knockdown and guide processing of the top five *SNCA* miRNAs in single- and triple- vMiX hairpin configurations

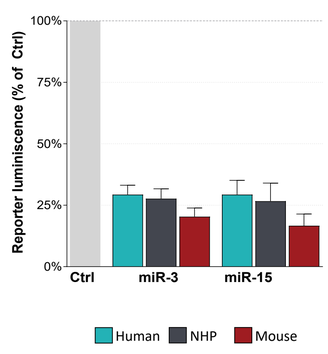


RNA was harvested from KELLY cells 48 h after plasmid transfection and analysed for **(A)** *SNCA* mRNA knockdown, expressed as % of a non-targeting miRNA control (n=3). Small RNA-seq (Illumina) was used to measure **(B)** Guide strand loading and **(C)** 5' end processing fidelity of the miRNAs (n=3). Threshold for ideal guide processing metrics is ≥ 90%. Data represented as mean + SD.

Cross-species target engagement

- Engagement of miRNAs against non-human *SNCA* mRNA orthologues, such as mouse and NHP, was initially predicted *in silico* based on complete miRNA sequence complementarity to those mRNA orthologues.
- Engagement of miR-3 and miR-15 against mouse *Snca* and NHP *SNCA* mRNA orthologues was subsequently validated *in vitro* using a dual-luciferase-reporter (DLR) assay (**Figure 4**).

Figure 4: Cross-species engagement of *SNCA* mRNA orthologues by miR-3 and miR15

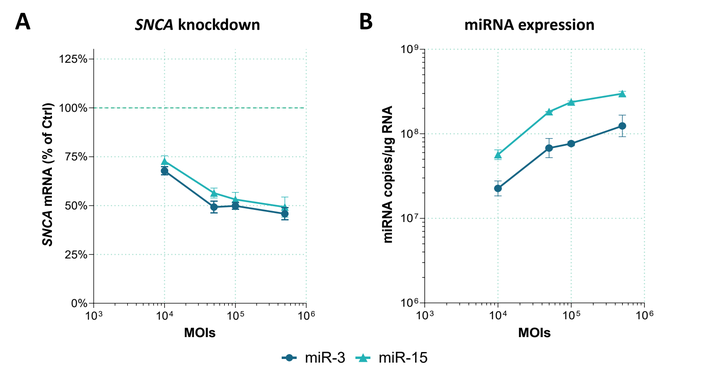


DLR-*SNCA* reporter plasmids encoding the target sequences for each *SNCA* mRNA orthologue were co-transfected into HEK293A cells with either miR-3 or miR-15 vMiX triple hairpin payloads. Firefly:Renilla luminescence ratios were measured 48 h post-transfection on a plate reader (n=3). Data represented as mean + SD.

In vitro activity of the top two AAV vMiX-*SNCA* miRNA constructs

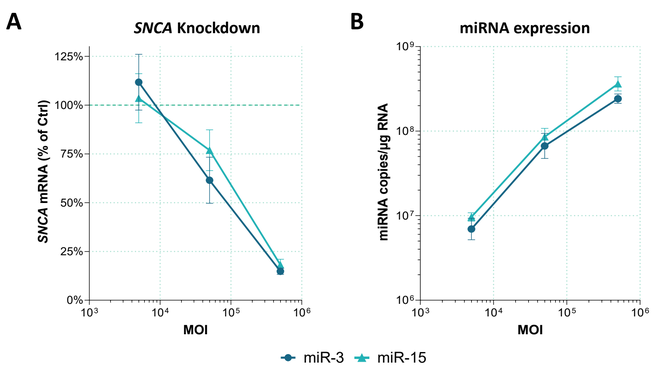
- The miR-3 and miR-15 vMiX triple hairpin payloads were vectorised into a BBB-crossing AAV capsid under the control of a ubiquitous promoter and evaluated in HeLa cells (**Figure 5**) and human iPSC-derived neurons (**Figure 6**).
- Both AAV constructs showed dose-dependent *SNCA* mRNA knockdown and miRNA expression, achieving approximately 50% and 80% knockdown at the highest MOIs in HeLa cells and iPSC-derived neurons, respectively.
- Although miR-3 and miR-15 showed similar *SNCA* mRNA knockdown, miR-15 had the better guide processing profile (**Figure 3B-C**) and was therefore selected as the lead vMiX-*SNCA* miRNA.

Figure 5: *In vitro* transduction of miR-3 and miR-15 AAVs in HeLa cells



HeLa cells were transduced with miR-3, miR-15 or negative control AAVs at MOIs of $1x10^4$ to $5x10^5$. Cells were harvested 72 h post-transduction and analysed for **(A)** *SNCA* mRNA knockdown, expressed as % of the negative control AAV, and **(B)** miRNA expression (n=3 per MOI group). Data represented as mean ± SD.

