# Discovery of TRACER AAV Capsids Escaping Pre-existing Neutralizing Antibodies

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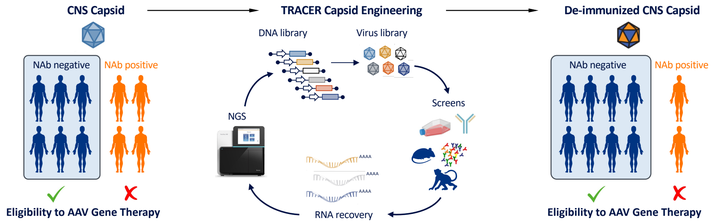
## INTRODUCTION

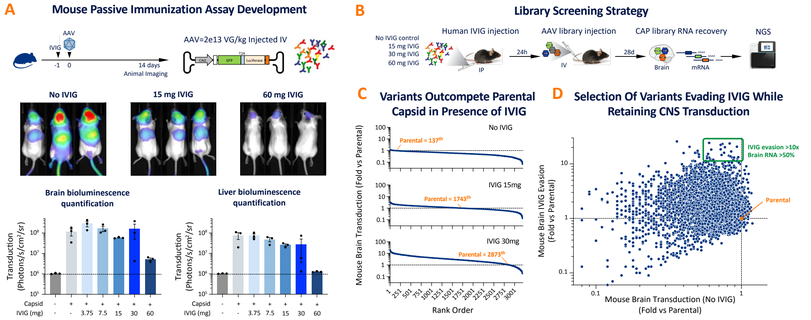
Adeno-Associated Virus (AAV) gene therapy shows great promise to treat a wide array of human genetic diseases. However, patient eligibility to receive AAV gene therapy is limited by the presence of pre-existing anti-AAV antibodies resulting from prior exposure to natural AAVs. These antibodies restrict AAV biodistribution and target tissue transduction, especially for systemically administered AAVs. Recent capsid engineering efforts have successfully improved AAV tissue tropism, and paved the way for enhancing other AAV properties.

In this study, we leveraged our proprietary RNA-driven TRACER evolution platform to detarget our 2nd generation AAV9-derived CNS tropic capsids from neutralizing antibodies (NAbs). We evolved immunogenic capsid surface regions, and screened the resulting libraries in vitro in presence of monoclonal NAbs as well as human intravenous immunoglobulin (IVIG) to maximize the clinical relevance. Capsid libraries were also screened in marmoset and in a mouse passive immunization model with human IVIG. We identified several capsid families with specific amino acids enriched by IVIG selection pressure at several mutation hotspots. In pooled capsid library assays, top candidates evaded IVIG up to 20-fold better than the parental capsid in mouse while retaining over 50% brain transduction of the parent capsid in mouse & marmoset. Individual validation of these capsids in mouse confirmed that top candidates evaded human IVIG 10 to 30-fold better than AAV9. Most importantly, these capsids were able to completely evade ~35% of neutralizing human serum samples. Remarkably, combining mutations from different capsid regions further improved NAb evasion, with seroprevalence decreasing from 60% down to 30% in a panel of 50 human serums.

