Evaluation of Cross-species Expression Across Four Species and Cellular Tropism of VCAP-102, an Engineered Blood-brain Barrier-penetrating AAV Derived Capsid from TRACER Platform Screens

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INTRODUCTION

The effectiveness of the blood-brain barrier (BBB) at preventing therapies from properly engaging with targets within the central nervous system (CNS) has resulted in the use of invasive delivery methods of adeno-associated viruses (AAVs). The TRACER (Tropism Redirection of AAV by Cell-type-specific Expression of RNA) platform has enabled the generation of engineered AAV variants that have greater BBB crossing efficiency and liver detargeting compared to parental AAV capsids. Previously, we have shown that VCAP-102 was superior to AAV9 in crossing the BBB after IV administration and had widespread CNS transduction and expression in mouse and Cynomolgus macaques. To evaluate the efficacy of VCAP-102 for preclinical studies in rodents and show potential translatability into humans, we performed VCAP-102 biodistribution and expression studies after IV administration in mice, marmosets, African Green Monkeys (AGM), and Cynomolgus Macaques.

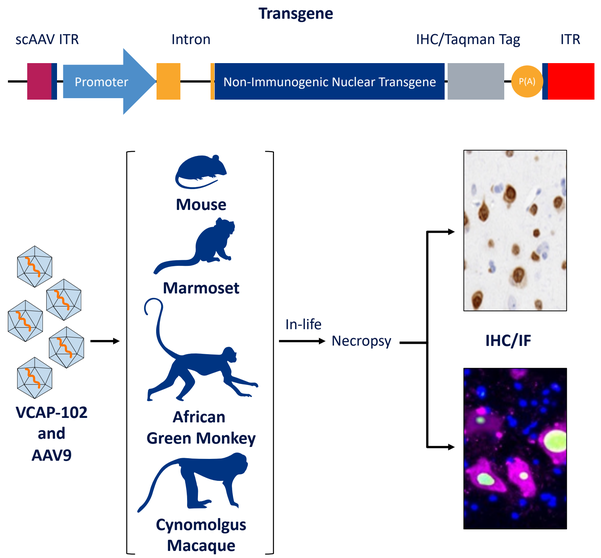
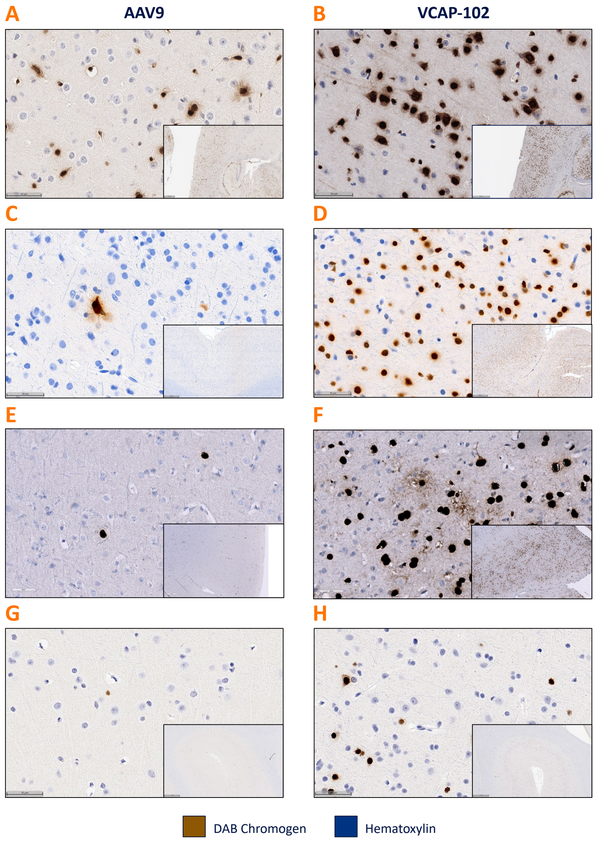
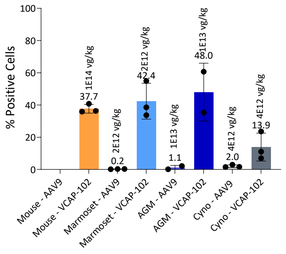


Figure 1. Expression of VCAP-102 and AAV9-transgene in Mouse, Marmoset, AGM, and Cynomolgus Macaque Cortex Using Immunohistochemistry



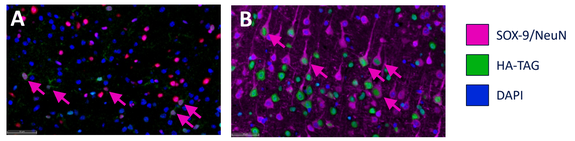
Transgene-HA expression in cortices of (**A**) AAV9 in mouse at 5E13 vg/kg, (**B**) VCAP-102 in mouse at 1E14 vg/kg, (**C**) AAV9 in marmoset at 2E12 vg/kg, (**D**) VCAP-102 in marmoset at 2E12 vg/kg, (**E**) AAV9 in AGM at 1E13 vg/kg, (**F**) VCAP-102 in AGM at 1E13 vg/kg, and transgene-Myc expression in cortices of cynomolgus macaque in (**G**) AAV9 at 4E12 vg/kg, (**H**) VCAP-102 at 4E12 vg/kg.