Figure 6: *In vitro* transduction of miR-3 and miR-15 AAVs in human iPSC-derived neurons

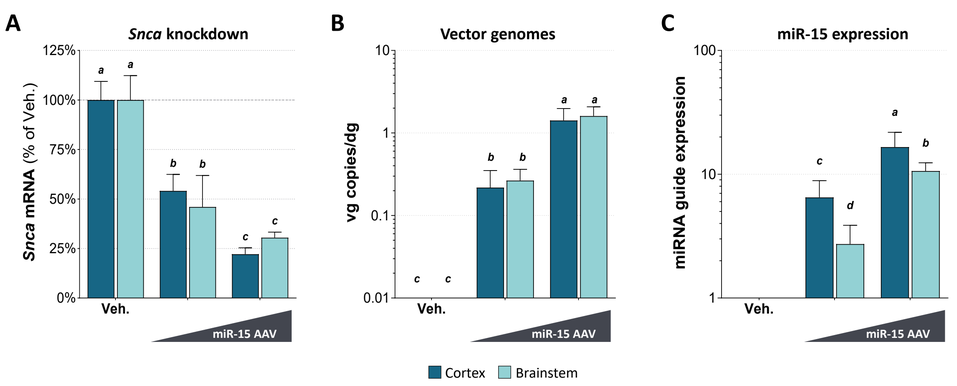


Human iPSC-derived neurons were transduced with miR-3, miR-15 or negative control AAVs at MOIs of $5x10^3$ to $5x10^5$. Cells were harvested 7 days post-transduction and analysed for **(A)** *SNCA* mRNA knockdown, expressed as % of the negative control AAV, and **(B)** miRNA expression (n=3-4 per MOI group). Data represented as mean ± SD.

In vivo proof of mechanism for the lead vMiX-*SNCA* miRNA

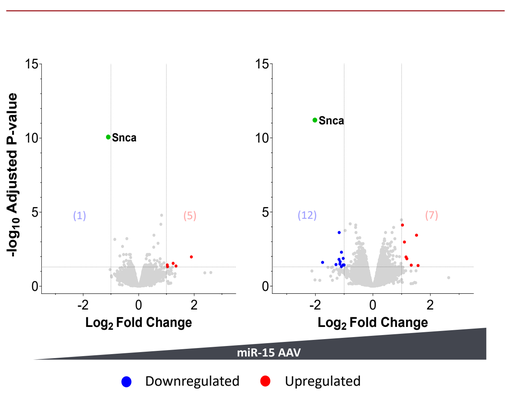
- The miR-15 AAV characterised *in vitro* was evaluated in a proof-of-mechanism study in mice at two doses.
- miR-15 demonstrated dose-dependent *Snca* mRNA knockdown in cortical and brainstem tissues, with corresponding vector biodistribution and miRNA expression (**Figure 7**).
- In the cortex, the transcriptional response to miR-15 AAV was largely restricted to *Snca* (**Figure 8**).

Figure 7: *In vivo* proof of mechanism for miR-15 AAV



Mice aged 10-12 weeks were administered miR-15 AAV at two doses or vehicle (veh.) intravenously by tail-vein injection (n= 8 per group; 3-4 of each sex). Tissues were collected 4 weeks post-injection and analysed for **(A)** *Snca* mRNA knockdown, expressed as % of veh.-treated animals, **(B)** vector genome (vg) biodistribution, and **(C)** miRNA expression, represented as miR-15 expression normalised to the expression of a housekeeping miRNA. Data represented as mean + SD.

Figure 8: Transcriptional response to miR-15 AAV in the cortex



Volcano plots of transcriptome-wide differential gene expression in the cortex of mice treated with miR-15 AAV relative to vehicle, determined by 3'-end polyA RNA-seq (Illumina; n= 3-4 per dose). Significance thresholds: Adjusted P ≤ 0.05 and |fold change| ≥ 2.

CONCLUSIONS

- The vMiX platform was successfully applied to silence *SNCA*, with miR-15 selected as the lead vMiX-*SNCA* miRNA.
- When vectorised into a BBB-crossing AAV capsid, miR-15 demonstrated dose-dependent *Snca* mRNA knockdown *in vivo* in CNS regions relevant to synucleinopathies, with a transcriptional response in the cortex largely restricted to *Snca.*
- These data support continued development of vMiX-*SNCA* as a single-dose *SNCA*-lowering gene therapy for Parkinson's disease and other synucleinopathies.

REFERENCES: [1]Calabresi P, et al. Cell Death Dis 2023;14:176. [2]Goedert M, et al. Nat Rev Neurol 2013;9:13–24. [3]Pitton Rissardo J, et al. Brain Sci 2025;15:1260. [4]Lim Y, et al. J Neurosci 2011;31:10076–10087. [5]Zharikov AD, et al. J Clin Invest 2015;125:2721–2735. [6]Cole TA, et al. JCI Insight 2021;6:e135633. [7]Elmer B, et al. Mol Ther 2026;34:1172–1186.

ABBREVIATIONS: **AAV:** Adeno-associated virus; **BBB:** Blood-brain barrier; **CNS:** Central nervous system; **dg:** Diploid genome; **iPSC:** induced pluripotent stem cell; **MOI:** Multiplicity of infection; **NHP:** Non-human primate

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