Engineered Cardioskeletal-Directed AAV Capsids That Detarget the Liver

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Introduction

- Adeno-associated virus (AAV)-mediated therapeutic treatments for musculoskeletal and cardiac indications require high systemic doses to achieve widespread therapeutic benefit. However, high vector doses have been associated with safety risks, as seen in clinical trials. The engineering of more precise and efficacious vectors, could lead to vectors that improve the safety profile for diseases that require a systemic delivery approach.
- Previously, we described an engineered capsid, AAV-SLB101, that has shown a 2-4x increase in muscle transduction and ~0.5x decrease in liver transduction compared to AAV9 in *DMD^{mdx}* and wild type mice, and non-human primates.
- Currently, we are using rational design and structure activity relationship (SAR)-like approaches to iteratively engineer and test additional novel AAV capsids using combinations of liver detargeting with muscle targeting modifications.
- Here we characterize AAV-SLB134 and screen additional novel capsids, which include modifications to enhance muscle targeting while detargeting the liver.

AAV-SLB134 Detargets the Liver in C57BL/6 Mice

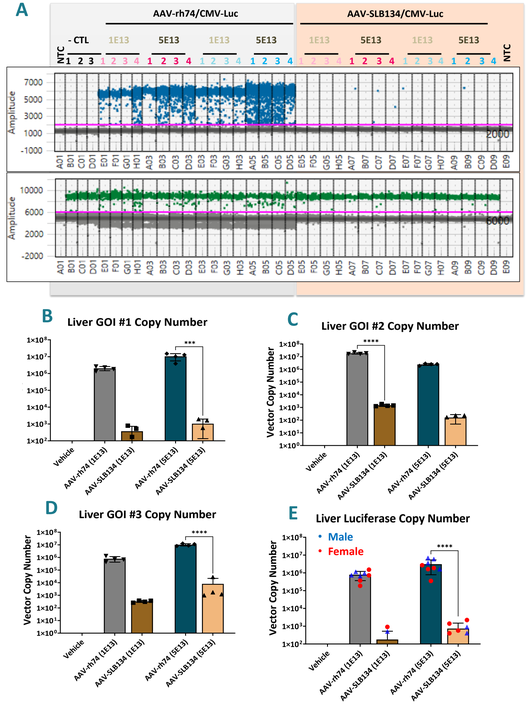

Figure 1. Liver biodistribution from C57BL/6 mice. Male and female mice were treated with AAV-rh74 or AAV-SLB134 encapsidating a CMV-Luciferase (CMV-Luc) reporter construct or three therapeutic transgenes.

A) Vector copy number (VCN) obtained via ddPCR for CMV-Luc treated groups shows liver detargeting in animals treated with the AAV-SLB134/CMV-Luc vector compared to the AAV-rh74/CMV-Luc vector in both male and female mice at 1E13 vg/kg and 5E13 vg/kg. NTC = non-template control; - CTL = Negative control (vehicle/AAV storage buffer).

The quantified VCN of each treatment group normalized to mouse Rpp30 is graphed for **B)** GOI #1, **C)** GOI #2, **D)** GOI #3, and **E)** CMV-Luc.

Statistics for each graph were determined by ordinary one-way ANOVA ($p < 0.05$, ** $p < 0.01$).*

Protein Expression Confirms AAV-SLB134 Liver Detargeting

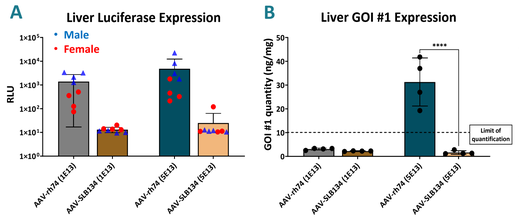

Figure 2. Protein expression in the liver of C57BL/6 mice was measured via luciferase relative light unit (RLU) and capillary-based immunoassays for the therapeutic GOI #1. **A)** AAV-SLB134 shows a decrease in luciferase expression over AAV-rh74 in the liver **B)** Statistically significant reduction in expression of GOI #1 from AAV-SLB134 versus AAV-rh74 was measured through the capillary-based immunoassay, as determined by ordinary one-way ANOVA (**** $p < 0.0001$).

