Effective knockdown of *ATXN2* following intracerebroventricular administration of AVB-205 construct in BAC-*ATXN2*-Q72 transgenic mice

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

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

AVIADOBIO

vMiX™ TST-01

OBJECTIVE

To develop a vectorized adeno-associated virus (AAV) gene silencing approach to modulate ataxin-2 (*ATXN2)* expression as a potential therapeutic approach for some neurodegenerative diseases.

INTRODUCTION

- Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by the degeneration of motor neurons in the brain and spinal cord, resulting in gradual loss of voluntary muscle control, paralysis, and fatal respiratory failure. Although familial forms of ALS exist, the multifactorial nature of sporadic ALS remains elusive.[1]
- Recent animal studies have shown that *Atxn2* deficiency disrupts the ataxin-2-TDP-43 interaction and ameliorates TDP-43 proteinopathy, a causative factor in ~97% of ALS and tau-negative frontotemporal dementia (FTD) cases.[2]
- Thus, *ATXN2* silencing offers a promising therapeutic strategy for neurodegenerative diseases caused by aberrant *ATXN2* expression and TDP-43 proteinopathies.

METHODS

In vitro guide selection

Assessment of *ATXN2* knockdown by guides following *in vitro* transfection

- Fifty-one guides with optimal guanine and cytosine content and predicted binding were cloned into plasmids and transfected into HEK293 cells.
- After 48h, *ATXN2* mRNA knockdown was assessed by luciferase activity. The knockdown detected by the 14 best guides was further assessed by quantitative polymerase chain reaction (qPCR).

Assessment of *ATXN2* knockdown by guides following *in vitro* transduction

- These 14 guides were cloned into the vMiX™ platform, inserted into AAV9 vectors, and transduced into HEK293 cells.
- *ATXN2* mRNA knockdown was assessed by qPCR.

In vivo lead candidate assessment

- The vector with the greatest *in vitro ATXN2* mRNA knockdown efficacy (AVB-205) was selected for *in vivo* evaluation in BAC-*ATXN2*-Q72 transgenic mice.
- AVB-205 (4 doses), vMiX.CT (Control vector; CONT.), or vehicle (PBS) were administered by intracerebroventricular (ICV) injection into neonatal heterozygous BAC-*ATXN2*-Q72 mice and their wild-type littermates.
- After 8 weeks, all mice were euthanized and *ATXN2*/*Atxn2* mRNA knockdown, miRNA guide expression, and vector genomes per cell (vg/cell) were evaluated in cortex and spinal cord by digital PCR (dPCR) and qPCR, respectively.

