SGT-001 Microdystrophin Gene Therapy for Duchenne Muscular Dystrophy

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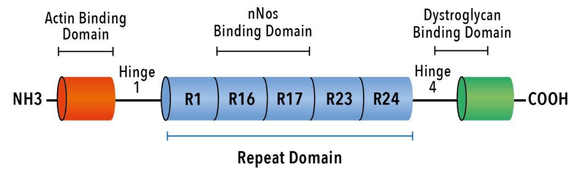
Introduction

Duchenne Muscular Dystrophy

- Duchenne muscular dystrophy (DMD) is a fatal neuromuscular disease caused by mutations in the *DMD* gene that lead to the absence of functional dystrophin protein
- Dystrophin stabilizes the dystrophin glycoprotein complex (DGC) at the sarcolemma and maintains local nitric oxide (NO) production by anchoring neuronal nitric oxide synthase (nNOS) to the DGC
- Without dystrophin, DGC members and nNOS lose sarcolemmal localization and show decreased overall protein levels and function
- As a result, muscles become susceptible to contraction-induced injury and functional ischemia, and break down over time
- Although the cause of DMD is well known, the largest challenges to developing a therapy are the size of the *DMD* gene (considered the largest protein-encoding gene in the human genome) and the need to deliver a therapy systemically to all muscles in the body

SGT-001



- SGT-001 is a recombinant adeno-associated virus serotype 9 (rAAV9) vector containing a microdystrophin transgene under the control of the muscle-specific CK8 promoter
- The microdystrophin transgene in SGT-001 maintains critical elements for dystrophin function, including the nNOS binding domain, while still fitting within AAV packaging limits
- Canine SGT-001 contains a canine-codon optimized microdystrophin
- SGT-001 is administered systemically by intravenous (IV) delivery to produce microdystrophin protein in skeletal and cardiac muscle

Scalable Manufacturing

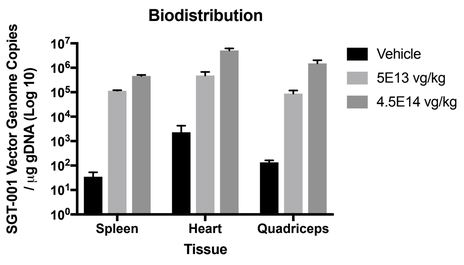
- SGT-001 manufacturing methodology evolved from an adherent cell-based method of research-grade production to a suspension culture method of clinical-grade production for increased scalability
- Suspension culture allows for production runs of hundreds to thousands of liters, which is essential to treat all patients amenable to therapy at potentially efficacious vector genome (vg)/kg dose levels

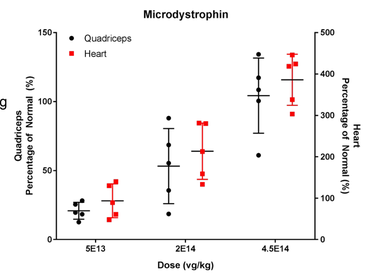
Preclinical Package

- Preclinical safety studies have been completed that show SGT-001 is well-tolerated at target dose levels
- Preclinical efficacy studies in small and large animal models of DMD show that a single IV dose of SGT-001 produces widespread, durable expression of microdystrophin in muscle tissues, with associated improvements in histology and functional measurements

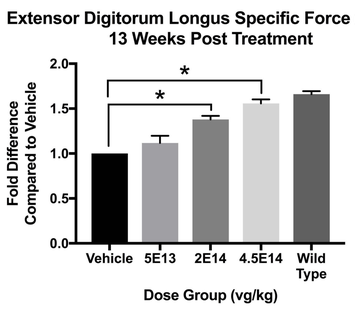
Results

SGT-001 Treated mdx Mice Show High Levels of Microdystrophin Expression and Rescued Muscle Function After 3 Months

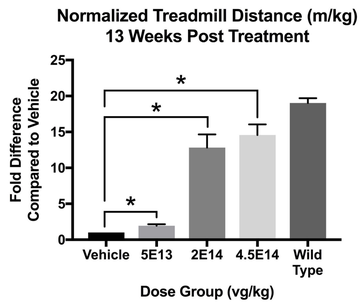




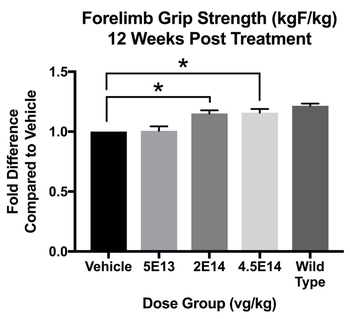
Data represent mean ± SEM
Animal numbers per dose group: n=5



