Engineering an AAV9-derived Muscle-Tropic Capsid to Evade Pre-Existing Human Neutralizing Antibodies

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INTRODUCTION

Adeno-Associated Virus (AAV)-based gene therapy represents a promising approach for addressing a wide spectrum of human genetic diseases. Yet, its clinical utility is currently limited by pre-existing anti-AAV antibodies that sometimes occur from previous exposure to natural AAVs. These neutralizing antibodies can compromise vector biodistribution and reduce transduction efficiency, particularly following systemic delivery. Recent advancements in capsid engineering have not only enhanced AAV tissue tropism but also opened avenues for improving other key AAV characteristics, such as immune evasion.

In this study, we leveraged our proprietary RNA-driven TRACER evolution platform to detarget Voyager's new AAV9-derived muscle-tropic capsid from neutralizing antibodies (NAbs).

We initially mutated antibody epitopes located on the surface of an AAV9-derived Voyager capsid, and screened the resulting libraries *in vitro* and *in vivo* in the presence of human intravenous immunoglobulin (IVIG). We then applied Machine-Learning / Artificial Intelligence models to design optimized capsid libraries for *in vivo* biopanning. This iterative strategy allowed us to generate "stealth" capsids able to evade neutralization by a significant amount of NAb-positive human sera, while retaining the tissue tropism properties of its parent.

Remarkably, these antibody-evading mutations were transferable to other AAV9-derived capsids with minimal impact on vector packaging or transduction properties. Introduction of these stealth mutations into Voyager's 1st generation muscle-tropic capsid resulted in a marked enhancement of human IVIG evasion in Mouse while largely preserving muscle tropism. Most importantly, these mutations conferred stronger antibody evasion properties than the recently published Muscovy Duck Parvovirus. This finding suggests that a small set of targeted mutations in AAV9 capsids may be sufficient to enable effective detargeting from human neutralizing antibodies, avoiding the possible shortcomings of non-mammalian AAV serotypes relative to tropism or manufacturing.

Figure 1. Leveraging Voyager's TRACER Platform to Disrupt Pre-Existing Antibody Epitopes from AAV Capsids

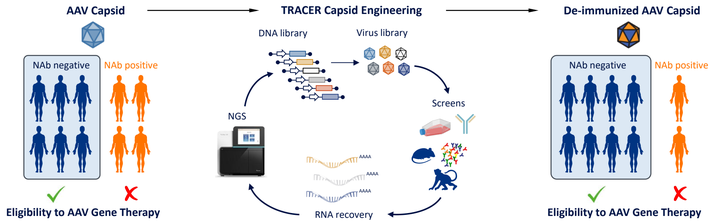
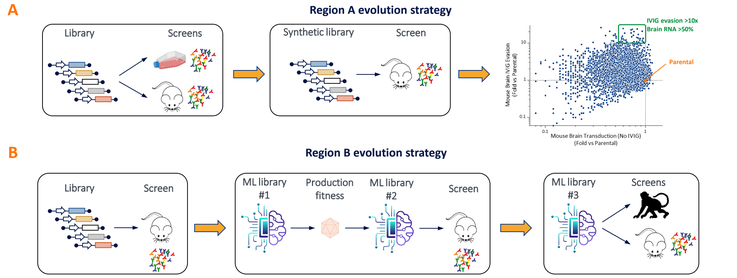
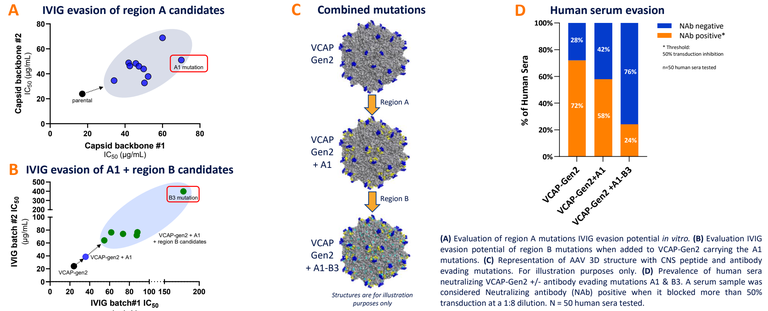