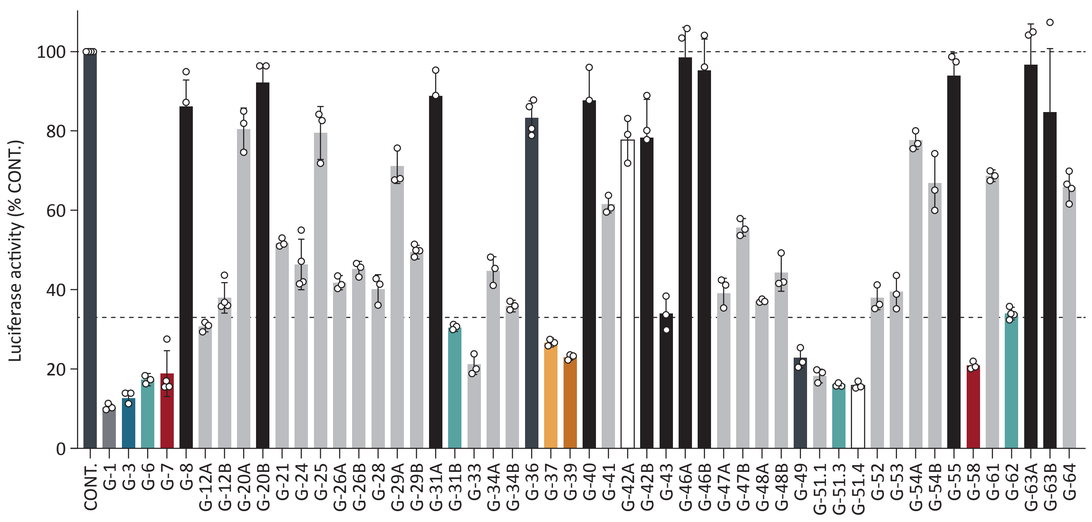
RESULTS

In vitro results

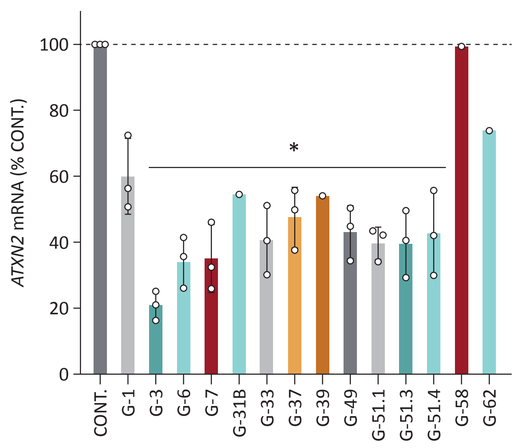
- Luciferase activity was quenched up to 79% in 14 out of 51 guide candidates tested (**Figure 1A**).
- Transfection (**Figure 1B**) and transduction (**Figure 1C**) experiments showed multiple effective guides; one guide G-3 (AVB-205) was selected to test further *in vivo*.

Figure 1: *In vitro* guide selection

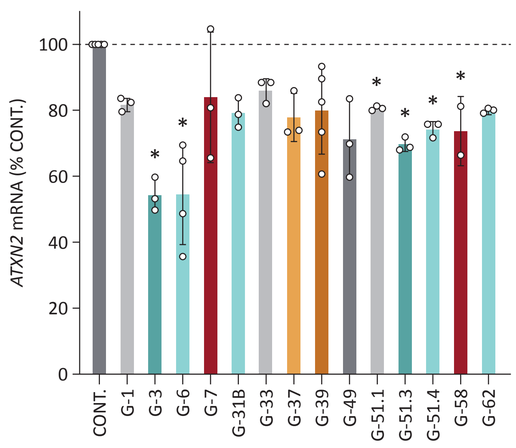
A) Luciferase Assay: HEK293 cell transfection



B) Plasmid transfection of HEK293 cells



C) Vector transduction of HEK293 cells



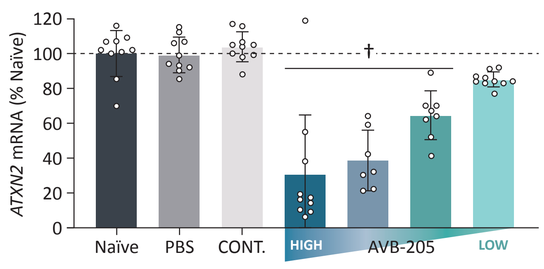
Mean ± SD. Statistical significance assessed by mixed effects analysis *p<0.05 (vs CONT.).

In vivo results

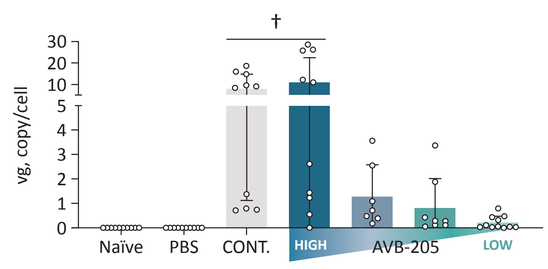
- *ATXN2* mRNA was knocked down by AVB-205 by up to 70% in a dose-dependent manner in the cortex of BAC-*ATXN2*-Q72 mice (**Figure 2A**).
- Between 1–10 vg/cell of AVB-205 in cortex yielded a significant *ATXN2* mRNA knockdown (on average: 29–70%; **Figure 2A–B**).
- *ATXN2* mRNA knockdown by AVB-205 was 25–30% in spinal cord yielded from 0.25 vg/cell (**Figure 2C–D**).