### Figure 1. Leveraging Voyager's TRACER Platform to Disrupt Pre-existing Antibody Epitopes from CNS Capsids

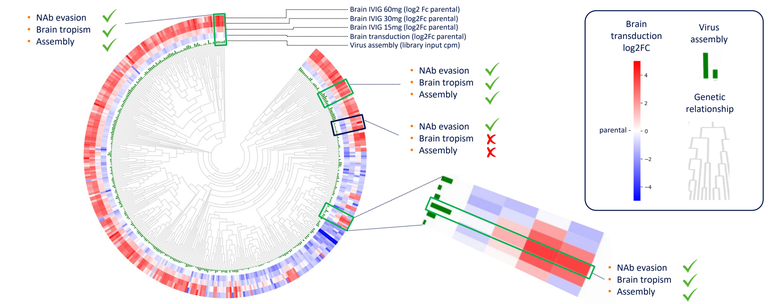



### Figure 2. Screen for Variants from Capsid Region A Evading Human IVIG in Mouse



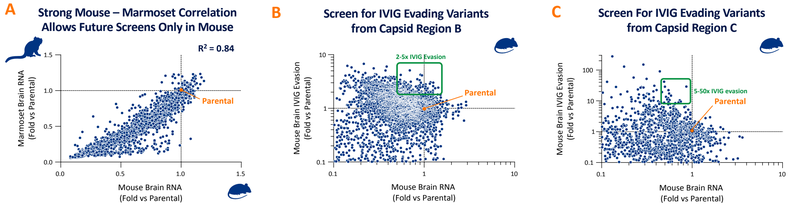

**(A)** Development of a passive immunization model in mouse with human IVIG. Mice were injected with 3.75 to 60mg IVIG intraperitoneally, then 24 hours later animals were dosed intravenously with 2e13 VG/kg rAAV expressing Luciferase and GFP under the control of a CAG promoter. Luciferase was measured at 14 days post-AAV injection in Brain & Liver to determine the optimal IVIG dose to use for library screening. **(B)** Library screening strategy focused on AAV capsid region A. The AAV library was injected into mice IV 24h post-immunization with human IVIG. Capsid RNA was recovered from Brain & quantified by NGS. **(C)** Capsid ranking for Mouse brain transduction in presence or absence of IVIG. Ranking of the parental is highlighted in each plot. **(D)** Comparison of library capsids brain transduction in Mouse in presence & absence of IVIG. Top IVIG evading capsids were identified as IVIG evading while retaining at least 50% of the parental capsid brain transduction properties.

### Figure 3. Antibody Evading Variants Cluster by Sequence Homology



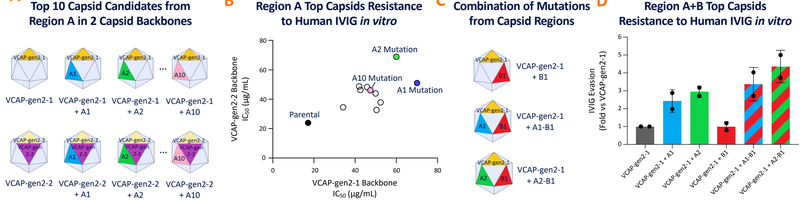

Circos plot representing major capsid clusters present in the screen, and their associated Mouse brain transduction properties expressed as log2 fold-change over parental capsid in presence or absence of multiple doses of human IVIG. Virus assembly expressed as NGS count per millions is shown to inform the ability of capsids to form intact virions. Capsids genetic relationships is represented at the center, and variants are clustered by sequence homology using linclust. Top 100 as well as bottom 100 clusters are shown in this figure.

