VecTabs®: Targeting misfolded and aggregated proteins with vectorized antibodies for the treatment of ALS and HD

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Introduction

Protein misfolding is a pathogenic feature of multiple neurodegenerative diseases, including Amyotrophic Lateral Sclerosis (ALS) and Huntington's Disease (HD). As the pathogenic mechanisms usually include both a loss of function and a gain of toxicity by misfolded and aggregated proteins, strategies are required to specifically target the toxic protein species whilst preserving the function of the native monomer.

97% of **ALS** patients show accumulation of misfolded TAR DNA-binding protein 43 (TDP43), which interferes with the translation of mitochondrial proteins at the neuromuscular junction (NMJ) resulting in ALS pathology. In **HD**, the CAG repeat expansion in Huntingtin (HTT) gene results in the translational of a mutant protein with an expansion of glutamines (polyQ). The mutant HTT protein aggregates in neuronal cells to form soluble oligomers and insoluble inclusions which induce toxicity and ultimately cell death.

Within VectorY, AAV-delivered intrabodies scFv's (VecTabs®) are developed that specifically recognize misfolded proteins (figure 1). We identified candidates that effectively interfere with aggregate formation, allowing selection of a lead candidate for further development of a gene therapy for the treatment of ALS and HD. Moreover, the biodistribution and expression of AAV-delivered VecTabs was demonstrated in the CNS of mice and pigs without inducing any severe adverse events.

VecTabs® mechanism of action

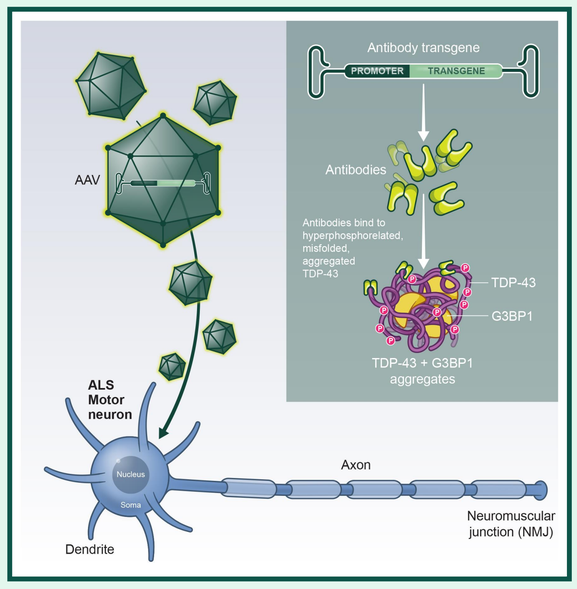

Figure 1: The VecTabs® technology combines state-of-the-art antibody engineering and AAV delivery, providing a strategy for efficient production of scFv as a long-term therapeutic for ALS and HD patients. VecTabs® specifically bind to pathological aggregates resulting in a specific and efficient reduction of toxic aggregates.

Preclinical development strategy

VectorY's preclinical development strategy

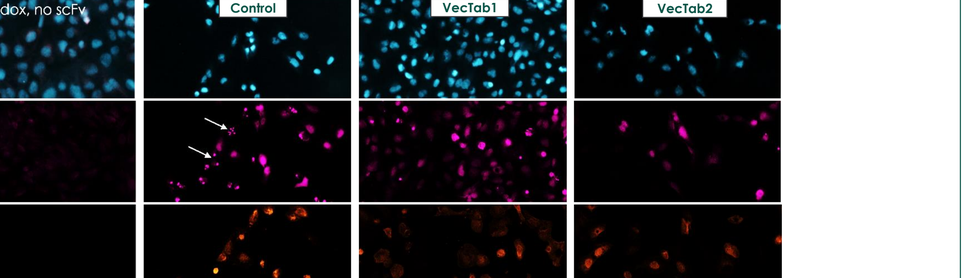

Figure 2: VecTabs® are designed and screening in a high throughput manner. The best performing candidates are tested in advanced, patient-specific cellular models for therapeutic efficacy. State of the art multi-omics analyses are conducted to evaluate therapeutic efficacy and off target effects. In vivo biodistribution and toxicity studies are performed in big animals, who are better resemble human physiology.