Figure 2: *ATXN2* mRNA knockdown by AVB-205 in BAC-*ATXN2*-Q72 mice

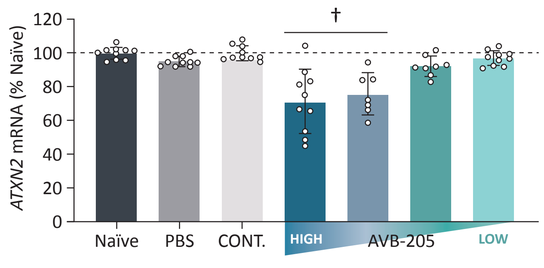
A) Cortex *ATXN2* mRNA



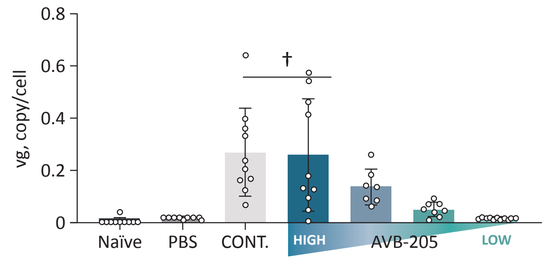
B) Cortex vector genomes



C) Spinal cord *ATXN2* mRNA



D) Spinal cord vector genomes

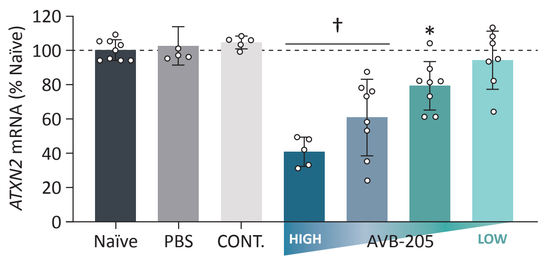


Mean ± SD. Statistical significance assessed by 1-way ANOVA followed by Dunnett's multiple comparisons test; †p<0.0001 (vs Naïve). Doses: 3-fold dilution between doses.

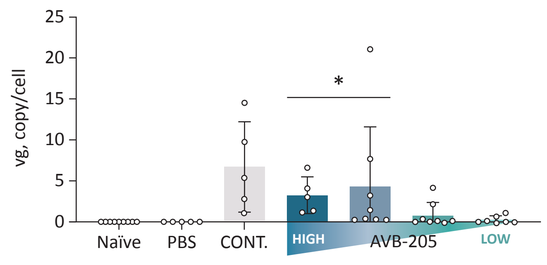
- AVB-205 achieved up to 59% *Atxn2* mRNA knockdown in a dose-dependent manner in the cortex of wild-type littermates (**Figure 3A**).
- On average, 5–7 vg/cell yielded a knockdown of 20–60% (**Figure 3B**).
- *Atxn2* mRNA knockdown by AVB-205 was 22%, yielded from 0.35 vg/cell in spinal cord (**Figure 3C–D**).

