The vMiX™ platform: Tailored miRNA gene therapy targeting molecular drivers of ALS and FTD

A. Ule,[1] R. Elsayed,[1] A. Gennari,[1] D. Ojutalayo,[1] L. Oliver,[1] Z. Walker,[1] Y. Bekele,[2] A. Abhilash,[2] P. Hosseini,[2] S. Correia,[2] P.M. Coan,[1] O. Brock,[2] C.J. Miranda,[1] R. Joubert,[1] J. Isaac,[1] D.Y. Lee,[1] Y.B. Lee,[1] C.E. Shaw[1,2], C. Andreassi[1]

[1]AviadoBio Ltd, London, UK; [2]UK Dementia Research Institute, King's College London, London, UK

AVIADOBIO

vMiX™

P0089

OBJECTIVE

To report the development of vMiX™, an adeno-associated virus vectorized gene silencing platform. vMiX™ expresses proprietary microRNAs (miRNAs) capable of down-regulating target messenger RNA (mRNA) levels as a potential therapeutic strategy for the treatment of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD).

INTRODUCTION

- ALS and FTD are neurodegenerative diseases with distinctive clinical manifestations, but common underlying genetics and pathology, including the presence of abnormal protein aggregates in the brain.
- The ataxin-2 (ATXN2) protein facilitates aggregation of TAR DNA-binding protein (TDP)-43, an RNA-binding protein that dysregulates mRNA splicing in ALS and tau-negative FTD.[1]
- Reduction of *ATXN2* mRNA levels has shown protective effects against TDP-43 toxicity both *in vitro* and *in vivo*, and is a potential therapeutic strategy for ALS and FTD.[2,3]
- Gain-of-function mutations in the superoxide dismutase 1 (*SOD1*) gene can also cause ALS due to its ability to cause SOD1 protein aggregation. Reduction of *SOD1* expression by mRNA silencing is a treatment strategy for familial ALS caused by autosomal dominant inheritance of the SOD1 gene.[4–6]
- miRNA sequences, delivered via a gene silencing platform, can engage the RNA-induced silencing complex leading to degradation or translational repression of specific mRNA sequences, such as *ATXN2* and *SOD1*, ultimately reducing protein expression (see **Poster P0944**).
- Generation of accurately targeting miRNAs is a prerequisite for the use of silencing approaches in gene therapy.