Figure 2. Quantification of VCAP-102 and AAV9-transgene Expression in Cortex of Mouse, Marmoset, AGM, and Cynomolgus Macaque



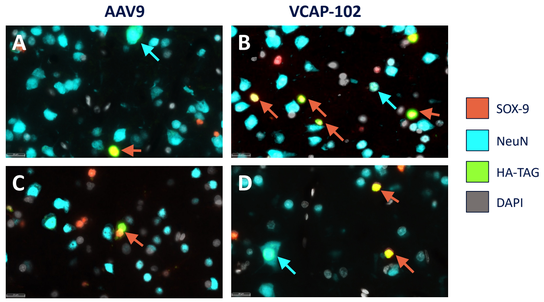
VCAP-102 and AAV9 mediated transduction is quantified by IHC analysis of transgene-tag positive cells (see Fig. 1) and expressed as %DAB+ cells in the cortices of mouse dosed with VCAP-102 and AAV9 at 1E14 vg/kg; marmoset dosed with VCAP-102 and AAV9 at 2E12 vg/kg; AGM dosed with VCAP-102 and AAV9 at 1E13 vg/kg, and cynomolgus macaque dosed with VCAP-102 and AAV9 at 4E12 vg/kg. Error bars indicate 1xSD. Quantification performed on HALO v3.6.4134.309.

Figure 3. Cellular Tropism of VCAP-102 in Cortex of Mouse



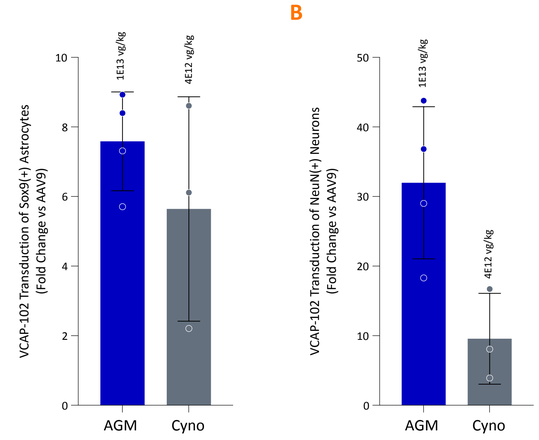
Immunofluorescence analysis of astrocytic (**A**) and neuronal (**B**) transduction by VCAP-102-transgene-HA in mouse cortex dosed at 1E14 via IV administration. Arrows indicate SOX-9/NeuN + HA-TAG co-staining.

Figure 4. Cellular Tropism of AAV9 and VCAP-102 in Cortex of AGM and Cynomolgus Macaques



Immunofluorescence analysis of astrocytic and neuronal transduction AGM by AAV9 (**A**) and VCAP-102 (**B**)-transgene-HA dosed at 1E13 vg/kg; cynomolgus macaque by AAV9 (**C**) and VCAP-102 (**D**)-transgene-HA dosed at 4E12 vg/kg via IV administration. Arrows indicate SOX-9/NeuN + HA-TAG co-staining.

Figure 5. Cell Type-Specific Quantification of VCAP-102 and AAV9-transgene Expression in Cortex of AGM, and Cynomolgus Macaque



VCAP-102- and AAV9-mediated transduction in cortex, quantified by IF analysis of transgene-tag positive cells (see Fig. 3 and 4). Data represents the percentage of Sox9(+) astrocytes (**A**) and NeuN(+) neurons (**B**) transduced by VCAP-102 in AGM (1E13 vg/kg dose) and cynomolgus macaque (4E12 vg/kg dose), normalized to AAV9. Error bars indicate SD. Quantification was performed on HALO v3.6.4134.309.

CONCLUSIONS

- Higher biodistribution and widespread expression of VCAP-102 Nuclear-HA in all the tested species as compared to AAV9 after IV administration.
- Immunofluorescent staining performed on mice, AGM, and Cynomolgus macaques confirmed that VCAP-102 Nuclear-HA is expressed in neurons and astrocytes of the cortex.
- Data confirm that VCAP-102 is superior to AAV9 in crossing the BBB across multiple species after IV administration, thus suggesting that VCAP-102 can be used for preclinical studies and may be useful for CNS- targeting indications.