AAV-SLB134 Targets C57BL/6 Mouse Heart

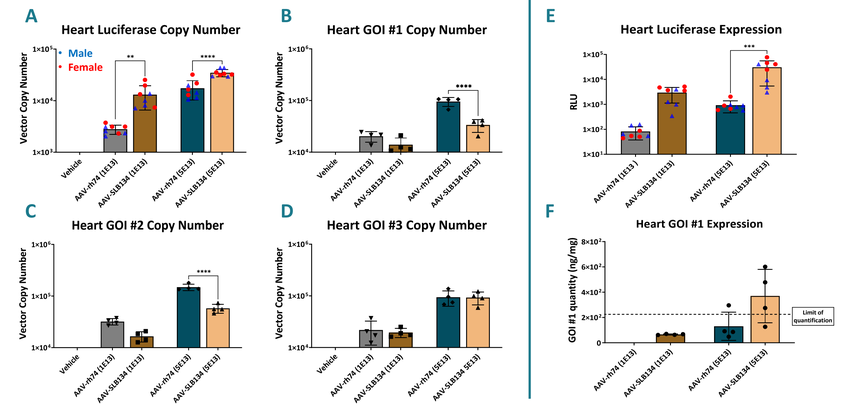

Figure 3. Heart biodistribution from C57BL/6 mice. The vector copy number was obtained via ddPCR for the mice treated with AAV-rh74 and AAV-SLB134 in the context **A)** CMV-Luc, **B)** GOI #1, **C)** GOI #2, and **D)** GOI #3. Protein expression from the delivered transgenes was assessed via **E)** a standard luciferase assay comparing the different dose regimen, and **F)** a capillary-based immunoassay for GOI #1 detection in the heart.
*Statistics for each graph were determined by ordinary one-way ANOVA (*** $p < 0.001$,* (**** $p < 0.0001$).

Expression with AAV-SLB134 in Supplementary Tissues

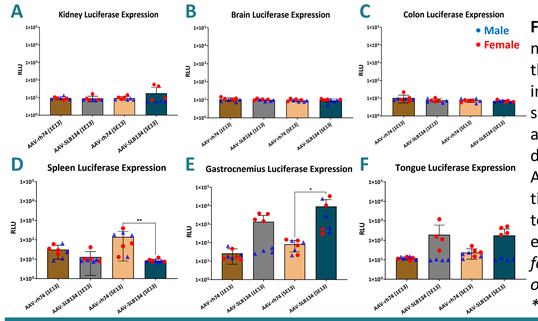

Figure 4. Luciferase expression was measured in additional tissues from the treated C57BL/6 mice. Expression in the **A)** kidney, **B)** brain and **C)** colon show similar levels across both capsids and in the **D)** spleen, there is a decrease in luciferase expression from AAV-SLB134. Of the additional muscle tissue tested, **E)** gastrocnemius and **F)** tongue, AAV-SLB134 has increased expression over AAV-rh74. *Statistics for each graph were determined by ordinary one-way ANOVA (* $p < 0.05$, ** $p < 0.01$).*

Novel AAV Capsids Detarget Liver Cell Line *In Vitro*

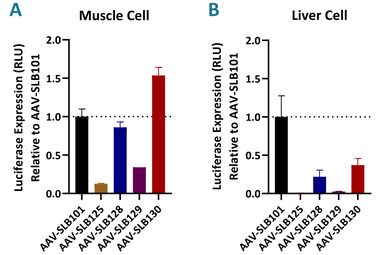

Figure 5. Assessing novel capsids *in vitro*. AAV capsids with liver detargeting modifications were tested via transduction in a muscle and liver cell line and compared to AAV-SLB101. Resulting luciferase expression from AAV-SLB128 and AAV-SLB130 show similar or increased signal in the **A)** muscle cell line, while decreasing expression in the **B)** liver cell line. Data is represented as fold change in comparison to AAV-SLB101.

Conclusions

- In this study, we tested an engineered novel capsid, AAV-SLB134, designed to be muscle targeting and liver detargeting.
- In C57BL/6 mice we demonstrated that compared to AAV-rh74, AAV-SLB134:
  1. *Significantly detargets the liver*
  2. *Targets the heart and other muscle tissues (gastroc & tongue)*
  3. *Results in similar or decreased expression in peripheral organs (kidney, brain, colon, and spleen)*
- Additional *in vivo* studies will help further assess AAV-SLB134 for cross-species compatibility and as a potential capsid candidate for AAV-mediated therapeutic treatments of musculoskeletal and cardiac indications.
- We are continuing to assess additional engineered novel capsids for liver detargeting capabilities *in vivo*, including AAV-SLB128 and AAV-SLB130.