CHARACTERIZATION OF NOVEL AAV VECTORS ENGINEERED FOR MUSCLE GENE DELIVERY

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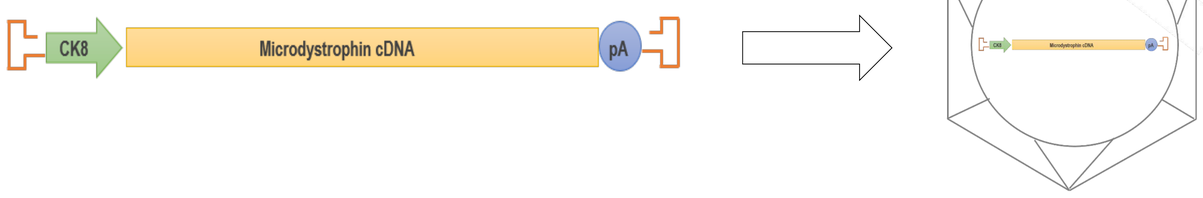
Introduction

- Recombinant adeno-associated viral (rAAV) vectors demonstrate great promise as the leading platform for *in vivo* gene delivery. A variety of rAAV vectors enable delivery to multiple tissues, including the muscular system, for the treatment of many genetic and other complex diseases. Select natural serotypes and engineered rAAV capsids exhibit enhanced widespread biodistribution to muscle, which could reduce the total dose required.
- In applications that require systemic administration of high doses of vectors, such as Duchenne muscular dystrophy (DMD), development of muscle-targeted capsids with a higher potency could improve safety and efficacy of gene transfer therapy.
- We utilized a rational design approach to generate a set of novel capsids predicted to have increased muscle tropism and transduction efficiency for the development of treatment for DMD.

AAV Vectors

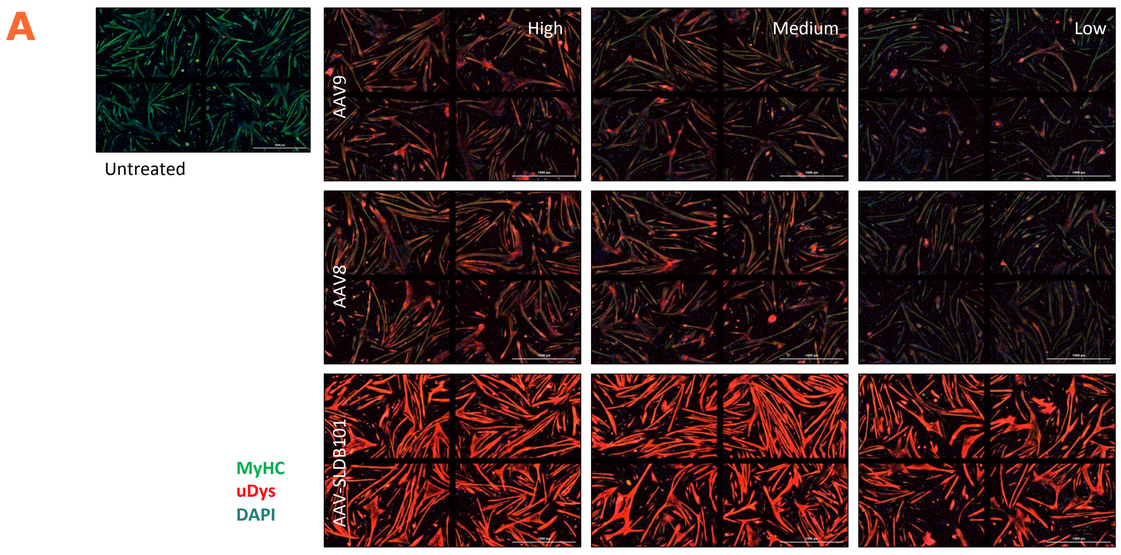
- Here we compare an expanded panel of these rationally designed capsids with the previously described AAV-SLB101 and natural serotype, AAV9, in *in vitro* assays to characterize binding, uptake and microdystrophin protein expression as a first step to identifying additional promising candidates.



- All capsids were packaged with a microdystrophin-expressing transgene, driven by the muscle-specific promoter CK8. In *in vivo* studies, no microdystrophin expression in peripheral tissues was detected, as expected for this promoter.

Results

In Vitro Assays Comparing AAV-SLB101 Capsid to AAV8 & AAV9



	Fold Change
AAV9	1.00
AAV-SLB101	10.91
AAV8	0.77

	Fold Change
AAV9	1.00
AAV-SLB101	4.01
AAV8	1.45

Figure 1. Muscle Cell Assays Comparing Microdystrophin Expression from AAV Capsids

A) C2C12 cells were transduced at three multiplicity of infections (MOIs) AAV9, AAV8 or novel capsid AAV-SLB101. At 96 hours post-transduction, cells were fixed and immunostained for microdystrophin expression. Representative immunofluorescent images are shown.