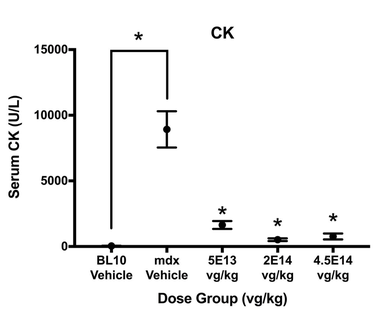
Data represent mean ± SEM
Animal numbers per dose group: Vehicle n=12, 5E13 n=10, 2E14 n=11, 4.5E14 n=10, Wild Type n=121
* p<0.05



Data represent mean ± SEM
Animal numbers per dose group: Vehicle n=12, 5E13 n=11, 2E14 n=12, 4.5E14 n=12, Wild Type n=12
* p<0.05

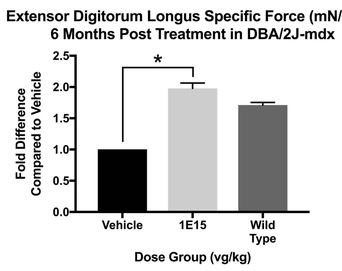


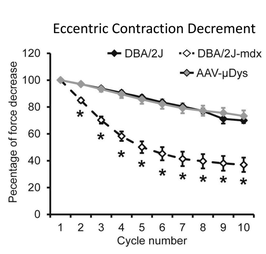
Data represent mean ± SEM
Animal numbers per dose group: Vehicle n=12, 5E13 n=11, 2E14 n=12, 4.5E14 n=12, Wild Type n=12
* p<0.05

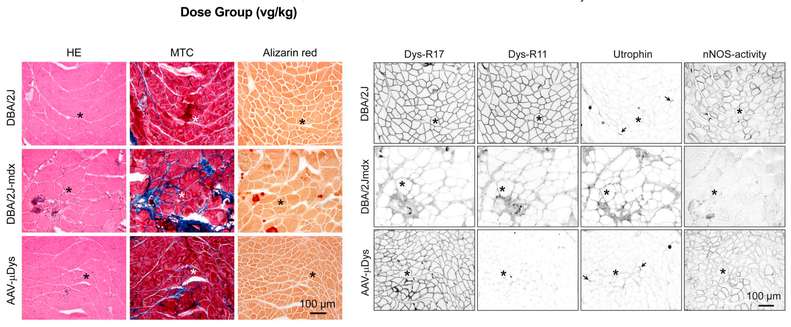


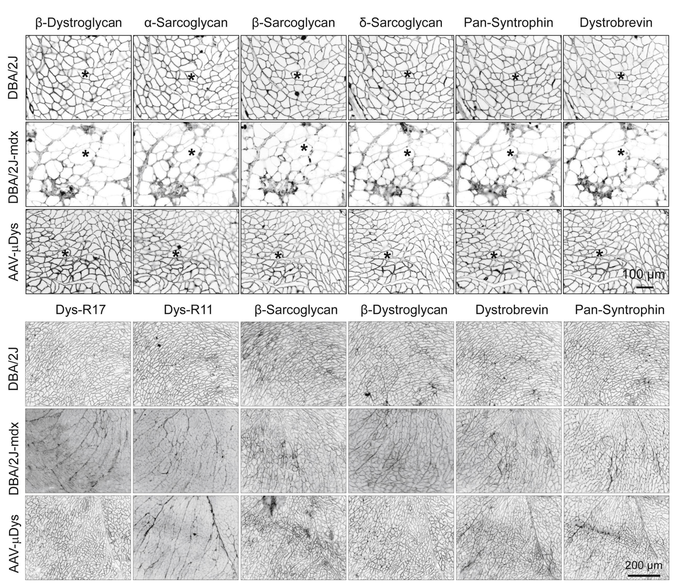
Data represent mean ± SEM
Animal numbers per dose group: Vehicle n=12, 5E13 n=11, 2E14 n=12, 4.5E14 n=12, Wild Type n=12
* p<0.05

DBA/2J-mdx Mice Treated with Canine SGT-001 Show Stabilization of DGC Members at the Sarcolemma, NO Production and Restored Muscle Function Near WT Levels After 6 Months