Results

VecTabs® reduce pathological aggregates in ALS models

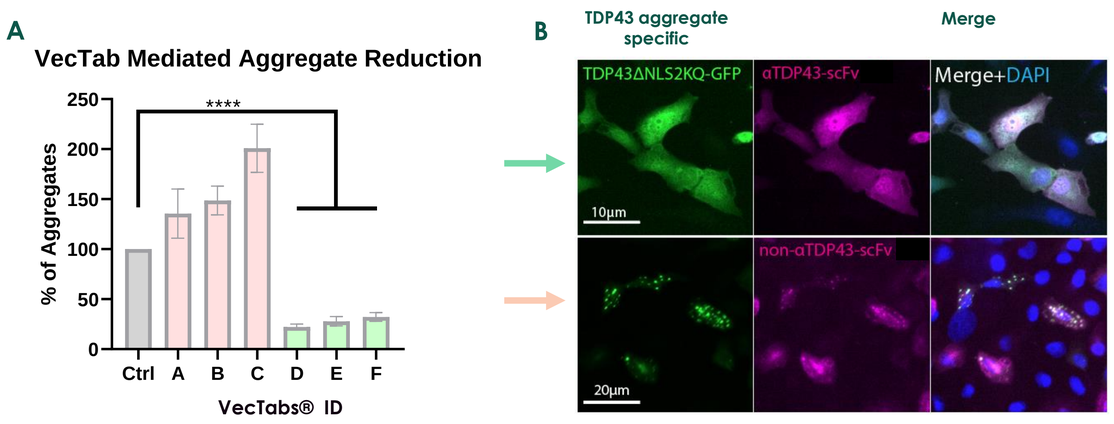

Figure 3: (A) Protein aggregate analysis using high-throughput quantitative aggregate imaging. Some VecTabs® (green bars) greatly reduce aggregate formation, whereas other VecTabs® (pink bars) have no effect or increase aggregate formation. (B) Images of U2OS cells transfected with aggregate-prone TDP43 (ΔNLS2KQ-GFP) and anti-TDP-43 VecTabs®. Images were taken and analyzed with the ImageXpress® Pico Automated Cell Imaging.

VecTabs® target HTT pathological inclusions in HD models

Figure 4: Images of U2OS cells expressing eGFP-Htt-Q97 HD upon 48 hr doxycycline induction and transfected with intrabody candidates. Images were taken and analyzed with the ImageXpress® Pico Automated Cell Imaging. Note: Errors indicate HTT aggregates that are reduced upon VecTab treatment.

AAV-delivered VecTabs® are expressed in iPSC-neuronal cells

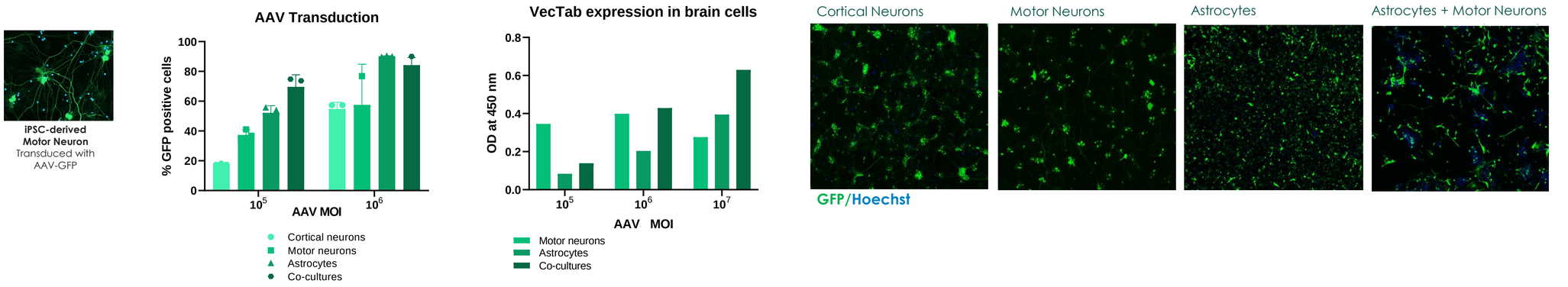

Figure 5: (A) Dose-dependent AAV transduction levels quantified by the percentage of GFP positive cells. (B) VecTabs® expression analysis by ELISA in disease affected cell types, showing a dose-dependent VecTabs® expression upon transduction. (C) Images of GFP expressing iPSC-derived brain cell types after AAV transduction. Images were taken and analyzed with the ImageXpress® Pico Automated Cell Imaging System.

