A Translational Assay for Ataxin-2 Related Drug Development Products

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BIO-16

OBJECTIVE

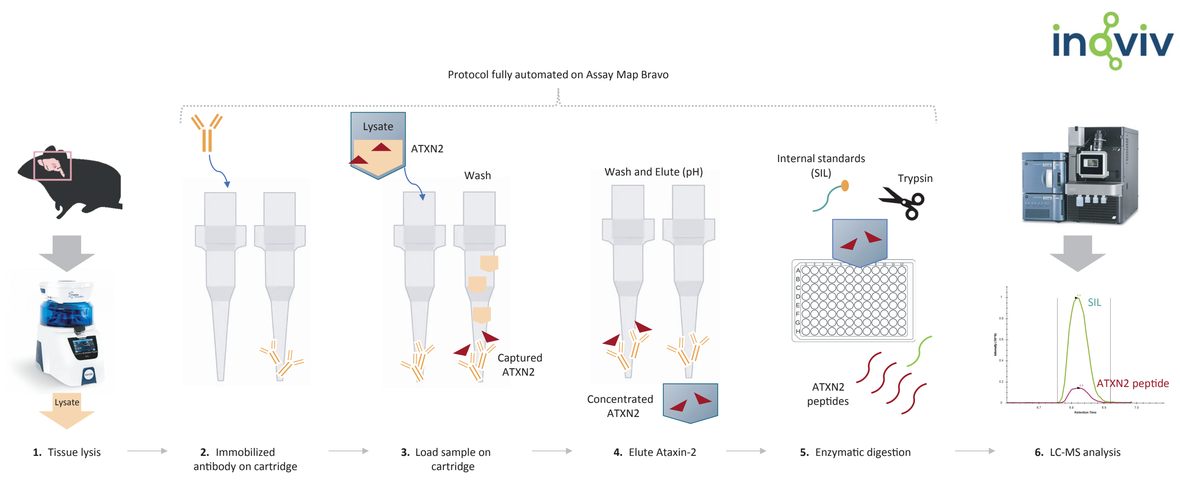
To develop a translational assay to discriminate and confidently measure human, mouse, pig, and non-human primate Ataxin-2 protein.

BACKGROUND

- Ataxin-2 (ATXN2) is a stress granule-associated protein related to TAR DNA-binding protein 43 (TDP-43) proteinopathies. Its mis-localization and intermediate poly-Q repeat expansions are associated with spinocerebellar ataxia type 2 (SCA2) and amyotrophic lateral sclerosis (ALS).[1] Knockout (KO) of ATXN2 homologs reduce TDP-43 toxicity in yeast, flies and transgenic (TG) mice.[2,3]
- Anti-sense oligonucleotides targeting ATXN2 given by intracerebroventricular (ICV) injection to TDP-43 TG mice reduce TDP-43 aggregation, improve motor function and increase survival after a single treatment at birth,[3] thus suggesting ATXN2 as a therapeutic target for ALS.
- ATXN2 is widely conserved across species. Commercially-available antibodies are not species-specific, therefore an assay able to discriminate and confidently measure mouse versus (*vs.*) human, pig, and non-human primate (NHP) proteins is crucial in pre-clinical studies.

METHODS

Figure 1: Immunoprecipitation mass spectrometry (IP-MS) workflow



- A mass-spectrometry (MS)-based method was designed to quantify the relative expression of human *vs.* mouse ATXN2 protein in a TG ATXN2 (hBACQ72[4]) mouse model. A cocktail of commercially available antibodies was used to immuno-precipitate (IP) mouse and human ATXN2 protein from tissue lysates from wild type (WT) and hBACQ72 (TG) mice (**Figure 1**).
- The identification and quantification of mouse *vs.* human ATXN2 protein was obtained by selecting specific peptides to target mouse, human/pig/NHP or pan-species regions of the full-length protein (referred to as Mouse, Human and Total respectively in **Figure 2** and **6**).

