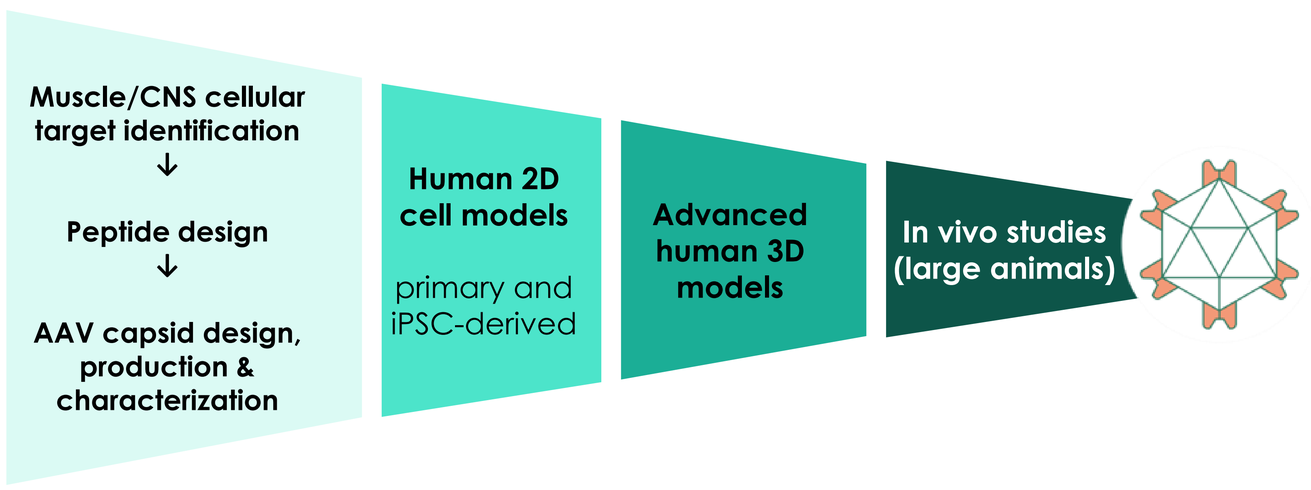
Next generation rAAV capsids show enhanced transduction efficiency in 2D and 3D human cellular models for neurodegenerative diseases

C Gallego[1, #], D Boogers[1], W Pos[1], M Sogorb[1], S Pasteuning[1], R van Dijk[1], F Sharifi [1], S Fick [1], M Kessler[1], A G Duarte[1], J Snapper[1], S Juhas[2], Z Ellederova[2], J Motlik[2], S van Deventer[1], P S Konstantinova[1], B Sanders[1]

[1] VectorY BV, Science Park 408, Matrix Innovation Center VI, 1098 XH Amsterdam, The Netherlands [2]Institute of Animal Physiology and Genetics, Libechov, Czech Republic [#] carmen.gallego@vectorytx.com

Introduction

AAVs are widely used for *in vivo* delivery of gene therapies but there is a need to improve their tissue tropism while avoiding off-target transduction of other tissues. To address this challenge, we have **engineered AAV capsids** presenting tissue-specific homing peptides, to target muscle and central nervous system (CNS). We have built a modular platform and selection pipeline that allows the identification and design of peptides through a rational design approach. The combination of capsid screening in relevant **human 2D and 3D in vitro models** together with biodistribution **in vivo studies in large animals** will help faster translation of these vectors to the clinic.

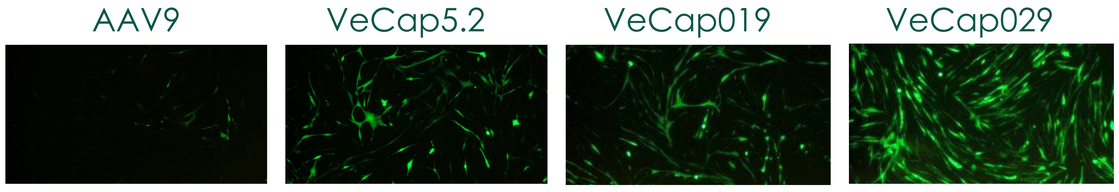


VectorY capsid pre-clinical pipeline for muscle and CNS capsids. The selection funnel goes from peptide design based on selected cellular targets to AAV production and characterization. Capsid screening is performed in human 2D and 3D models as well as large animals for in vivo delivery.