Figure 3: *Atxn2* mRNA knockdown by AVB-205 in wild-type littermate mice

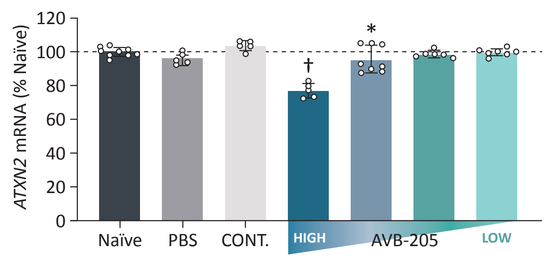
A) Cortex *Atxn2* mRNA



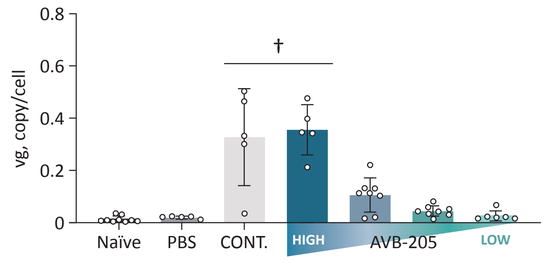
B) Cortex vector genomes



C) Spinal cord *Atxn2* mRNA



D) Spinal cord vector genomes

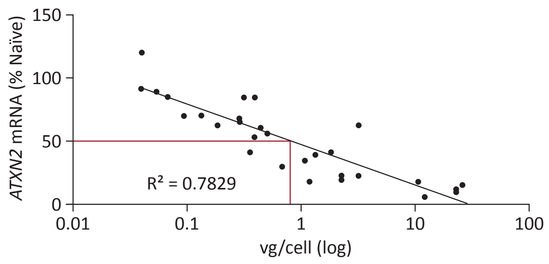


Mean ± SD. Statistical significance assessed by 1-way ANOVA followed by Dunnett's multiple comparisons test, *p<0.05, †p<0.0001 (vs Naïve). Doses: 3-fold dilution between doses.

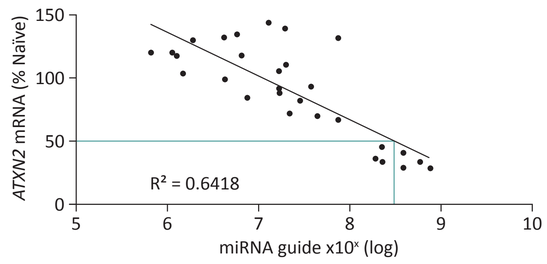
- A strong negative correlation was found between *ATXN2* mRNA expression in cortex and vg/cell (p<0.005; **Figure 4A**) or miRNA guide expression (p<0.0001; **Figure 4B**).
- *ATXN2* mRNA knockdown of 50% was achieved by transduction in the cortex of 0.8 vg/cell and expression of $2.5x10^8$ miRNA guides (per µg RNA; **Figure 4A–B**).

Figure 4: Relationship between vg/cell, guide, and *ATXN2* mRNA

A) vg/cell vs *ATXN2* knockdown



B) miRNA vs *ATXN2* knockdown



CONCLUSIONS

- **Taken together, these data provide support for further testing of AVB-205 in neurodegenerative disease models to evaluate phenotype modification and product development for ALS and some forms of FTD.**

REFERENCES: [1]Masrori & Van Damme, 2020; [2]Becker et al 2017.

ABBREVIATIONS: **AAV:** adeno-associated virus; **ALS:** amyotrophic lateral sclerosis; **ANOVA:** analysis of variance; ***ATXN2*:** Ataxin-2; **BAC-*ATXN2*-Q72 mice:** transgenic mice expressing human *ATXN2*; **CONT.:** control vector; **dPCR:** digital polymerase chain reaction; **FTD:** frontotemporal dementia; **G:** miRNA guide candidate; **ICV:** intracerebroventricular; **mRNA:** messenger RNA; **miRNA:** microRNA; **PBS:** phosphate-buffered saline; **qPCR:** quantitative polymerase chain reaction; **SD:** standard deviation; **TDP-43:** TAR DNA-binding protein 43; **vg:** vector genome; **vMiX™:** miRNA silencing platform

ACKNOWLEDGMENTS & DISCLOSURES: This study was funded by AviadoBio Ltd. **PMC, RJ, ZW, ED, NS, LR, CA, AA, LI, CJM, JI,** and **CES** are employees and shareholders of AviadoBio Ltd. **OB** and **NAMN** are employees of King's College London. **RJ**, **CS**, **DYL** and **YBL** are named in patents related to the vMiX™ platform.

Presented at the 35th International Symposium on ALS/MND | 6–8 December 2024 | Montreal, Canada