Use of adeno-associated virus (AAV) vectors in non-human primates is complicated by host immune responses that can limit transgene expression. Here we compared the expression of anti-SIV antibody (ADA) responses against the rhesus macaque derived, anti-SIV antibody ITS01 when delivered by five different AAV capsids. We first observed that ITS01 expression was three-fold more efficiently in mice from AAV vectors in which heavy and light-chain genes were separated by a P2A ribosomally skipping peptide, compared with those bearing F2A or T2A peptides. We then measured the pre-existing neutralizing antibody responses against the five different AAV vectors (AAV1, AAV8, AAV9, AAV-NP22, AAV-KP1) and found similar concentrations of ITS01 (247 µg/mL, n=3, and 162 µg/mL, n=3, respectively), whereas the remaining capsids expressed an average of 35-47 µg/mL. Notable, ADA responses against ITS01 were only observed in one of the 15 animals. Overall, our data suggest that the AAV9 capsid and the P2A peptide can improve expression of antibodies from muscle tissue.

**AAV transfer plasmid encoding anti-SIV antibody, ITS01**

- ITS01 is an anti-SIV antibody isolated from a rhesus macaque infected with SIVmac251 (Walton RD et al., PLoS Pathogens, 2016).
- Transfer plasmid encoding ITS01 utilizes a CASI promoter (Balazs AB et al., Nature, 2011), a codon-optimized coding region of ITS01, a 2A peptide to generate both ITS01 heavy and light chains, a shortened WPRE, and an SV40 polyadenylation signal sequence (Figure 1A).
- It was encoded to use the rhesus macaque IgG1Fc with the MA28L/N5495 amino acid substitutions to improve FcγR affinity and half-life.

**Rhesus macaque selections for NHP study based on pre-existing neutralizing antibodies**

- ITS01 is anti-SIV antibody isolated from a rhesus macaque infected with SIVmac251 (Walton RD et al., PLoS Pathogens, 2016).
- Transfer plasmid encoding ITS01 utilizes a CASI promoter (Balazs AB et al., Nature, 2011), a codon-optimized coding region of ITS01, a 2A peptide to generate both ITS01 heavy and light chains, a shortened WPRE, and an SV40 polyadenylation signal sequence (Figure 1A).
- It was encoded to use the rhesus macaque IgG1Fc with the MA28L/N5495 amino acid substitutions to improve FcγR affinity and half-life.

**AAV vectors for NHP study express ITS01**

- Recombinant ITS01 antibody utilizing an F2A, P2A, or T2A peptide was produced through transient transfection of HEK293T cells and purified using Protein A columns.
- All versions of the ITS01 antibody bound SIV/HIV gp120 with similar affinity, regardless of which 2A peptide was used (Figure 1B).

**Figure 1. Schematic and characterization of ITS01 expressed from an AAV transfer plasmid. (A) Schematic of an AAV transfer plasmid encoding ITS01. (B) ITS01 expression is driven by a CASI promoter and utilizes a 2A peptide to generate both the heavy and light chains. (B) Purified recombinant antibody binds SIV/HIV gp120 with similar affinity regardless of which 2A peptide was used for FcγR affinity and half-life.

**Summary & Recommendations**

- Codi optimize the transgene.
- Use a 2A peptide when encoding an antibody.
- Inoculate with a dose around 2.0–5.0 x 10^11 vg/kg.
- Spread the inoculation over at least 8 sites.
- An AAV9 capsid is suitable replacement for AAV1.

**Acknowledgements**

We would like to thank Meredith Davis Gardner and Jesse Weber for their technical assistance. We would like to thank the staff of the Wisconsin Primate Research Center for managing the NHP part of the study. We would like to thank Mark Kay and Katja Pelkonen for lending us the AAV-NP22 and AAV-KP1 expression plasmids and advice for the study. We would like to thank Guangming Gao and the staff of the UMass Vector Core for producing the AAV vectors for the NHP part of the study. This project was supported by the International AIDS Vaccine Initiative (IAVI) through the funding from the Division of AIDS.