Results

2D models

AAV capsids show improved muscle tropism in 2D primary myotubes and 3D muscle micro-tissues

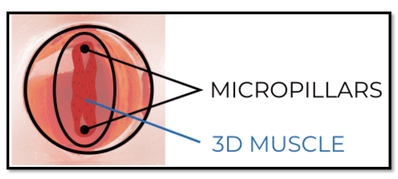


Capsid	VeCap5.2	VeCap019	VeCap029
GFP mRNA expression (vs AAV9)	8.51 x	7.75 x	49.4 x

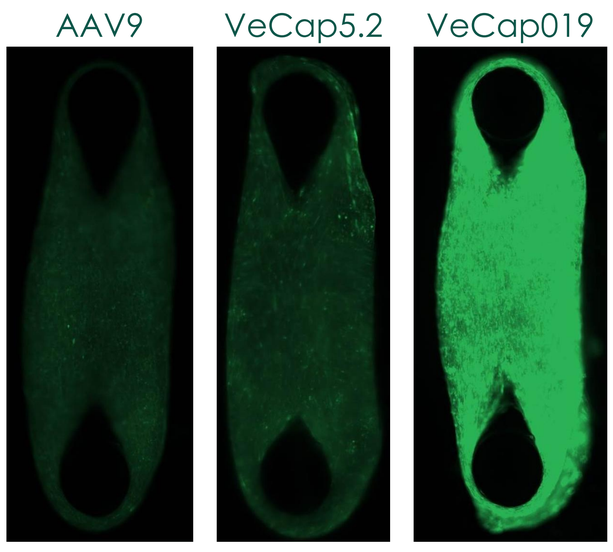
Figure 1. Enhanced transduction of human myotubes with engineered capsids. Primary human myoblasts were differentiated into myotubes and transduced with VeCap5.2 (VectorY's chimeric AAV) and muscle-targeting capsids at MOI 10^6. AAV9 was used as a control. Image acquisition was performed 7 days post transduction and cell pellets were subsequently collected for mRNA extraction and measurement of GFP expression levels (normalized to housekeeping gene average).

3D models

Figure 2. 3D muscle micro-tissues as a screening platform. Human myotubes are seeded around built-in micropillars that serve as anchor points for the 3D muscle culture, which were then transduced at MOI 10^6 with engineered capsids. Image acquisition was done 7 days post transduction.



Capsid	GFP intensity (vs AAV9)
VeCap5.2	1.4 x
VeCap019	4.6 x



AAV capsids cross the blood-brain barrier and transduce human astrocytes on the brain side of a human 3D microfluidic system

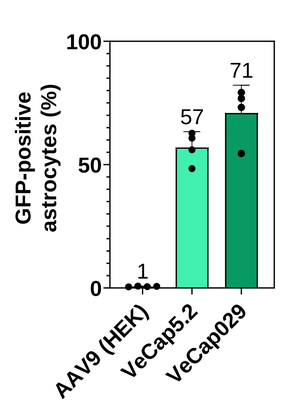


Figure 4. Improved transduction of human astrocytes with engineered capsids. Human iPSC-derived spinal astrocytes were seeded in 96-well format AAV capsids (AAV9 as control, VeCap5.2 (VectorY's chimeric AAV) and CNS-targeting capsids at MOI 10^6. Representative pictures were taken at day 7 post transduction and percentage of transduced astrocytes was calculated as GFP-positive cells versus total nuclei count in determined areas.

