Systemic Gene Transfer with AAVrh74.MHCK7.SGCB Increased β-sarcoglycan Expression in Patients with Limb Girdle Muscular Dystrophy Type 2E

Louise R. Rodino-Klapac[1,3], Eric R. Pozsgai[1,3], Sarah Lewis[1,3], Danielle A. Griffin[1,3], Aaron S. Meadows[1,3], Kelly Lehman[1], Katie Church[1], Linda Lowes[1], Jerry R. Mendell[1,2]

[1]Center for Gene Therapy, The Research Institute at Nationwide Children's Hospital, Columbus, Ohio, USA; [2]Department of Pediatrics and Neurology, The Ohio State University, Columbus, OH, USA, The Ohio State University, Columbus, OH, USA; [3]Sarepta Therapeutics, Inc., Cambridge, MA, USA

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Many LGMDs Are Caused by Defects in the Sarcolemmal Proteins That Comprise the Dystrophin-associated Protein Complex

- **Sarcoglycans** prevent muscle damage during contraction[1]
  - Mutations in any of the following 4 subunits of the sarcoglycan complex lead to muscular dystrophy[1]
    - **β-sarcoglycan**
    - **α-sarcoglycan**
    - **γ-sarcoglycan**
    - **δ-sarcoglycan**
  - Sarcoglycan deficiency leads to dystrophin deficiency[1]
- **Dysferlin** and **anoctamin-5** support muscle membrane repair[2]
  - Failed muscle repair leads to chronic muscle degeneration

SCHEMATIC OF SARCOLEMMA*

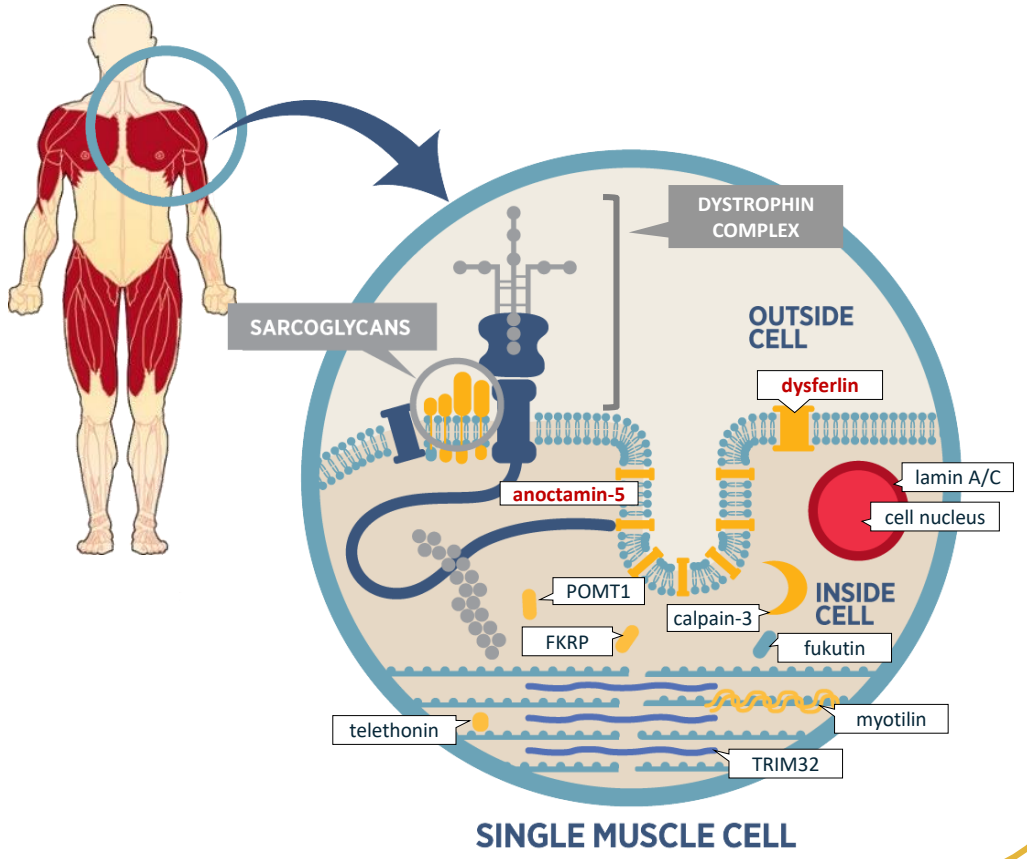
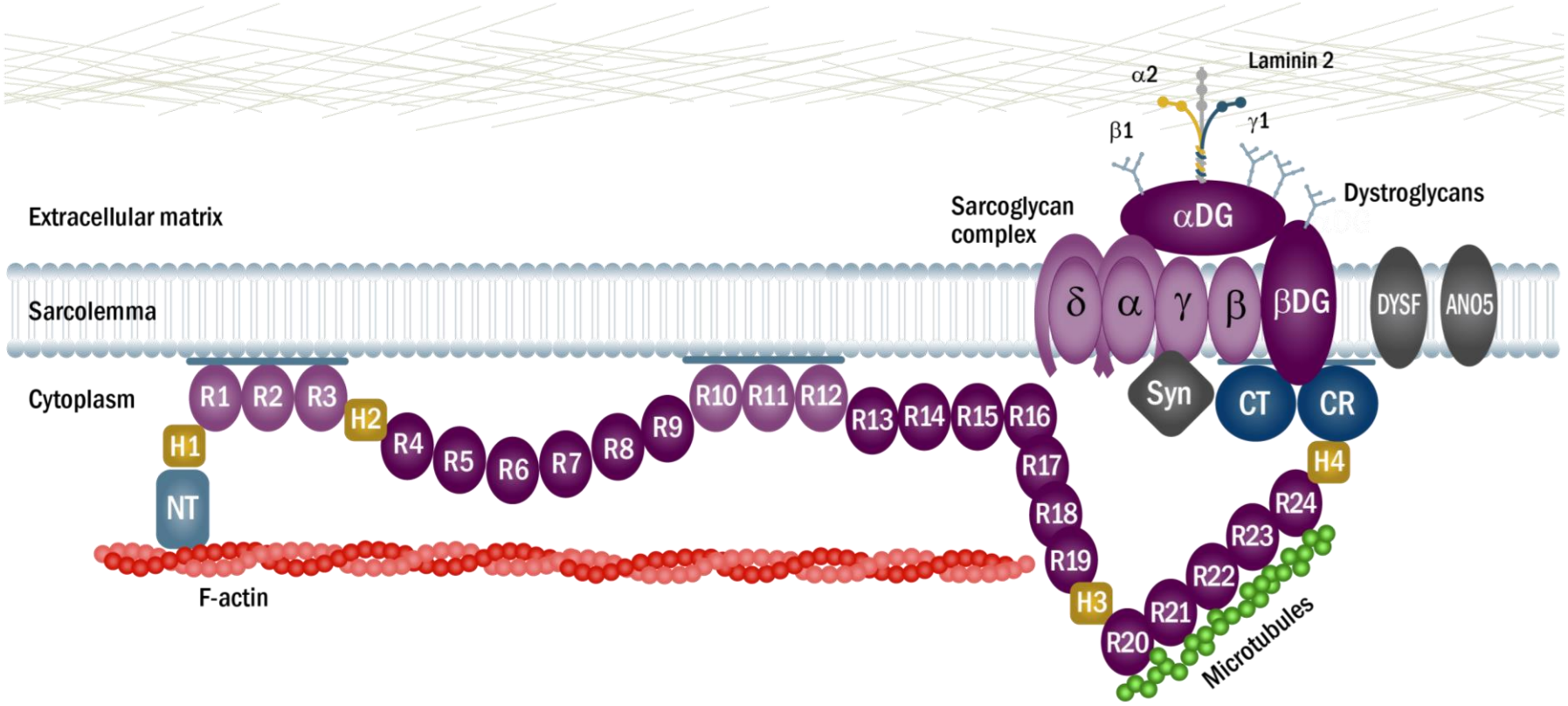