RESULTS

Figure 2: Examples of peptides selected using tissues from WT and TG mice

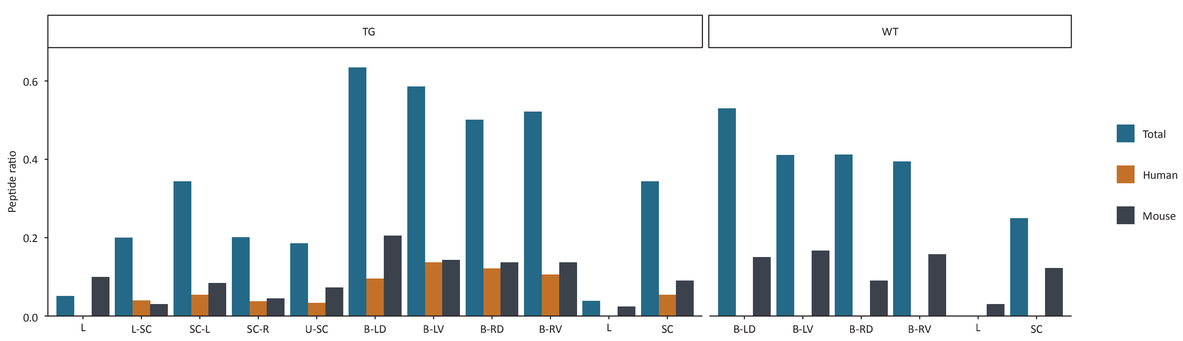
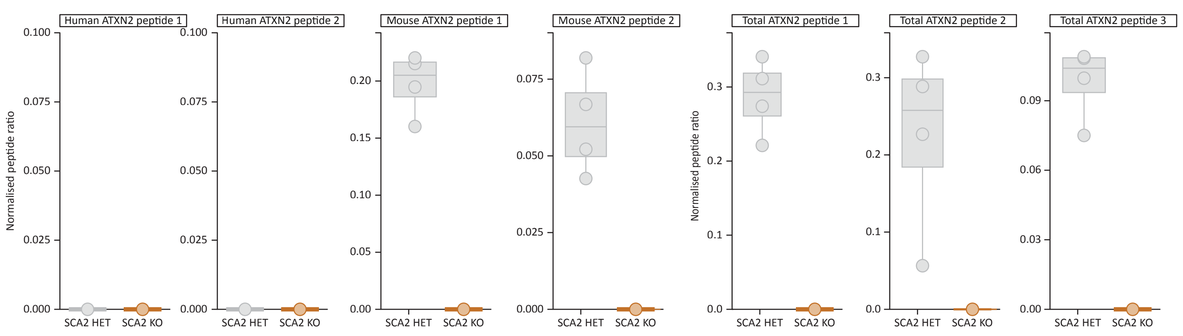


Figure 2 shows the peptide ratio for the three groups of peptides in tissues excised from TG and WT mice: liver (L); upper and lower spinal cord (U-SC and L-SC); different area of the brain (B) including sub-cortex left and right (SC-L and SC-R).

Figure 3: Peptides selected are confirmed to be highly species-specific



- Specificity of the selected peptides was tested in brain tissue from SCA2 KO (murine ATXN2 KO line) and SCA2 genetically heterozygous (HET) mice (**Figure 3**).
- The IP-MS assay developed was confirmed to be species-specific.
- hBACQ72 mice were treated with three different doses of AviadoBio's AVB-205, an AAV9 vector expressing an miRNA targeting Ataxin-2. vMiX Neg was used as a negative control matching the highest dose of AVB-205 (see poster **TST-01**).
- Right brains were used for protein analysis; left brains were used for vector genome (vg)/cell determination by quantitative polymerase chain reaction (qPCR) and mRNA analysis by digital PCR of mouse and human Ataxin-2.
- A naïve group was used to account for any effect of the ICV procedure on ATXN2 protein expression (**Figure 4**).