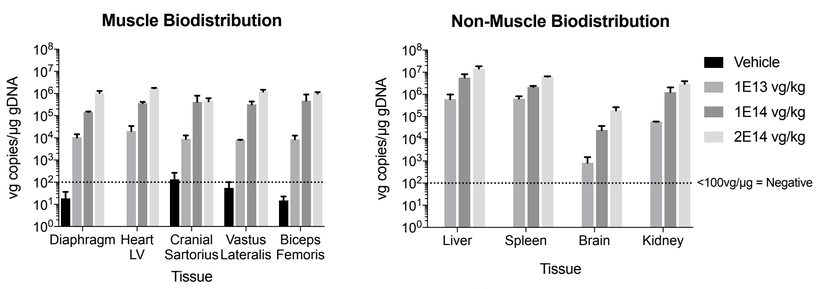


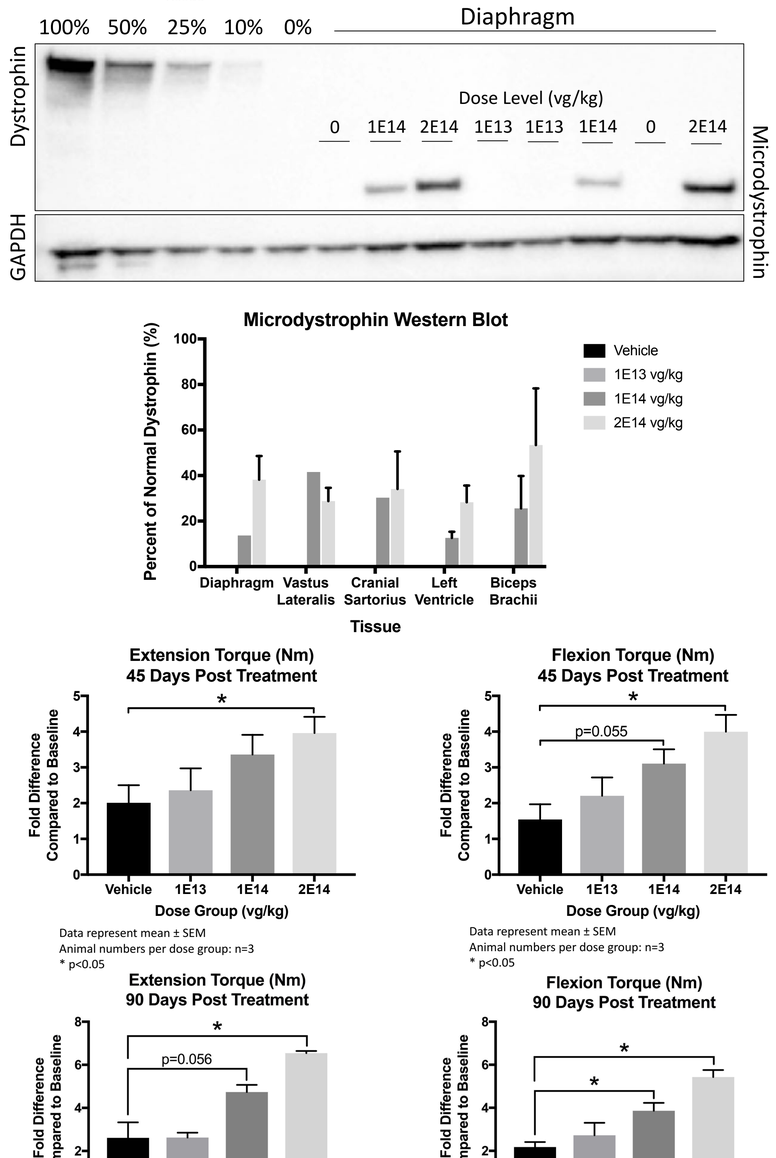






GRMD Canine Models of DMD Treated with Canine SGT-001 Display Bodywide Microdystrophin Expression in Muscle and Significantly Improved Muscle Function