*Image adapted from the MDA website. Causes/Inheritance. www.mda.org/disease/limb-girdle-muscular-dystrophy/causes-inheritance. Accessed December 12, 2018.

1. McNally EM. The Sarcoglycans. Landes Bioscience 2000-2013. www.ncbi.nlm.nih.gov/books/NBK6317/. Accessed December 16, 2018. 2. Liewluck T, Milone M. *Muscle Nerve.* 2018;58(2):167-177.

The Dystrophin-Associated Protein Complex (DAPC)

- The relationship between dystrophin and the DAPC is both intricate and dependent
- The dystrophin complex **stabilizes the plasma membrane** of striated & cardiac muscle cells
- Loss of function **mutations** in the genes encoding dystrophin, or the associated proteins, **trigger instability of the plasma membrane and myofiber & cardiomyocyte loss**



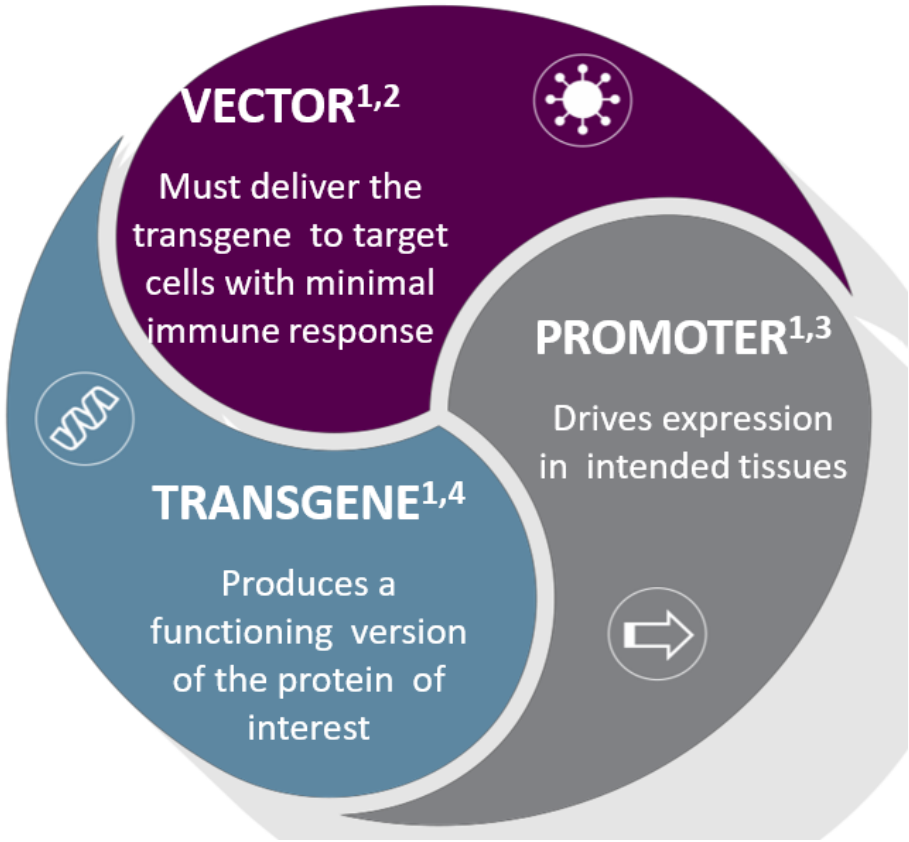
nNOS, neuronal nitric oxide synthase.