B) C2C12 cells were transduced with the same AAV at the low MOI only. Cells were harvested 96 hours after transduction and microdystrophin expression was measured. The data shown are normalized to AAV9 and fold change is indicated in the table. AAV-SLB101 has significantly higher microdystrophin protein expression than AAV9 ($p < 0.0001$). Statistics are determined by ordinary one-way ANOVA.

C) Patient-derived DMD cells were transduced with the same AAV at the low MOI only. Cells were harvested 72 hours after transduction and microdystrophin expression was measured. The data shown are normalized to AAV9 and fold change is indicated in the table. AAV-SLB101 has significantly higher microdystrophin protein expression than AAV9 ($p < 0.0001$). Statistics are determined by ordinary one-way ANOVA.

In vivo Comparison of Novel Capsid AAV-SLB101 to AAV9

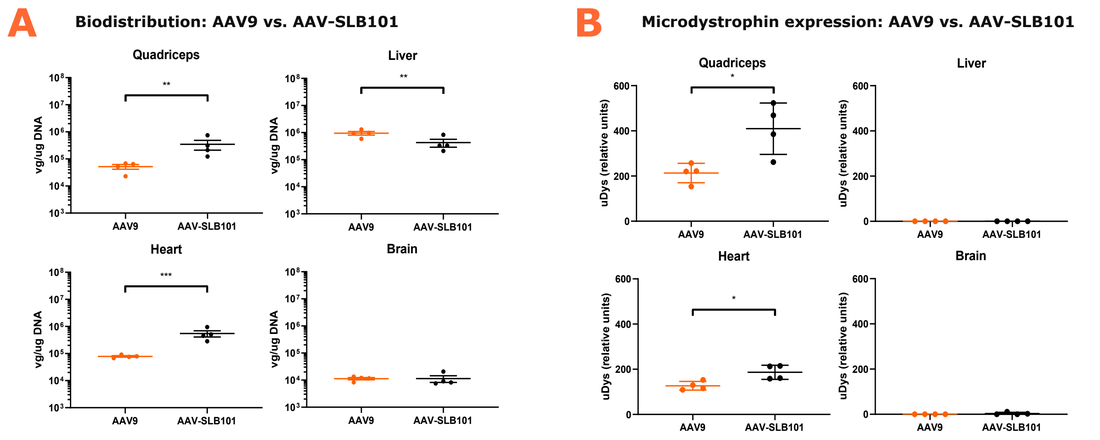


Figure 2. AAV-SLB101 Compared to AAV9 After Intravenous Delivery

DMD^{mdx} mice, 5-6 weeks old, were systemically injected AAV9 and AAV-SLB101 at a dose of 1E14 vg/kg. The mice were necropsied 4 weeks post injection and tissues were harvested for quantification of vector biodistribution and microdystrophin expression. Statistics in all panels are determined by individual Welch's t tests in comparison to AAV9.

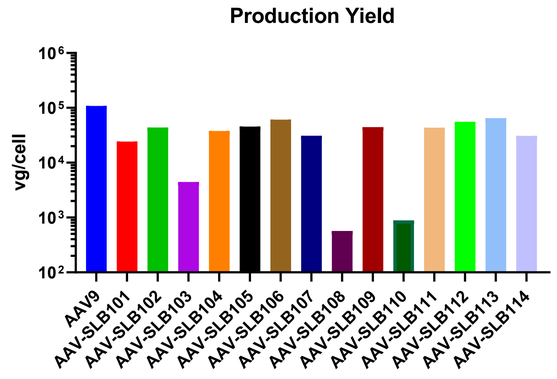
A) Tissue biodistribution. AAV-SLB101 has significantly higher vector genomes in heart ($p < 0.001$) and quadriceps ($p < 0.01$), and significantly lower vector genomes in liver ($p < 0.01$) than AAV9. The data shown are mean ± SD.

B) Microdystrophin protein expression. AAV-SLB101 has significantly higher microdystrophin expression in heart and quadriceps ($p < 0.05$). Neither capsid results in microdystrophin expression in peripheral tissues, as expected due to the use of a muscle-specific promoter. The data shown are mean ± SD.