Data represent mean ± SEM
Animal numbers per dose group: n=3
* p<0.05

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* p<0.05

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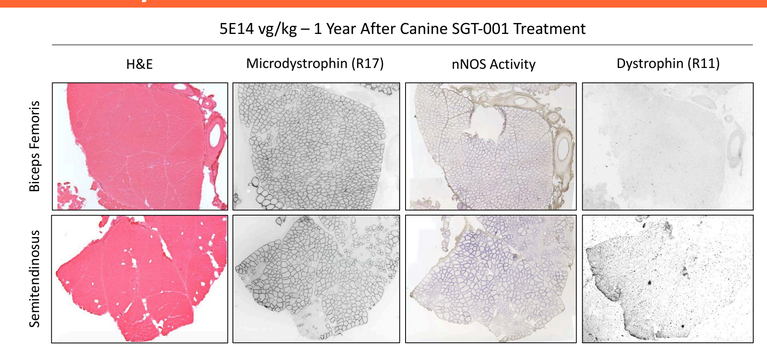
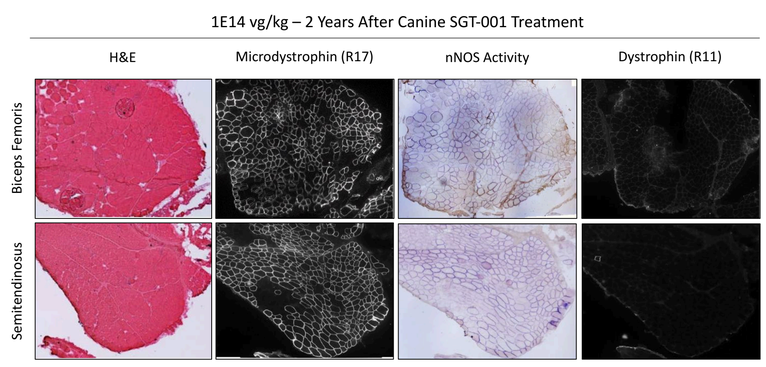
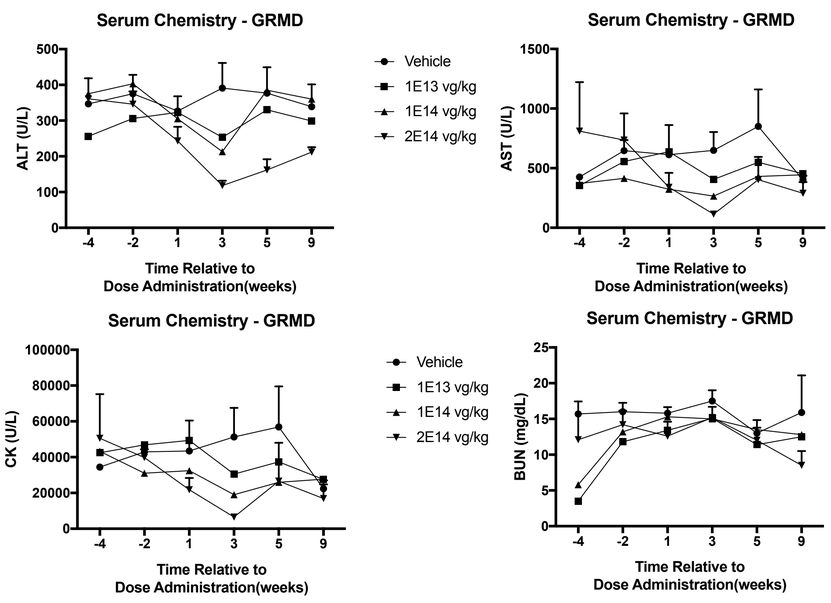
Animal numbers per dose group: n=3
* p<0.05

Microdystrophin Expression Persists Over 2 Years After a Single Systemic Administration of Canine SGT-001

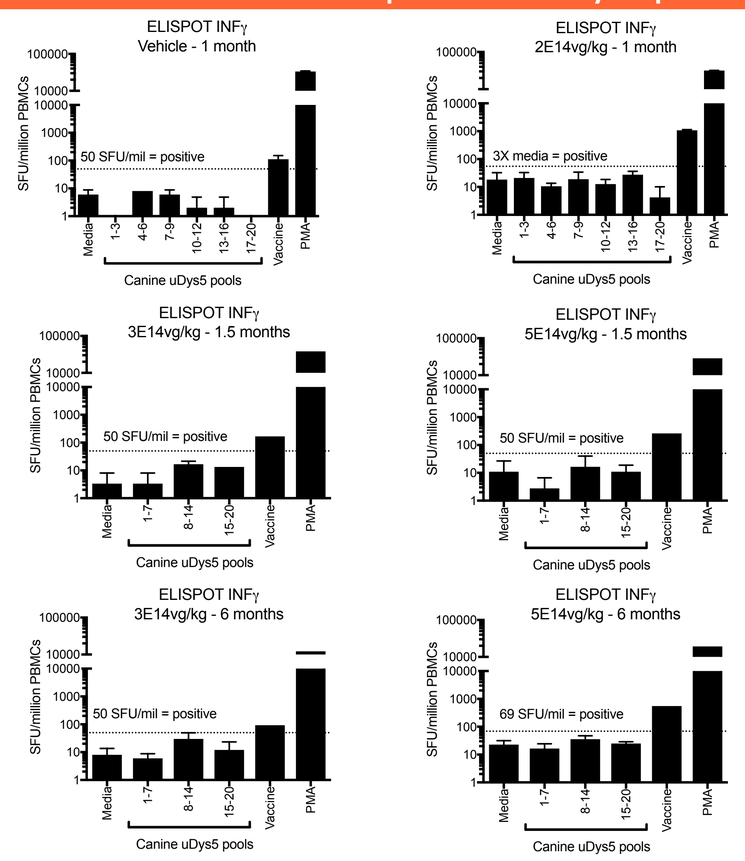




GRMD Animals Treated with Canine SGT-001 Do Not Display Adverse Test Article Related Changes in Serum Chemistry Parameters



At Doses Up To 5E14 vg/kg of Canine SGT-001, Canine Models of DMD Show No ELISPOT Response to Microdystrophin



Conclusions

- SGT-001 systemic administration results in widespread microdystrophin expression across target muscle tissues in a dose-dependent manner
- Microdystrophin expression corresponds with improvements in overall muscle histology and function in both small and large animal models of DMD
- SGT-001 mediated microdystrophin expression is durable, and persists for at least 2 years after administration of canine SGT-001
- Canine models of DMD treated with canine SGT-001 do not show signs of clinical pathology or an immune response to microdystrophin by ELISPOT analysis
- Data suggest SGT-001 may be a suitable candidate for DMD therapy