Image adapted from Fairclough RJ, et al. *Nat Rev Genet*. 2013;14(6):373-378.

Adeno-associated Virus (AAV)-Mediated Gene Transfer

- Adeno-associated viruses (AAV) are small, non-enveloped viruses that, unlike adenoviruses, have not been associated with human disease.[5,6]
- Several AAV serotypes have been identified, each with a different tissue tropism. This allows for specific tissue targeting with AAV-mediated gene therapies.[5]
- Delivers transgenes via nonintegrating, stable, extrachromosomal episomes to the nucleus, thereby limiting the risk of insertional mutagenesis seen with other viral vectors.[5]

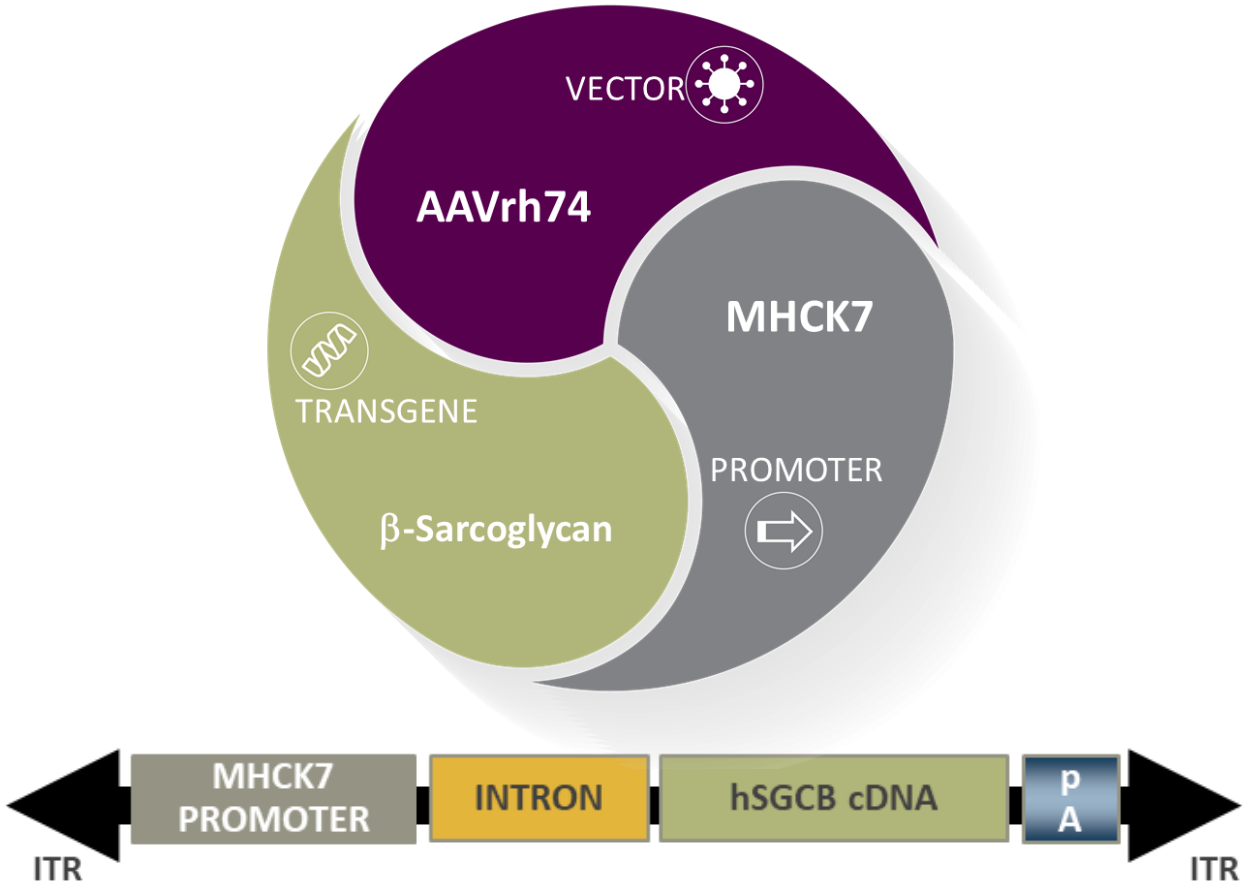
Essential Components of AAV Gene Therapy