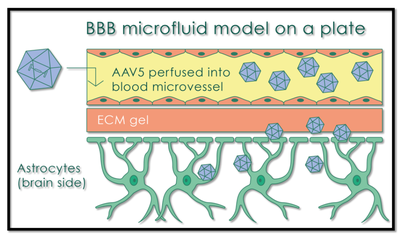
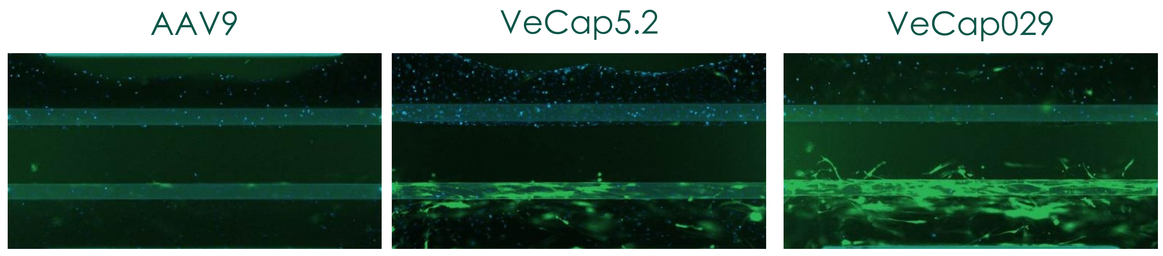


Figure 4. AAV capsid cross the blood-brain barrier (BBB) and transduce human astrocytes with higher efficiency. The BBB model consists of tubule of human brain endothelial cells in the top channel (blood side), an collagen-based gel (ECM) and human astrocytes on the bottom channel (brain side). This microfluidic device is perfused with AAV capsids at at MOI 10^6 Image acquisition was done 7 days post transduction and percentage of transduced cells is calculated for a region of interest in the brain side.



Capsid	AAV9	VeCap5.2	VeCap029
GFP-positive cells in brain compartment	2.4 %	30 %	47 %

In vivo

Intracisternal delivery of engineered capsids transduces different CNS regions

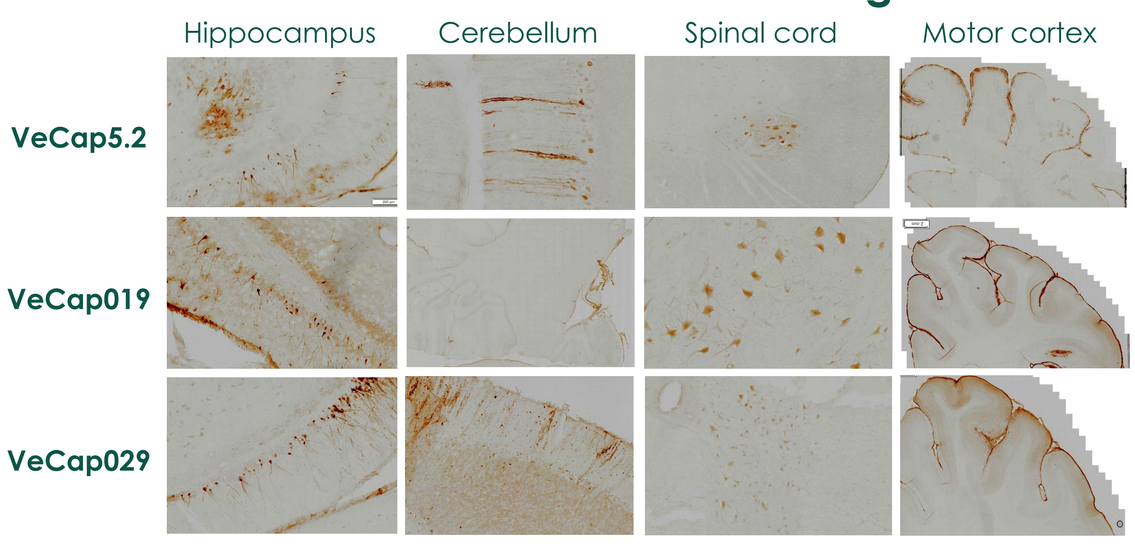


Figure 5. Biodistribution of engineered capsid in different CNS regions after intracisternal injection. Adult (9-month) mini-pigs were injected with AAV capsids in the cisterna magna and were monitored for 4 weeks, after which they were sacrificed. Here, images of GFP immunohistochemistry in different areas of CNS are shown.

Summary and perspectives

- Through rational design, new engineered AAV capsids have been successfully generated using baculovirus-based production system which is compatible with industrial scale production processes.
- These novel AAV capsids show improved cell tropism compared to AAV9 in human in vitro models. They are also able to transduce different CNS regions after in vivo delivery.
- Further screening and validation in disease models will provide insights into their relevance as gene therapy vectors for neurodegenerative diseases (see poster **P#353**).