Figure 2. Directed Evolution of AAV Capsid Regions A & B to Identify NAb Evading Mutations



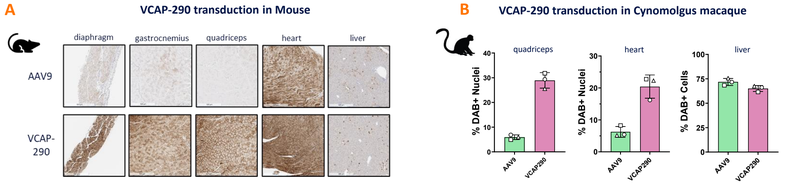
For more details about our machine learning approach, see Daniel Cox's poster #2027 on Wednesday, May 13th, 5:00 p.m. – 6:30 p.m. ET

Figure 3. Stealth Mutations Improve Human IVIG Evasion and Reduce NAb Seroprevalence



(A) Evaluation of region A mutations IVIG evasion potential *in vitro*. **(B)** Evaluation IVIG evasion potential of region B mutations when added to VCAP-Gen2 carrying the A1 mutations. **(C)** Representation of AAV 3D structure with CNS peptide and antibody evading mutations. For illustration purposes only. **(D)** Prevalence of human sera neutralizing VCAP-Gen2 +/- antibody evading mutations A1 & B3. A serum sample was considered Neutralizing antibody (NAb) positive when it blocked more than 50% transduction at a 1:8 dilution. N = 50 human sera tested.

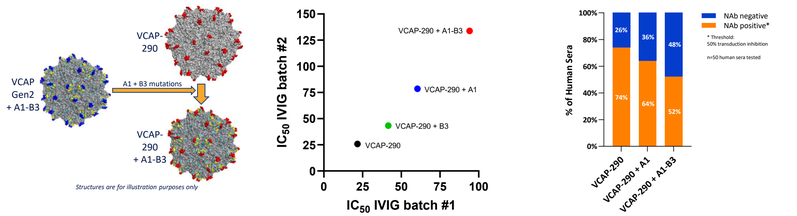
Figure 4. VCAP-290 is a Novel AAV9-derived Cross-Species Muscle Capsid



For more information about the discovery & characterization of VCAP-290, see Tyler Moyer's oral presentation on Friday, May, 15th, 8:00 a.m. – 9:45 a.m. ET

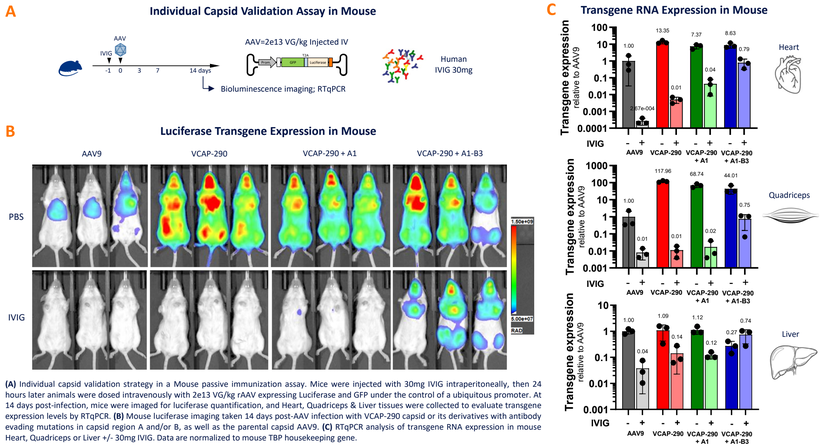
(A) Mice were injected intravenously with 2.5e13 VG/kg rAAV expressing GFP under the control of a ubiquitous promoter. At 14 days post-infection, tissue samples were collected to evaluate transgene expression levels by Immunohistochemistry (IHC). **(B)** Cynomolgus macaques were injected intravenously with a pool of multiple capsids (4e12 VG/kg per capsid) – each packaged with a histone transgene carrying a unique tag. At 30 days post-infection, tissue samples were collected and transgene expression was measured using IHC.