Acknowledgments

- Barry Byrne – University of Florida Powell Gene Therapy Center
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- Joe Kornegay – Texas A&M University
- Michael Lawlor– Medical College of Wisconsin

Quantification of Microdystrophin and Correlation to Circulating Biomarkers

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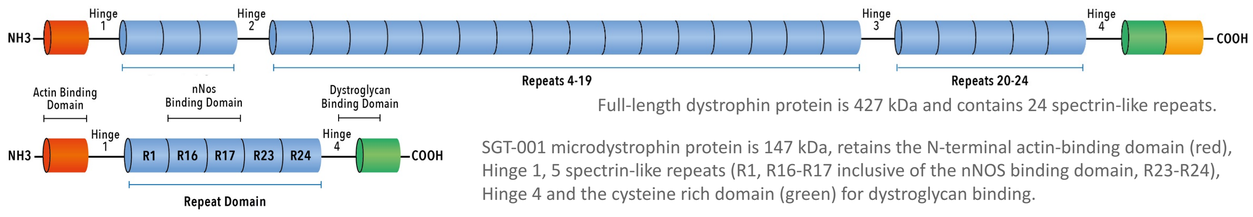
Introduction

- Solid Biosciences is developing SGT-001 for the treatment of Duchenne muscular dystrophy (DMD)
- SGT-001 is a rAAV9 vector containing a muscle-specific promoter that drives the expression of a 147 kDa human microdystrophin protein
- Quantification of microdystrophin levels provides an objective read-out of the drug mechanism of action and enables dose-optimization (dose-selection) in clinical trials
- Microdystrophin protein levels can be bridged to clinical outcomes, and thus serve as a surrogate outcome measure for predicting subsequent clinical benefit
- Quantification of microdystrophin was performed by Western blot (WB) and Mass Spectrometry (MS) with membrane localization visualized by IF in two dystrophic animal models treated with SGT-001
- All assays demonstrated robust and dose-dependent microdystrophin protein expression that correlated to improved functional efficacy
- Non-invasive biomarker discovery was performed on the SomaLogic platform

SGT-001

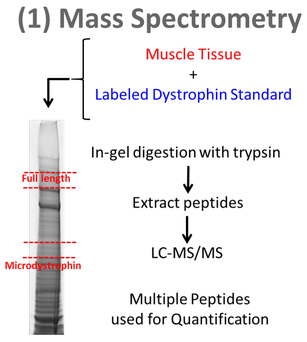


Full-length dystrophin protein is 427 kDa and contains 24 spectrin-like repeats.

SGT-001 microdystrophin protein is 147 kDa, retains the N-terminal actin-binding domain (red), Hinge 1, 5 spectrin-like repeats (R1, R16-R17 inclusive of the nNOS binding domain, R23-R24), Hinge 4 and the cysteine rich domain (green) for dystroglycan binding.

Accurate Quantification of Dystrophin

(1) Mass Spectrometry



(2) Western Blot

- Muscle proteins are extracted, separated by SDS-PAGE, transferred to a membrane and probed with an antibody reactive to full length and microdystrophin proteins.
- Each blot contains a 5-pt full length dystrophin calibration curve, R2>0.9
- Microdystrophin protein levels are reported as a percentage of normal based on a regression analysis of the control curve.

(3) Histology and Immunofluorescence

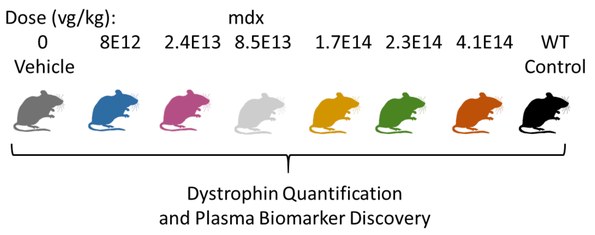
Muscle tissues are evaluated by H&E and IF stained using a microdystrophin antibody to visualize membrane localization, and IF stained with an antibody to full length dystrophin to differentiate between microdystrophin fibers and revertant fibers.