Intrathecal injection of VecTabs® results in dose-dependent expression in spinal cord of rodents and pigs

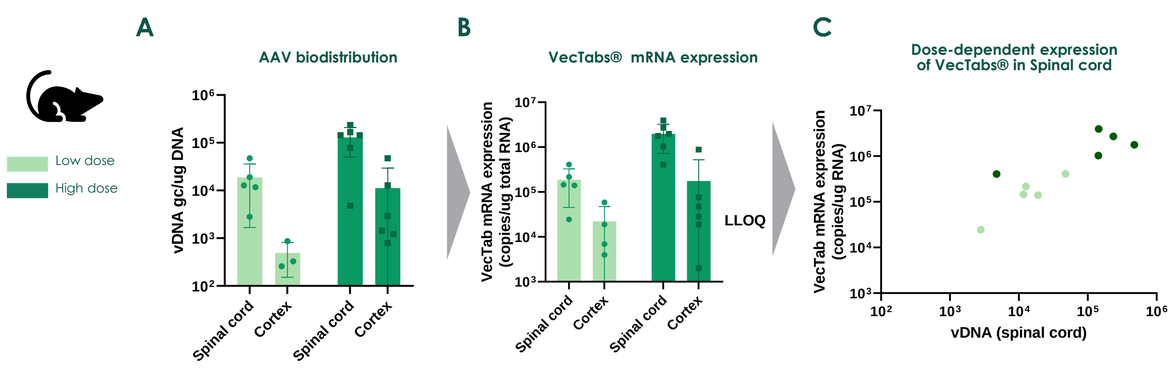

Figure 6: VecTabs® biodistribution in mice at 5 weeks after intrathecal administration of low dose and high dose AAV-VecTabs®. (A) Dose-dependent AAV vDNA levels in spinal cord and cortex quantified by qPCR. (B) Dose-dependent expression levels of VecTabs® mRNA in spinal cord and cortex quantified by RT-qPCR. (C) Dose-dependent correlation between AAV vDNA and VecTabs® mRNA expression in spinal cord of mice.

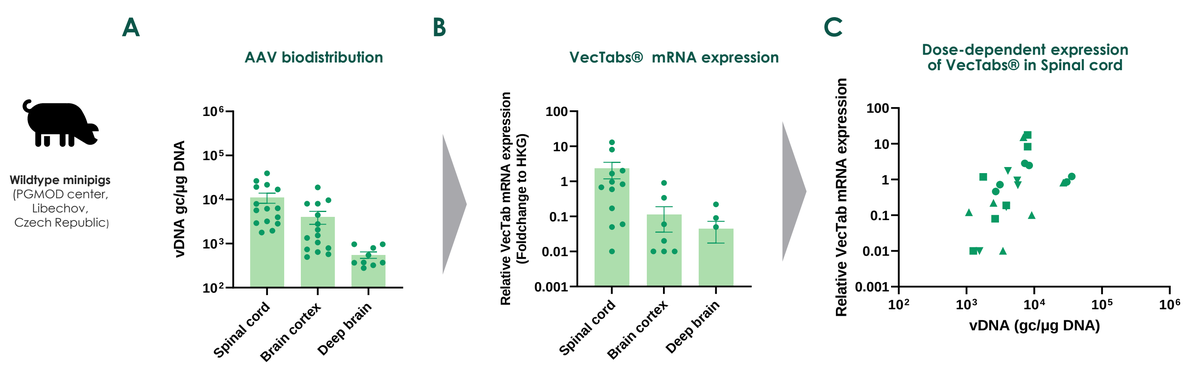

Figure 7: VecTabs® biodistribution in pigs at 8 weeks after intrathecal administration of low dose AAV-VecTabs® . (A) Levels of AAV vDNA in spinal cord, brain cortex and deep brain areas quantified by qPCR. (B) VecTabs® relative mRNA expression in spinal cord, cortex and deep brain areas quantified by duplex RT-qPCR. (C) Dose-dependent correlation between AAV vDNA levels and VecTabs® mRNA expression in spinal cord of pigs.

Conclusions

- **VecTab®** candidates have been identified that specifically reduce pathological **TDP43** and **HTT** aggregates in U2OS cells.
- Successful (dose-dependent) AAV transduction and VecTabs® expression were demonstrated in iPSC-derived models.
- Biodistribution of **VecTabs®** was shown in mice and pigs revealing a high transduction in the CNS, which highly correlated with VecTab® expression.

ACKNOWLEDGMENTS
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