Figure 5. Transfer of Stealth Mutations to the Muscle Capsid VCAP-290



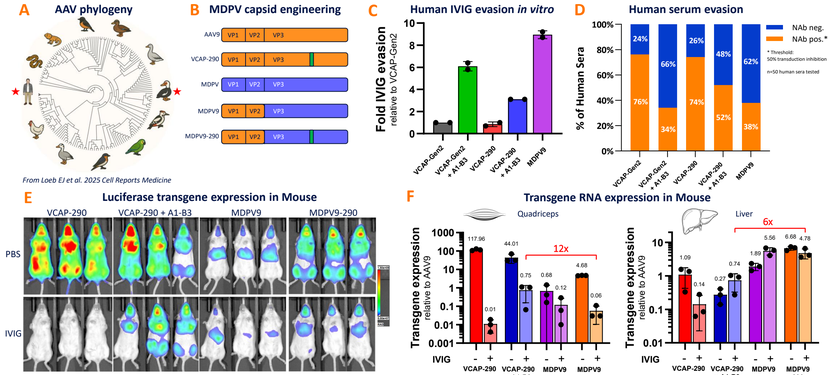
(A) Representation of AAV 3D structures illustrating the transfer of stealth mutations from VCAP-Gen2 to VCAP-290. For illustration purposes only. **(B)** Evaluation of region A & B mutations IVIG evasion potential in vitro. **(C)** Prevalence of human sera neutralizing VCAP-290 +/- antibody evading mutations A1 & B3. A serum sample was considered Neutralizing antibody (NAb) positive when it blocked more than 50% transduction at a 1:8 dilution. N = 50 human sera tested.

Figure 6. Stealth Mutations Improve VCAP-290 Human NAb Evasion While Retaining Most Muscle Tropism



(A) Individual capsid validation strategy in a Mouse passive immunization assay. Mice were injected with 30mg IVIG intraperitoneally, then 24 hours later animals were dosed intravenously with 2e13 VG/kg rAAV expressing Luciferase and GFP under the control of a ubiquitous promoter. At 14 days post-infection, mice were imaged for luciferase quantification, and Heart, Quadriceps & Liver tissues were collected to evaluate transgene expression levels by RTqPCR. **(B)** Mouse luciferase imaging taken 14 days post-AAV infection with VCAP-290 capsid or its derivatives with antibody evading mutations in capsid region A and/or B, as well as the parental capsid AAV9. **(C)** RTqPCR analysis of transgene RNA expression in mouse Heart, Quadriceps or Liver +/- 30mg IVIG. Data are normalized to mouse TBP housekeeping gene.

Figure 7. Comparison of Stealth Capsids to Naturally NAb Evading Muscovy Duck Parvovirus (MDPV)



(A) AAV phylogenetic tree illustrating the divergence between Human and Muscovy Duck isolates. From Loeb EJ et al. 2025 Cell Reports Medicine. **(B)** MDPV capsid was engineered to transduce Mammalian cells by swapping the VP1-2 domain from AAV9. MDPV9 capsid was then engineered by transferring the muscle tropic peptide from VCAP-290. **(C)** Comparison of human IVIG evasion potential between multiple capsids. Data are normalized to VCAP-Gen2. **(D)** Prevalence of human sera neutralizing various capsids. A serum sample was considered NAb positive when it blocked more than 50% transduction at a 1:8 dilution. N = 50 human sera tested. **(E)** Mouse luciferase imaging taken 14 days post-AAV infection with various capsids at 2e13 VG/kg +/- 30mg human IVIG injected intraperitoneally 24h prior. **(F)** RTqPCR analysis of transgene RNA expression in Mouse Quadriceps or Liver +/- 30mg IVIG. Data are normalized to mouse TBP housekeeping gene.

CONCLUSIONS

- We identified a 2nd generation Voyager AAV muscle capsid with mutations conferring evasion from pre-existing anti-AAV9 NAbs.
- Stealth modifications of existing muscle capsids could potentially increase the number of patients eligible for muscle-targeted gene therapies.