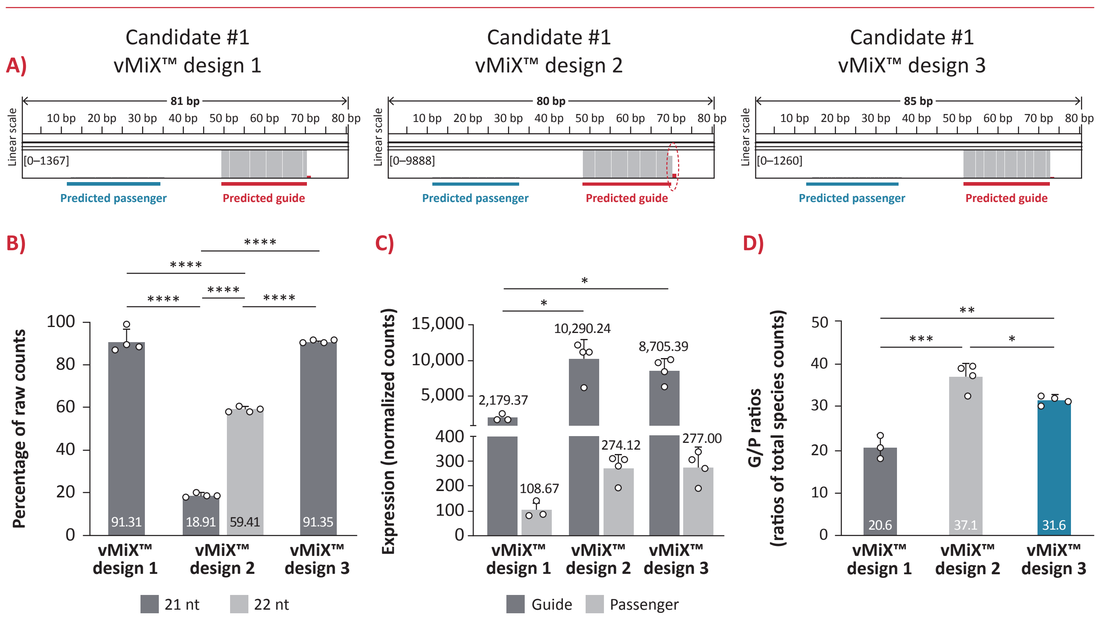
METHODS

- A battery of miRNA candidates targeting *ATXN2* or *SOD1* mRNAs was developed and the impact of sequence variations on the miRNA maturation process was assessed.
- Next Generation Sequencing technologies were employed to assess the effectiveness of several different sequences on:
  - the cleavage precision, guide sequence, and guide to passenger (G/P) ratio of candidate miRNAs;
  - the silencing efficiency of the candidate miRNAs;
  - the expression of endogenous miRNA repertoire.
- The species-specificity of the miRNA processing was assessed by conducting parallel experiments in human and mouse cell lines, and by comparing the results of *in vitro* and *in vivo* studies. For analysis of miRNA processing, aligned reads from BAM files were loaded in Integrative Genomics Viewer (IGV) for visualization. Counting was performed using command line by looking at all reads that aligned/mapped to the relevant sequence region (guide *vs* passenger).
- For analysis of differential miRNA expression, DESeq2 package in RStudio was employed to quantify expression and to test for statistical significance.

RESULTS

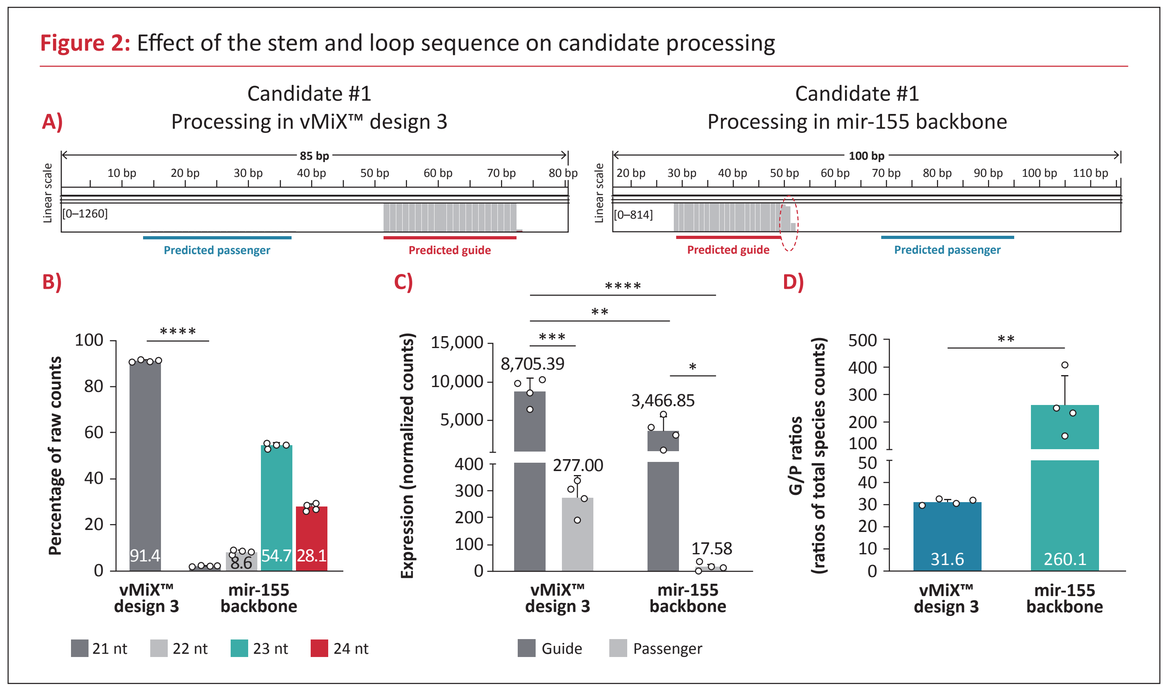
- Different stem sequences can affect either processing precision of the guide and/or G/P ratio (**Figure 1**).
- Despite a good G/P ratio, a number of guides of variable length were observed when the candidate was expressed using a mir-155 backbone that carries different stem and loop sequences compared to vMiX™ (**Figure 2**).
- Changes in the sequence of the miRNA duplex had a large effect on the precision of guide processing and the G/P ratio expressed by the candidates (**Figure 3**).
- Expression of guide species and G/P ratios were consistent in different cell types, animal species and different expression modalities (transfection *vs* transduction) (**Figure 4A–B**).
- Accurate processing conjugated with high expression levels resulted in a significant dose-response relationship between guide levels and target (human *ATXN2*) knockdown in a hBACQ72 transgenic mouse model (**Figure 4C–D**).
- Minimal alterations to the expression of endogenous miRNAs were observed in the spinal cord of B6SJL-SOD1(G93A) mice transduced with candidate #2 or #3 in optimized vMiX™ design 3 (**Figure 4E–G**).