Figure 4: No change in protein level after ICV injection

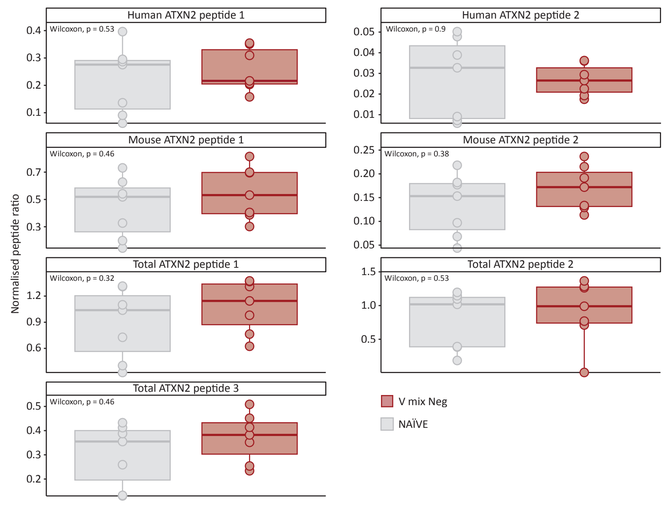
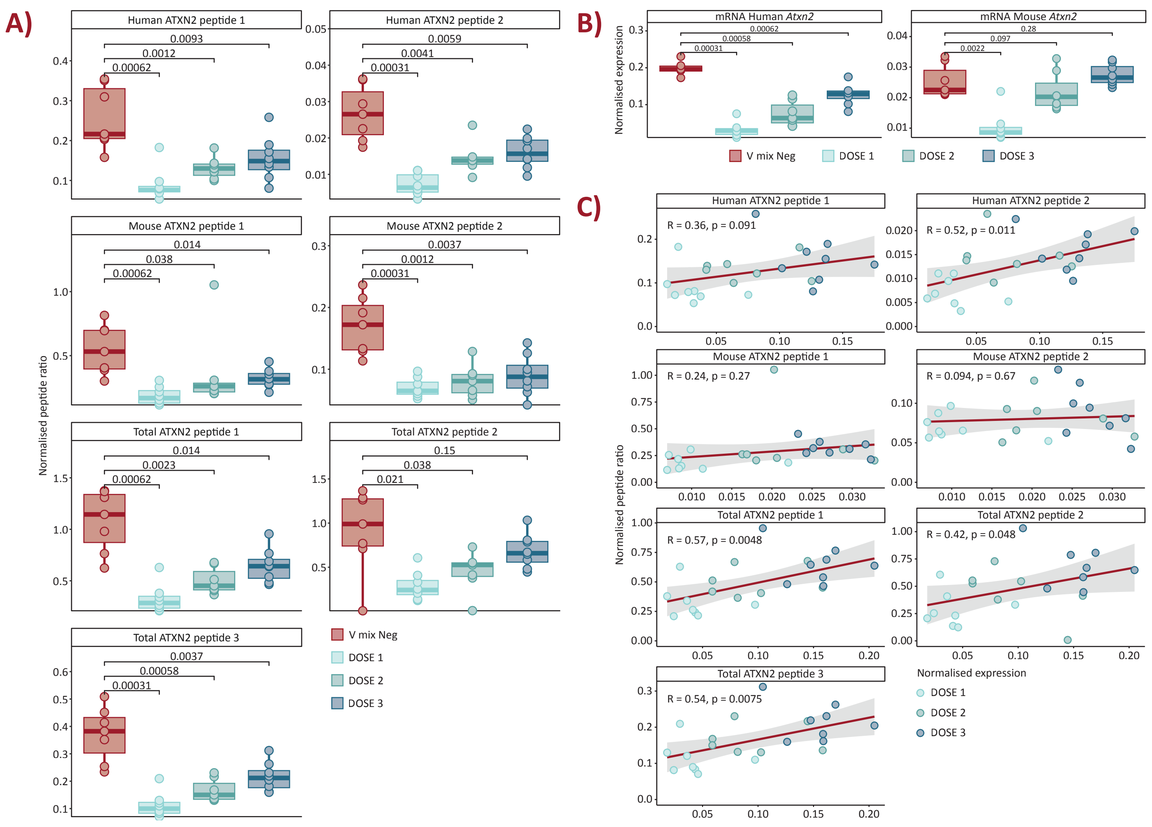
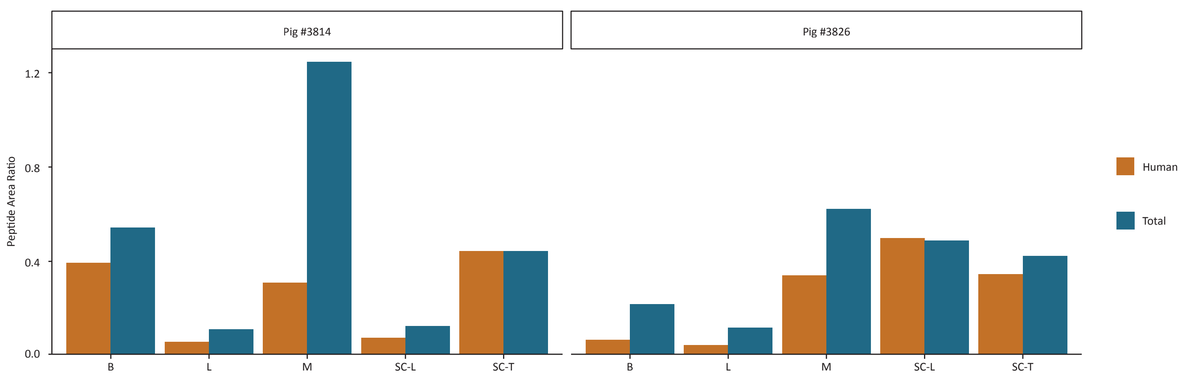