1. Naso MF, et al. BioDrugs. 2017;31(4):317-334. 2. US National Library of Medicine, Lister Hill National Center for Biomedical Communications. Genetics Home Reference. Help me Understand Genetics: *Gene Therapy*. Bethesda, Maryland: 2013. https://ghr.nlm.nih.gov/primer/therapy/genetherapy. Accessed August 29, 2018. 3. Zheng C, Baum J. *Methods Mol Biol*. 2008;434:205-219. 4. Chamberlain K, et al. *Hum Gene Ther Methods*. 2016;27(1):1-12. 5. Balakrishnan B, Jayandharan GR, *Curr Gene Ther*. 2014; 14:1-15. 6. Atchison RW et al. Science 1965; 149(3685): 754-6.

scAAVrh74.MHCK7.hSGCB for the Treatment of LGMD2E (β-sarcoglycanopathy)

scAAVrh74.MHCK7.hSGCB



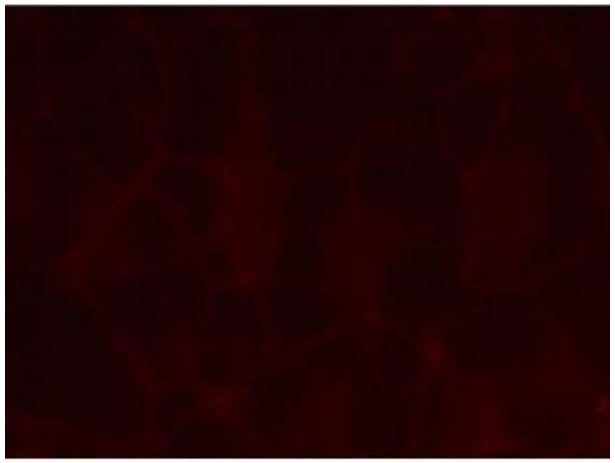
- **The scAAVrh74.MHCK7.hSGCB construct was designed to restore functional β-sarcoglycan to muscle cells**
  - **AAVrh74** provides superior systemic delivery, including to cardiac muscle
  - The **MHCK7** promoter allows for robust and widespread β-sarcoglycan expression
  - **β-sarcoglycan** data at $5x10^{13}$ vg/kg highlights the unique attributes of the AAVrh74/MHCK7 construct to target cardiac and skeletal muscle

ITR, inverted terminal repeat; hSGCB, human β-sarcoglycan; pA, poly-A tail

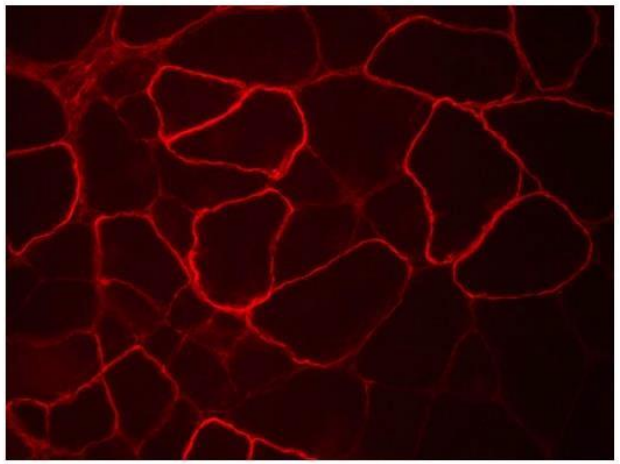
Pre-Clinical Models Correlated Expression and Function