Figure 1: Effect of the stem sequence on candidate processing



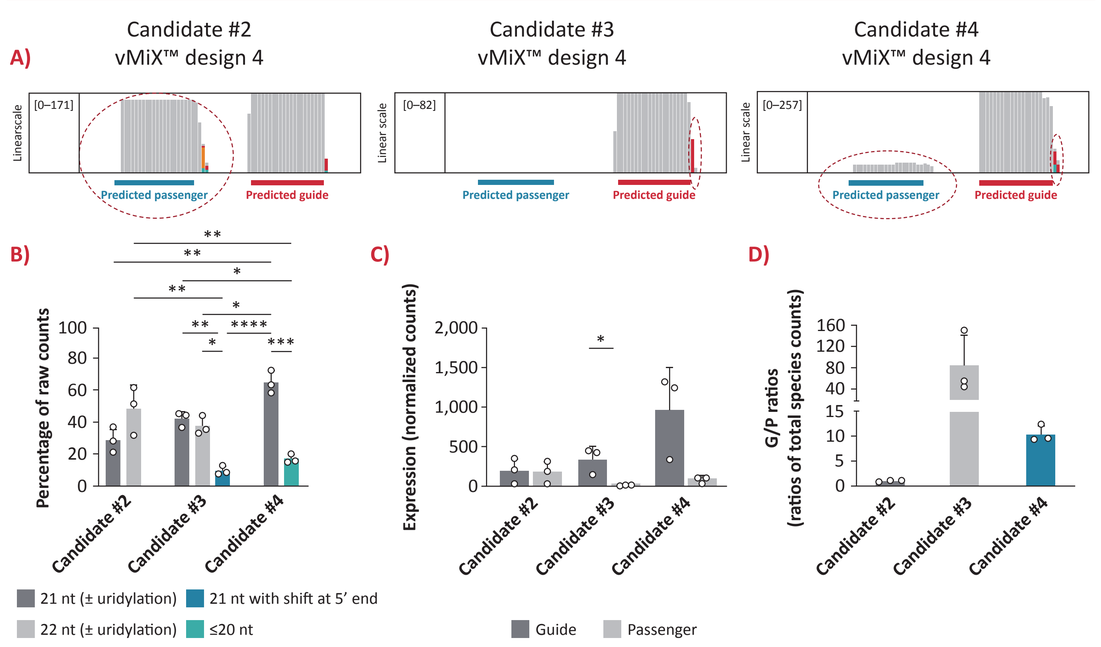
A) IGV alignments of reads obtained from the sequencing of WT mouse brain samples transduced with candidate #1 in three different vMiX™ designs (1, 2 and 3) by intrathalamic injection (1×10^9 vg/hemisphere). Position of the sequences corresponding to expected guide and passenger are indicated. **B, C)** Expression of guide species according to nucleotide (nt) length quantified as % of total raw counts **B)** or normalized reads counts **C)** in the same samples. **D)** G/P ratios of total species counts in the same samples. Only major species (>10%) are shown. Data are presented as mean ± SD. One-way ANOVA Tukey's post-hoc test. *p<0.05, **p<0.005, ***p<0.001, ****p<0.0001.