Results

Microdystrophin expression is dose responsive and correlates with functional improvements and biomarker response in two animal models of DMD

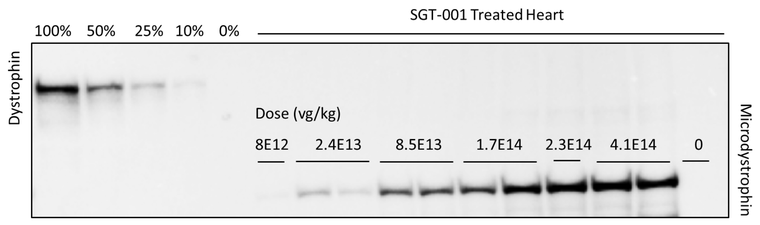
mdx Mouse Model

Study Design



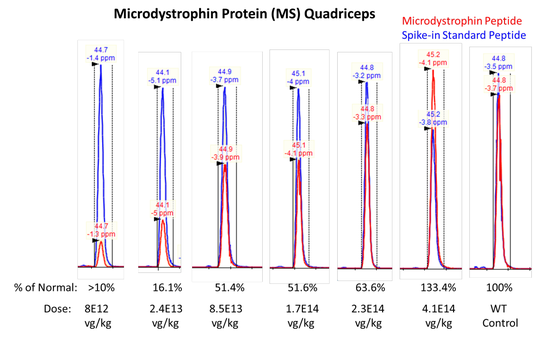
mdx mice, 6 weeks old, were systemically administered vehicle or SGT-001 at doses ranging 8E12 vg/kg to 4.1E14 vg/kg (n=4 per group) on Day 1. Terminal necropsy was performed on Day 29.

Western Blot

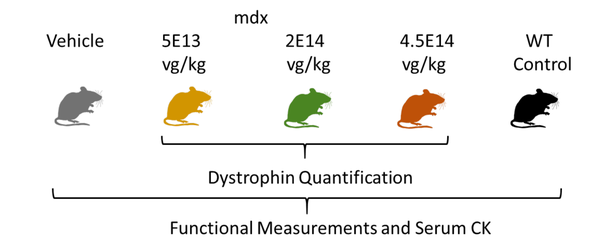


Microdystrophin protein was quantified in heart and quadriceps muscles from all study animals by WB and MS demonstrating the utility of MS based quantification of microdystrophin.

Mass Spectrometry

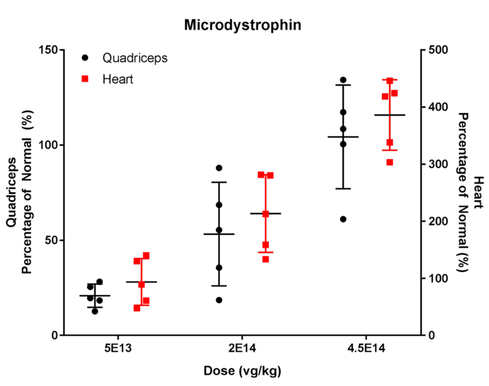


Study Design

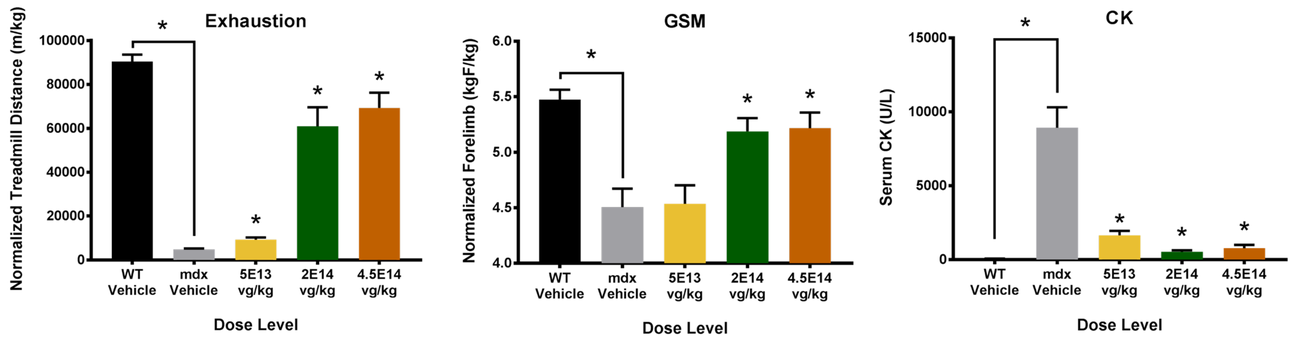


mdx mice, 6 weeks old, were systemically administered vehicle or SGT-001 at doses 5E13 vg/kg, 2E14 vg/kg or 4.5E14 vg/kg (n=5-12 per group) on Day 1. Terminal necropsy was performed at 13 weeks.

Mass Spectrometry



Functional Measurements and Serum CK

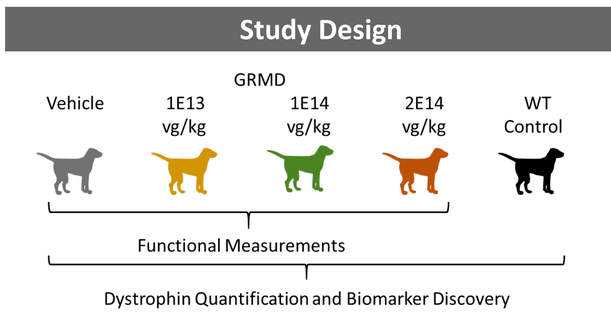


Total distance (13-week timepoint) and Absolute force (12-week timepoint) normalized to bodyweight. Data are presented as mean ± SEM. * p<0.05, adjusted, n=11-12

Serum creatine kinase levels, 13-week timepoint. Data are presented as mean ± SEM. * p<0.05, adjusted, n=7.