≥20% β-sarcoglycan expression leads to increased function

Pretreatment

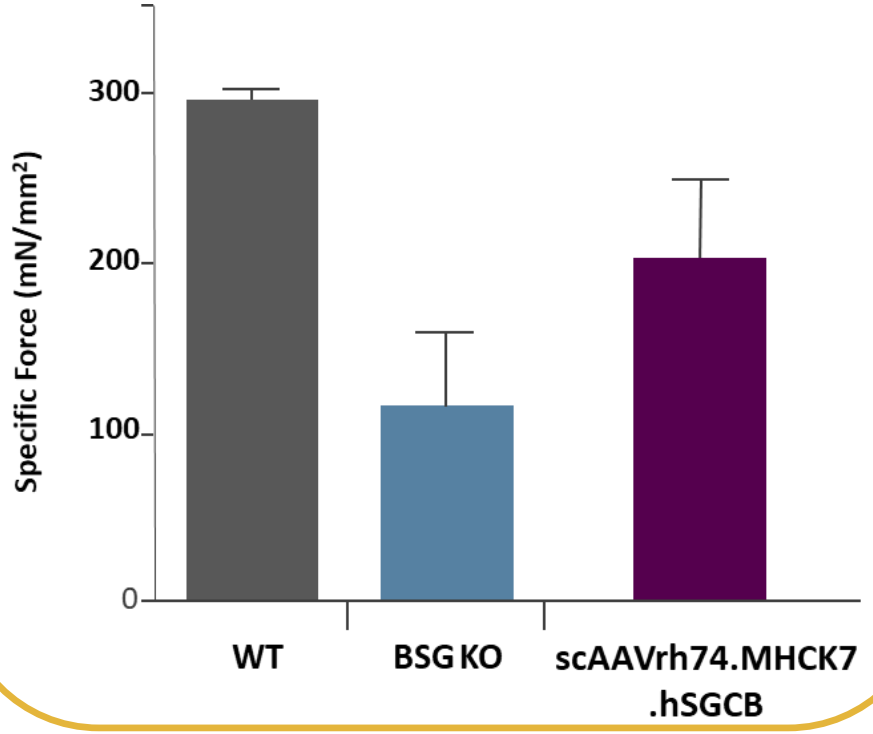


Post Treatment



$5x10^{12}$ vg/kg scAAVrh74.MHCK7.hSGCB

Function

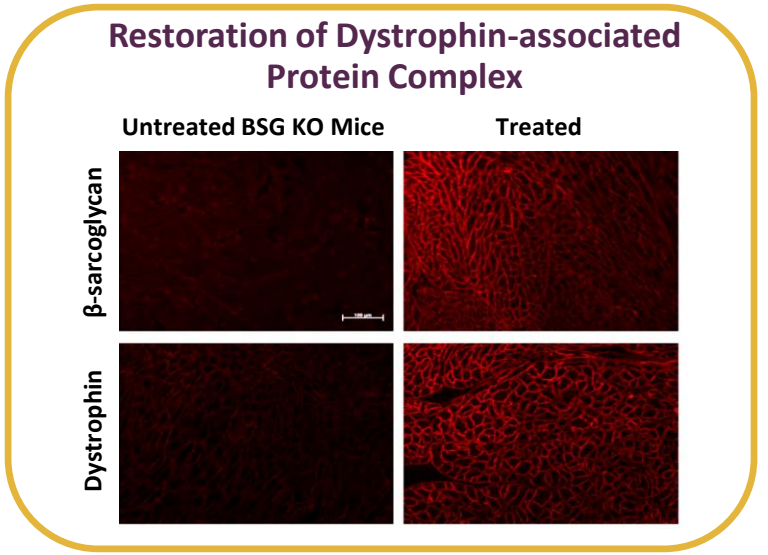


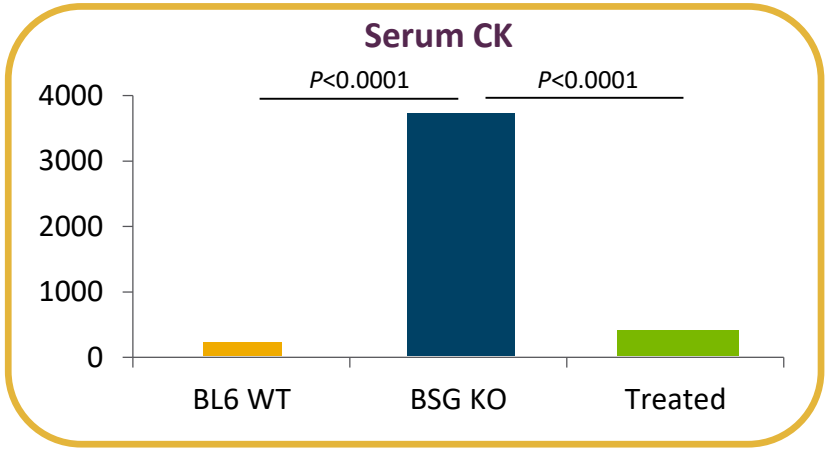
Pozgai ER, et al. *Mol Ther*. 2017;25(4):855-869.

Systemic Delivery of scAAVrh74.MHCK7.hSGCB Reconstitutes Full-Length β-sarcoglycan in Mouse Studies

- This led to 98.1% transgene expression across all muscles, which was accompanied by improvements in histopathology
  - >95% expression in the diaphragm and heart
- Serum CK levels were reduced following treatment by 85.5%
- Diaphragm force production increased by 94.4%
- Kyphoscoliosis of the spine was significantly reduced by 48.1%
- Overall ambulation increased by 57%
- No safety issues were observed in pre-clinical safety studies

These findings provided the justification to move scAAVrh74.MHCK7.hSGCB to clinical trial





Pozsgai ER, et al. *Mol Ther*. 2017;25(4):855-869.

LGMD (β-Sarcoglycanopathy) Phase I/II Study

Cohort 1 (N=3)

Phase I/II Open-Label Trial Design in Subjects with LGMD2E (β-sarcoglycanopathy)

- **Up to 9 subjects with LGMD2E**
  - Cohort 1: 3 subjects; 4-15 years of age, $5x10^{13}$ vg/kg scAAVrh74.MHCK7.SGCB
- **Inclusion criteria**
  - A confirmed SGCB mutation in both alleles
  - Negative for AAVrh74 antibodies
  - >40% of Normal 100 meter walk test
- **60-day muscle biopsy**
- **Prednisone 1 day prior to gene transfer, 30 days 1 mg/kg, taper**
- **Primary endpoints**
  - Expression: ≥20% β-sarcoglycan positive fibers
  - Safety
- **Secondary endpoints, including:**
  - Decrease in CK

LGMD2E Subject Demographics at Baseline

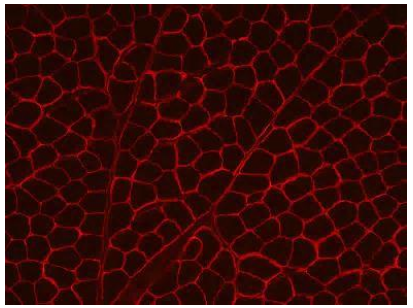
Subject	Age (years)	Mutation	Weight (kg)	CK Levels at Baseline (U/L)
1	13	Exon 3	55	10,727
2	4	Exon 4	17	12,826
3	13	Exon 3	50	10,985