Figure 2: Effect of the stem and loop sequence on candidate processing



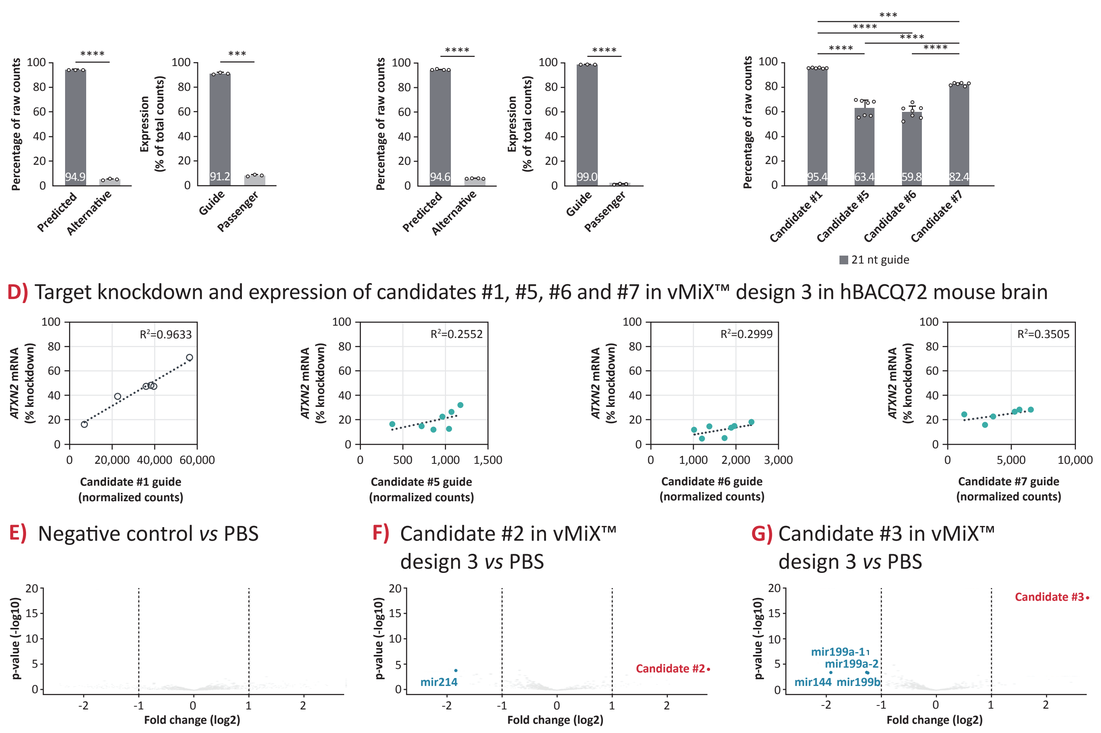
A) IGV alignments of reads obtained from the sequencing of WT mouse brain samples transduced with candidate #1 flanked by alternative loop and stem sequences (design 3 *vs* mir-155 backbone) by intrathalamic injection (1×10^9 vg/hemisphere). Position of the sequences corresponding to the predicted guide and passenger are indicated. **B)** Expression of guide species according to nt length quantified as % of total raw counts. Only major species (>2%) are shown. Data are presented as mean ± SD. Unpaired t-test ****p<0.0001. **C)** Normalized expression of total guide and passenger species. Data are presented as mean ± SD. One-way ANOVA Tukey's post-hoc *p<0.05, **p<0.005, ***p=0.0001, ****p<0.0001. **D)** G/P ratios of total species counts. Data are presented as mean ± SD. Unpaired t-test,**p<0.01.

Figure 3: Effect of the duplex sequence on the candidate processing



A) IGV alignments of reads obtained from the sequencing of WT mouse brain samples transduced with candidates #2–4 flanked by identical loop and stem sequences by intrathalamic injection (2.5×10^9 vg/hemisphere). Position of the sequences corresponding to expected guide and passenger are indicated. **B–C)** Expression of guide species according to nt length quantified as % of total raw counts **B)** and normalized expression **C)** of total guide and passenger species. Only major species (>2%) are shown. Data are presented as mean ± SD. **B)** One-way ANOVA Tukey's post-hoc *p<0.05, **p<0.005, ***p<0.001, ****p<0.0001. **C)** Unpaired t-test *p<0.05. **D)** G/P ratios of total species counts in the same samples. Data are presented as mean ± SD.