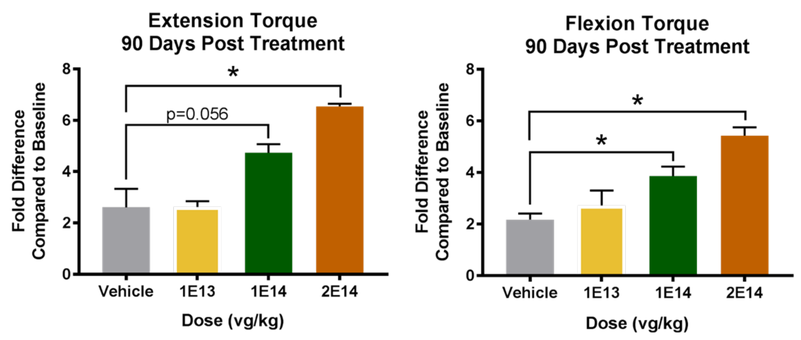
GRMD Dog Model

Study Design



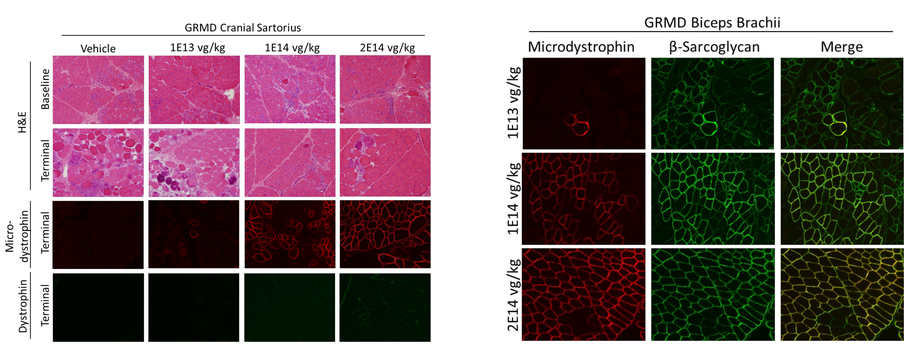
Juvenile GRMD dogs, 90 days old, were divided into four groups and administered a single systemic intravenous (IV) infusion on Day 1 of vehicle, or SGT-001 at 1E13 vg/kg, 1E14 vg/kg or 2E14 vg/kg (n=3 per group) and terminal necropsy performed on Day 90.

Functional Measurements

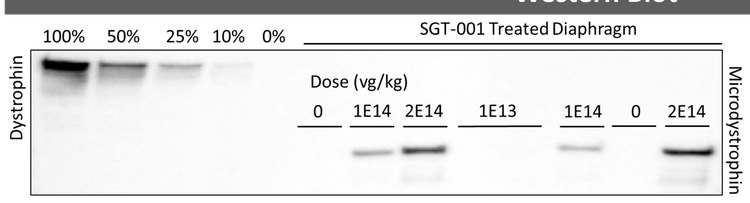


Analysis of torque produced by extension and flexion of the tibiotarsal joint. *p<0.05