Figure 5: Ataxin-2 down-regulation at the protein and mRNA level



- IP-MS method has the sensitivity to demonstrate a dose-response correlation in terms of almost complete knockdown (KD) of both human and mouse Ataxin-2 at the highest dose tested, and down-regulation of expression at the lower doses (**Figure 5A**; p-values highlighted above the bars).
- KD of both human and mouse Ataxin-2 mRNA follow the same dose-response pattern (**Figure 5B**); statistical significance achieved with all peptides but one tested (**Figure 5C**).
- KD of Ataxin-2 correlates with vg/cell in mouse brain tissue (see poster **TST-01**).

Figure 6: Examples of peptides selected using tissues from Yucatan mini-pigs



- To prove the translatability of this IP-MS assay for pre-clinical studies in drug development, the same antibody cocktail used to precipitate mouse tissue was then tested in tissues from two Yucatan mini-pigs. Two Human peptides and two Total peptides were then evaluated in muscle (M), liver (L), spinal cord lumbar (SC-L), spinal cord thoracic (SC-T) and brain (B) slices; results are shown in **Figure 6**.

CONCLUSIONS

- **We have successfully developed an assay able to discriminate and measure mouse *vs.* human Ataxin-2 using an IP-MS method.**
- **This method is sensitive, reproducible and has been orthogonally tested using dPCR, demonstrating that changes at protein level in the expression of species-specific Ataxin-2 can be measured and correlate with gene expression changes.**
- **Since conserved peptides have been identified in pig and NHP, this method should be readily transferable to species suitable for good laboratory practice (GLP) toxicology studies.**

REFERENCES: [1]van den Heuvel DMA, *et al*. Trends Mol Med 2014;20:25–35; [2]Elden AC, *et al*. Nature 2010;466:1069–75; [3]Becker LA, *et al*. Nature 2017;544:367–71; [4]Dansithong W, *et al*. PLoS Genet 2015;11:e1005182.

ABBREVIATIONS: **AAV9:** adeno-associated virus serotype 9; **ALS:** amyotrophic lateral sclerosis; **ATXN2:** Ataxin-2; **dPCR:** digital polymerase chain reaction; **GLP:** good laboratory practice; **HET:** heterozygous; **ICV:** intracerebroventricular; **IP-MS:** immuno-precipitation combined with mass spectrometry; **KD:** knockdown; **KO:** knockout; **LC-MS:** liquid chromatography mass-spectrometry; **miRNA:** micro RNA; **mRNA:** messenger RNA; **NHP:** non-human primate; **SCA2:** spinocerebellar ataxia type 2; **TDP-43:** TAR DNA-binding protein 43; **TG:** transgenic; **vg:** vector genome; **WT:** wild-type

ACKNOWLEDGEMENTS & DISCLOSURES: The authors are extremely grateful to Prof. SM Pulst for the donation of the brain tissue from the SCA2 line. This study was funded by AviadoBio Ltd. 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 Calum Suggett, MSc, of Costello Medical, UK. **ZW, JF, NS, ED, CS, CM, JI** and **AE** are employees and shareholders of AviadoBio Ltd. **BR, ROC, JB** and **IZ** are employees of Inoviv. **BCM, NMN** and **OB** are an employees of King's College London (SRA contract sponsored by AviadoBio).