Figure 4: Expression and processing of vMiX™ *in vitro* and *in vivo*, and the effects on the endogenous miRNAs



A–B) Expression of guide species according to nt length (left) and G/P ratios (right) of total guide and passenger species quantified as % or ratios of total raw counts in HEK293 cells samples transfected **A)** or mouse brain transduced **B)** by intrathalamic injection (1×10^9 vg/hemisphere) with candidate #1 in vMiX™ design 3. Data are presented as mean ± SD. Paired t-test, ***p=0.0001, ****p<0.0001. **C–D)**: Expression of 21nt guide species **C)** and correlation between amount of guide expressed and target knockdown **D)** in the mouse brain of hBACQ72 transgenic mice transduced with either candidate #1, #5, #6, or #7 in vMiX™ design 3 by intrathalamic injection (1×10^9 vg/hemisphere). **C)** Data are presented as mean ± SD. One-way ANOVA, ***p<0.001, ****p<0.0001. **D)** Linear regression analysis of candidate guide expression (normalized counts) against % of human *ATXN2* mRNA knockdown observed in individual samples (candidate #1: R2 > 0.95, p< 0.005; candidates #5–7: NS). *ATXN2* mRNA levels were quantified by RT-dPCR and normalized by mouse Gapdh expression. **E–G)**: Volcano plots of fold change and statistical significance for miRNAs differentially expressed in the B6SJLSOD1G93A mouse spinal cord following subpial injection (5×10^9 vg) of vectors expressing either a negative control, or candidate #2 or #3. Comparisons between treatments and the control PBS condition are shown. Cut-off values of log2-fold change between −1 and +1 (dotted lines) and p<0.05 were applied. Grey indicates NS genes, blue indicates statistically significant down-regulated genes, red indicates statistically significant up-regulated genes.

CONCLUSIONS

These analyses, along with rational candidate design, have endowed the vMiX™ platform with nucleotide-precise targeting abilities, developing a versatile, robust and ready-to-use miRNA-based gene therapy approach for potential application in ALS and FTD (see Posters P0167 and P0280 for more information).

REFERENCES: [1]Elden AC. Nature 2010;466:1069–75; [2]Becker LA. Nature 2017;544:367–71; [3]Alejandra Zeballos MC. Nat Commun 2023;14:6492; [4]Kim G. Neuron 2020;108:822–42; [5]Van Zundert B. Neurosci Lett 2017;636:32–9; [6]Bravo-Hernandez M. Nat Med 2020;26:118–30.

ABBREVIATIONS: **ALS:** amyotrophic lateral sclerosis; **ANOVA:** analysis of variance; ***ATXN2*:** Ataxin-2; **bp:** base pairs; **FTD:** frontotemporal dementia; **IGV:** Integrative Genomics Viewer; **mRNA:** messenger RNA; **miRNA:** microRNA; **PBS:** phosphate-buffered saline; **nt:** nucleotide; **NS:** non-significant; **SD:** standard deviation; ***SOD1*:** superoxide dismutase 1; **WT:** wild type

ACKNOWLEDGEMENTS & DISCLOSURES: This study was funded by AviadoBio Ltd. **CA, RE, AU, AG, DO, LO, ZW, YB, AA, PH, SC, PC, CM, RJ, JI,** and **CS** are employees and shareholders of AviadoBio Ltd. **OB** is an employee of King's College London. The authors meet criteria for authorship as recommended by the International Committee of Medical Journal Editors (ICMJE). Medical writing and editorial support was provided by David Morgan, PhD, and Oliver Palmer, BSc (Hons), of Costello Medical, UK, and funded by AviadoBio Ltd. **RJ** is named in patents related to vMiX™ platforms, *ATXN2* and *SOD1* miRNA therapy for ALS. **CA** has received grants/contracts from University College London and Wellcome Trust.