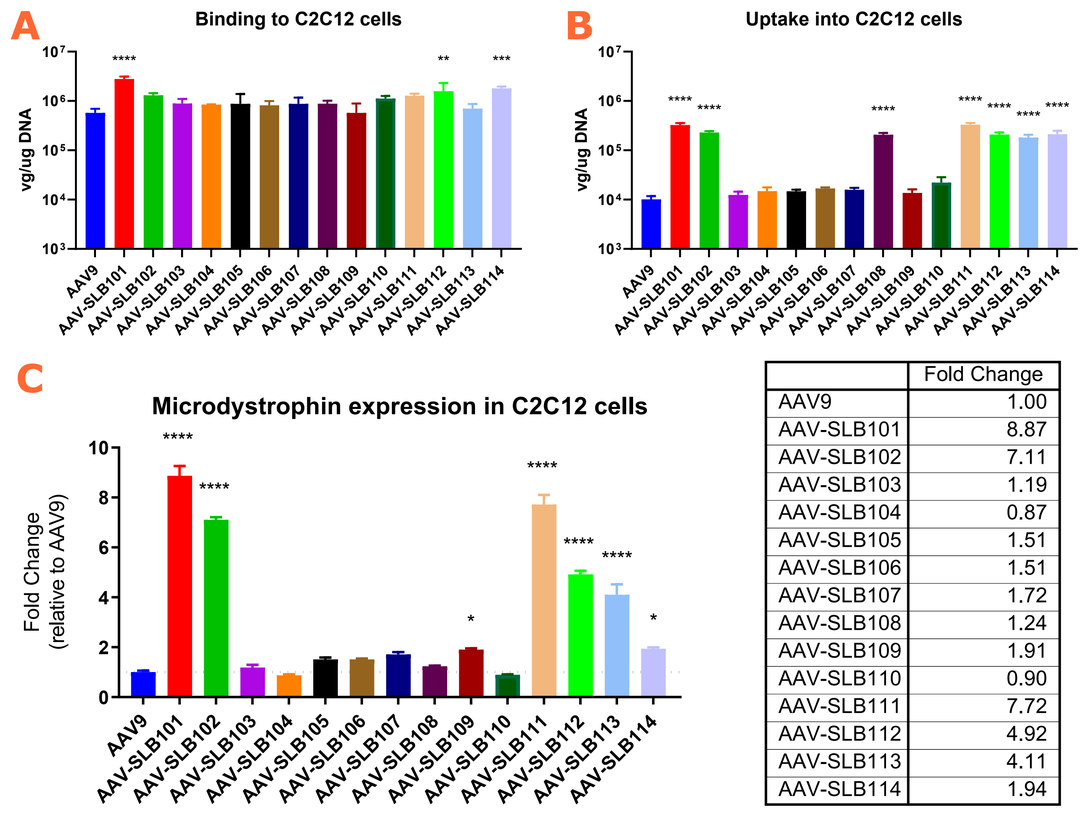
Production of Additional Novel Capsids

Figure 3. Production Yield of Novel Capsids Compared to AAV9

Expanded panel of novel AAV capsids were compared to AAV9 and AAV-SLB101 for AAV yield from triple transfection of adherent 293T cells and purification via step-wise iodixanol ultracentrifugation.



In vitro Characterization of Additional Novel Capsids and Comparison to AAV9 and AAV-SLB101



	Fold Change
AAV9	1.00
AAV-SLB101	8.87
AAV-SLB102	7.11
AAV-SLB103	1.19
AAV-SLB104	0.87
AAV-SLB105	1.51
AAV-SLB106	1.51
AAV-SLB107	1.72
AAV-SLB108	1.24
AAV-SLB109	1.91
AAV-SLB110	0.90
AAV-SLB111	7.72
AAV-SLB112	4.92
AAV-SLB113	4.11
AAV-SLB114	1.94

Figure 4. *In vitro* Characterization of Additional Novel Capsids for Comparison to AAV9 and AAV-SLB101 in C2C12 Cells

A) Quantification of AAV capsid binding to the cell surface of C2C12 cells was measured by qPCR of DNA isolated after 1 hour of incubation at 4°C. Significantly more binding to C2C12 cells by AAV-SLB101 ($p < 0.0001$), 112 ($p < 0.01$) and 114 ($p < 0.001$) than AAV9 was observed. Statistics are determined by ordinary one-way ANOVA.

B) Quantification of uptake of AAV into C2C12 cells was measured by qPCR of DNA isolated after 1 hour of incubation at 4°C followed by 1 hour at 37°C. Significantly more uptake by C2C12 cells of AAV-SLB101, 102, 108 and 111-114 than AAV9 ($p < 0.0001$) was observed. Statistics are determined by ordinary one-way ANOVA.

C) C2C12 cells were transduced AAV9, AAV-SLB101 and thirteen additional novel capsids. Cells were harvested 96 hours after transduction and microdystrophin expression was measured. The data shown are normalized to AAV9 and fold change is indicated in the table. AAV-SLB101, 102, 111, 112 and 113 had the highest microdystrophin protein expression over AAV9 ($p < 0.0001$), with AAV-SLB109 and 114 resulting in only slightly higher expression than AAV9 ($p < 0.001$). Statistics are determined by ordinary one-way ANOVA.

Conclusions

- A novel capsid, AAV-SLB101, showed superior transduction efficiency in comparison to AAV9 in *in vitro* assays in both mouse and DMD human skeletal muscle cells.
- These *in vitro* results translated to increased biodistribution and microdystrophin protein expression *in vivo* in both quadriceps & heart, and decreased biodistribution to liver, in comparison to AAV9.
- An expanded panel of novel capsids identified two more candidates of interest, AAV-SLB102 and AAV-SLB111, that look similar to AAV-SLB101 in *in vitro* assays for binding, uptake and microdystrophin protein expression in C2C12 cells. These will be further characterized in a future *in vivo* experiment.