Histology and Immunofluorescence

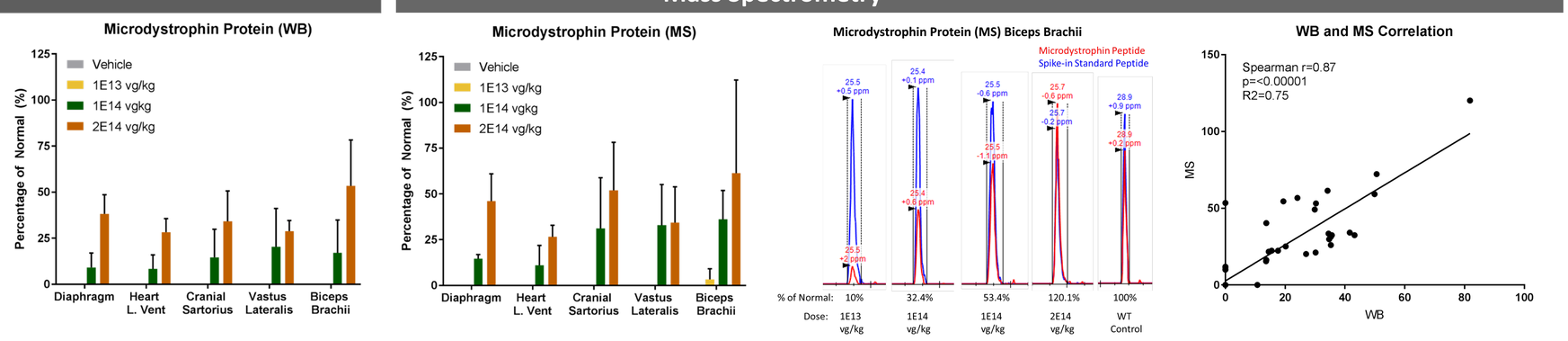


Western Blot



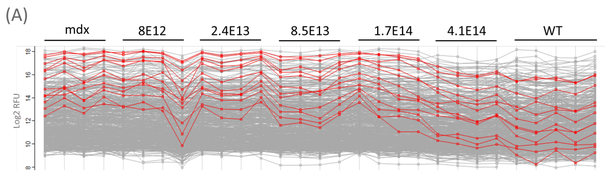
Microdystrophin protein was quantified in 5 muscles from all study animals by WB and MS and showed a strong correlation between the assays. H&E demonstrated improved histology with treatment. IF demonstrated proper membrane localization as well as increased β-sarcoglycan expression.

Mass Spectrometry

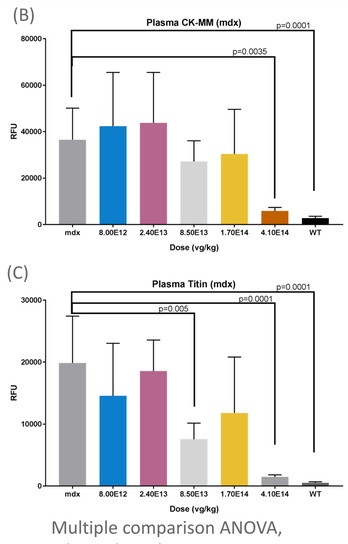


Exploratory Biomarkers-SomaLogic Platform

The majority of biomarkers identified in natural history studies are elevated in DMD compared to healthy controls, and decrease over time as muscle mass is lost. These biomarkers were confirmed in our two animal models of DMD (mdx, GRMD) compared to WT controls and responded efficaciously to SGT-001 treatment.

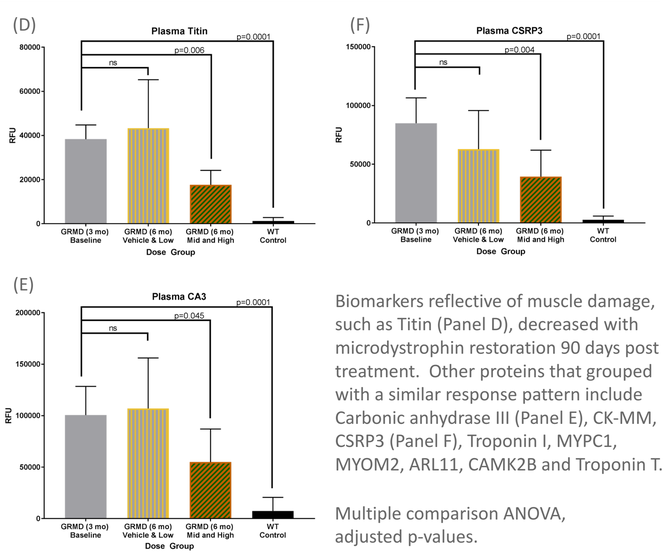


Creatine kinase M-type (Panel B) levels decreased in response to SGT-001 treatment at 28-days post treatment in mdx mice. Other proteins that grouped with a similar dose-response pattern (Panel A) include Triosephosphate isomerase, PURA1, Transketolase, TITIN (Panel C), Semenogelin, LDH-H 1, GPDA, VAPA, CAN3 and NHLC3.



Multiple comparison ANOVA, adjusted p-values.

GRMD Dog



Biomarkers reflective of muscle damage, such as Titin (Panel D), decreased with microdystrophin restoration 90 days post treatment. Other proteins that grouped with a similar response pattern include Carbonic anhydrase III (Panel E), CK-MM, CSRP3 (Panel F), Troponin I, MYPC1, MYOM2, ARL11, CAMK2B and Troponin T.

Multiple comparison ANOVA, adjusted p-values.

Conclusions

- SGT-001 treatment results in a dose-dependent production of microdystrophin protein
- Microdystrophin protein is membrane localized and increased levels improves muscle histology and β-sarcoglycan expression
- Microdystrophin protein expression is readily quantified by Western Blot and Mass Spectrometry assays
- Microdystrophin expression correlates to improved functional outcome
- Microdystrophin protein expression results in concomitant biomarker alterations reflective of improved functional outcomes
- Preclinical data support SGT-001 for the treatment of Duchenne muscular dystrophy