### Figure 4. Screen for Additional Antibody Evading Variants in Two Other Capsid Regions



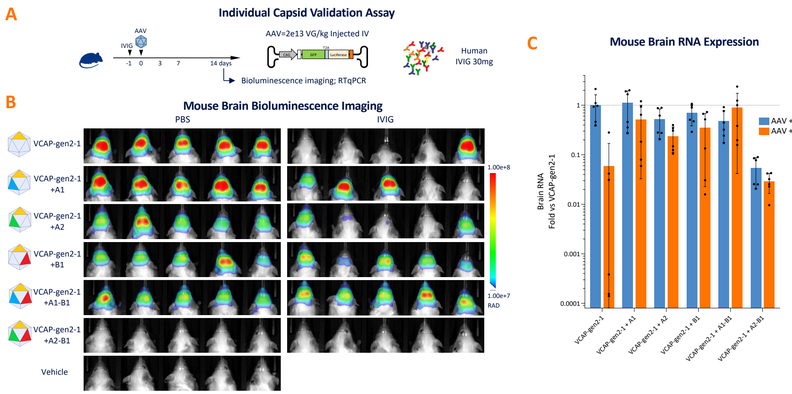

**(A)** Comparison between region A library capsids brain transduction properties in Mouse & Marmoset. Results are expressed as fold-change versus the parental capsid. Correlation coefficient $R^2$ is indicated in the figure top right corner. **(B)** Comparison between mouse brain transduction of a library of capsids focused on region B in presence & absence of IVIG. Top IVIG evading capsids were identified as IVIG evading while retaining at least 50% of the parental capsid brain transduction properties. **(C)** Comparison between mouse brain transduction of a library of capsids focused on region C in presence & absence of IVIG. Top IVIG evading capsids were identified as IVIG evading while retaining at least 50% of the parental capsid brain transduction properties.

### Figure 5. Single Capsid Evaluation of Top Candidates *in vitro* with Mutations in Capsid Regions A & B



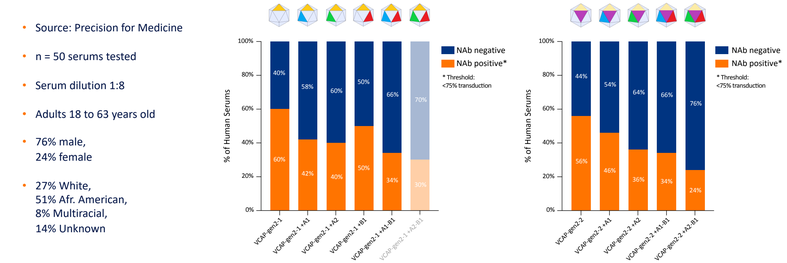

**(A)** Schematic representation of the top10 capsid mutations from region A, added into the capsid backbones of VCAP-gen2-1 (top) or VCAP-gen2-2 (bottom). Each number from A1 to A10 represents a series of mutations specific to each antibody evading variant. **(B)** Comparison of the human IVIG concentration required to inhibit 50% of HeLa cells transduction ($IC_{50}$) from capsid VCAP-gen2-1 vs VCAP-gen2-2 +/- antibody evading mutations. **(C)** Schematic representation of capsid mutations from region B or both regions A & B added into the capsid backbone of VCAP-gen2-1. Each number A1, A2 or B1 represents a series of mutation specific to each antibody evading variant. **(D)** IC50 from 2 batches of human IVIG at inhibiting antibody evading variants from 1 or 2 regions in the backbone of VCAP-gen2-1.

### Figure 6. Antibody Evading Mutations Increase VCAP-gen2-1 Resistance to Human IVIG in Mouse




**(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 CAG promoter. At 14 days post-infection, mice were imaged for luciferase quantification in the brain, and brain tissue was collected to evaluate transgene brain expression levels by RTqPCR. **(B)** Mouse brain luciferase imaging taken 14 days post-AAV infection with VCAP-gen2-1 capsid or its derivatives with antibody evading mutations in capsid region A and/or B. **(C)** RTqPCR analysis on transgene RNA expression in mouse brain +/- 30mg IVIG. Data are normalized to mouse TBP housekeeping gene.

### Figure 7. Antibody Evading Mutations Increase Potential Patient Eligibility by Over 50%




**(A)** Details & demographics about human serum sample used for this study. **(B)** Prevalence of human serums neutralizing VCAP-gen2-1 +/- antibody evading mutations. A serum sample was considered Neutralizing antibody (NAb) positive when it blocked more than 25% transduction at a 1:8 dilution. **(C)** Prevalence of human serums neutralizing VCAP-gen2-2 +/- antibody evading mutations.

## CONCLUSIONS

- Overall, this study identified a 3rd generation of bioengineered AAV capsids with mutations conferring evasion from pre-existing neutralizing antibodies while retaining the improved CNS tropism of their 2nd generation parent
- These novel capsids have the potential to significantly improve a key bottleneck in AAV treatments by increasing patient eligibility to receive AAV gene therapies.