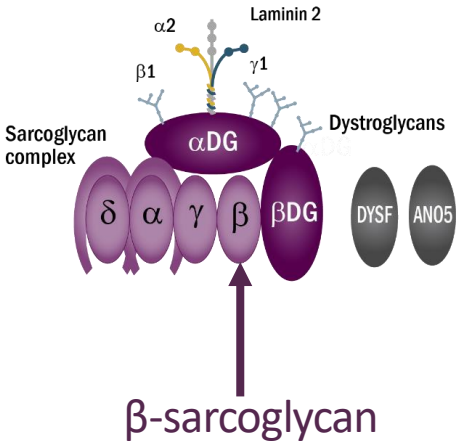
Exons 3-6 encode the extracellular domain and Semplicini 2015 paper showed mutations in these exons led to severe phenotype with cardiomyopathy and complete absence or severely reduced expression.[1]

1. *Semplicini C*, et al. Neurol. 2015;84(17):1772-1781

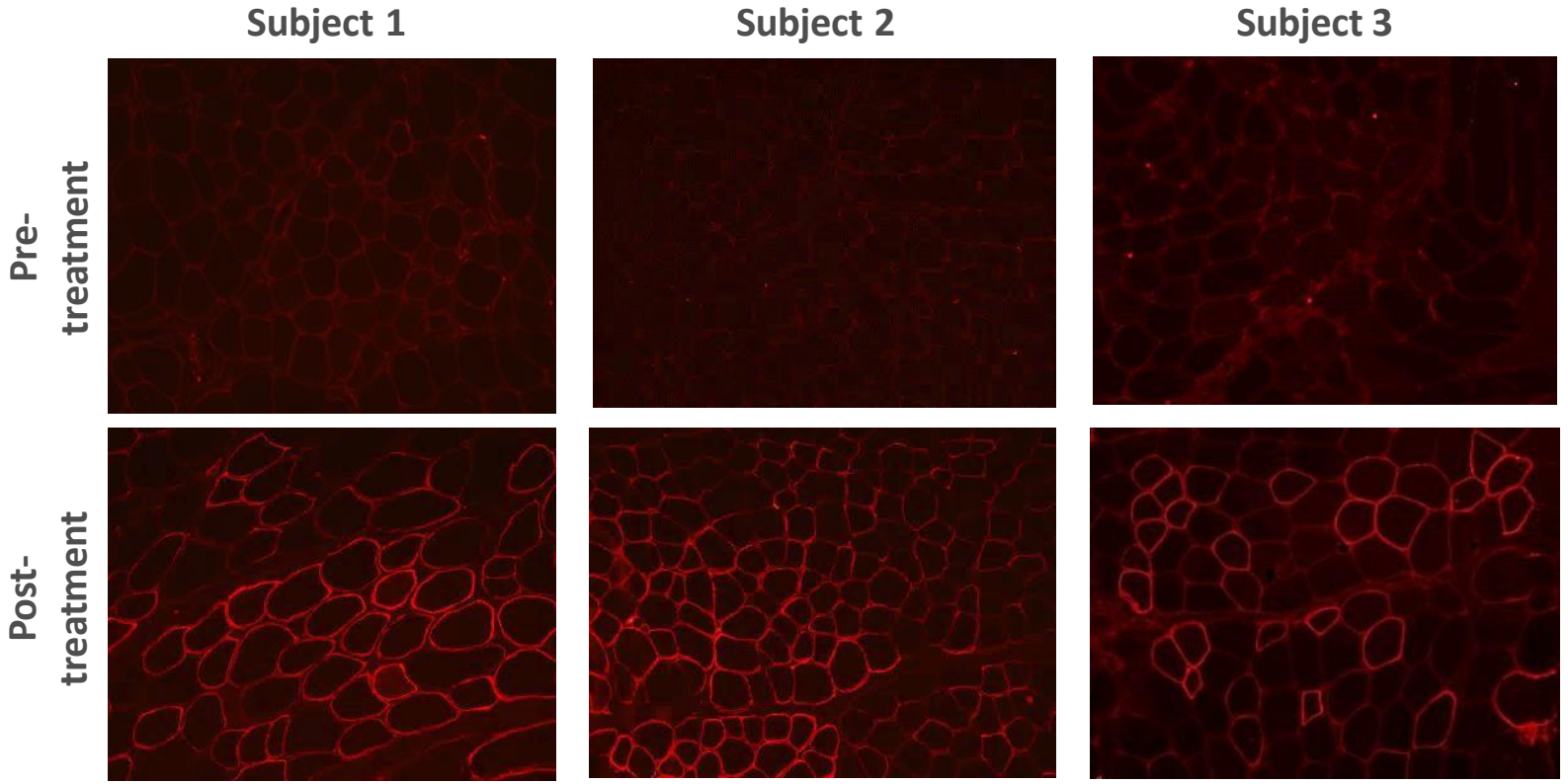
Robust β-sarcoglycan Expression In Muscle Biopsies In All 3 Subjects at a Dose of $5X10^{13}$ vg/kg

Normal Control





β-Sarcoglycan Expression (IHC)



	Percentage of SGCB-positive Fibers	Intensity
Mean (n=3)	51%	47%

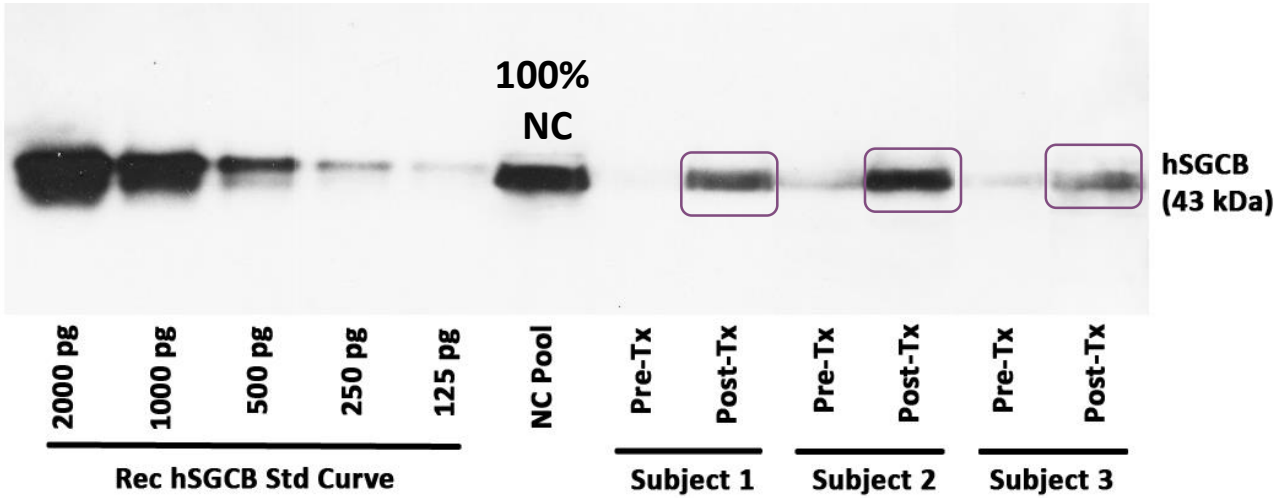
NCT03652259; Data on file, Sarepta Therapeutics Inc

Robust β-sarcoglycan Expression in Muscle Biopsies in All 3 Subjects at a Dose of $5x10^{13}$ vg/kg

Subject	Percentage of SGCB-Positive Fibers	Mean Intensity
1	63%	47%
2	49%	57%
3	42%	38%
Mean	**51%**	**47%**

NCT03652259; Data on file, Sarepta Therapeutics Inc

Robust and Consistent β-sarcoglycan Expression in All 3 Subjects As Measured by Western Blot Post-treatment



Pre-Tx Below LOQ
LOQ = 5% NC

	Mean β-sarcoglycan Expression (N=3) vs Normal
Mean	36.1%

The gene transfer delivers full-length β-sarcoglycan

NCT03652259; Data on file, Sarepta Therapeutics Inc

LOQ = Limit of Quantification, NC = Normal Control

Robust and Consistent β-sarcoglycan Expression in All 3 Subjects as Measured by Western Blot Post-Treatment

Subject	Mean β-Sarcoglycan Expression (N=3) vs Normal
1	34.7%
2	39.2%
3	34.5%
Mean	**36.1%**

The gene transfer delivers full length β-sarcoglycan

NCT03652259; Data on file, Sarepta Therapeutics Inc

The Optimized Vector and Promoter Provided Robust Expression at $5X10^{13}$ vg/kg

Vector Genome Number

	Vector Copies/µg DNA	Copies per Nucleus
Mean (n=3)	8.4E04	0.60

Beta-Sarcoglycan Expression (IHC)

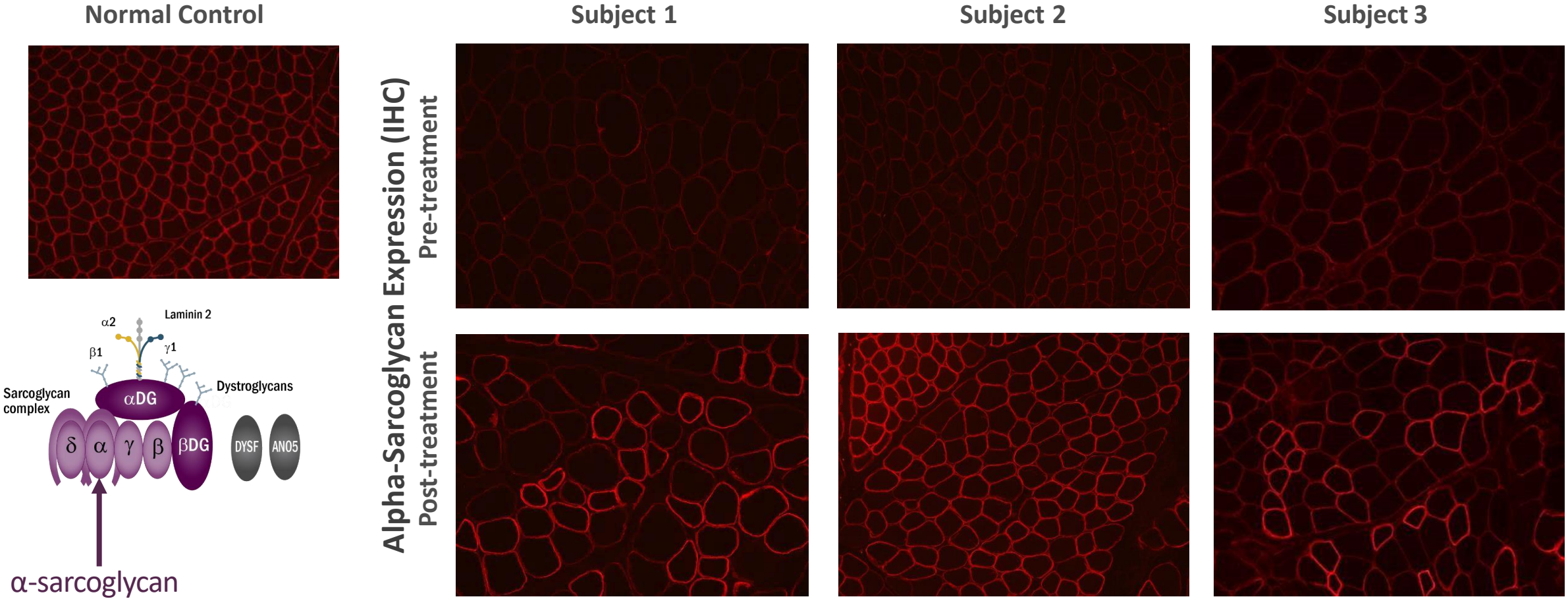
	Percentage of β-sarcoglycan-positive Fibers	Intensity
Mean (n=3)	51%	47%

Beta-Sarcoglycan Expression (Western Blot)

	Percent of Normal
Mean (n=3)	36.1%

NCT03652259; Data on file, Sarepta Therapeutics Inc

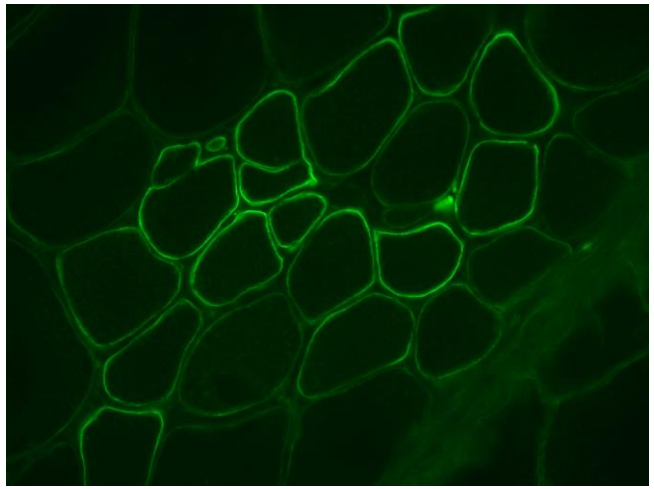
Robust β-sarcoglycan Expression Significantly Upregulated Sarcoglycan Complex at a Dose of $5x10^{13}$ vg/kg



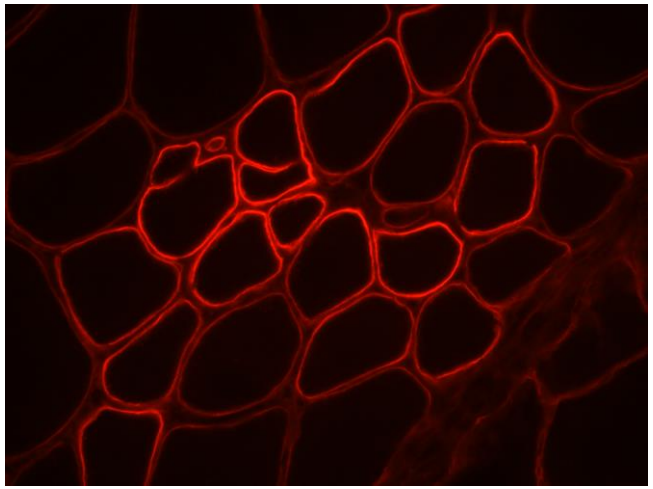
NCT03652259; Data on file, Sarepta Therapeutics Inc

β-Sarcoglycan Gene Transfer Restores Sarcoglycan Complex to the Membrane

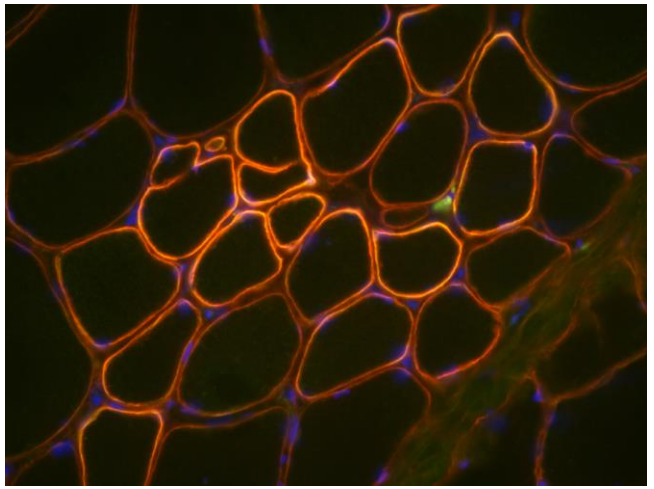
β-sarcoglycan



α-sarcoglycan



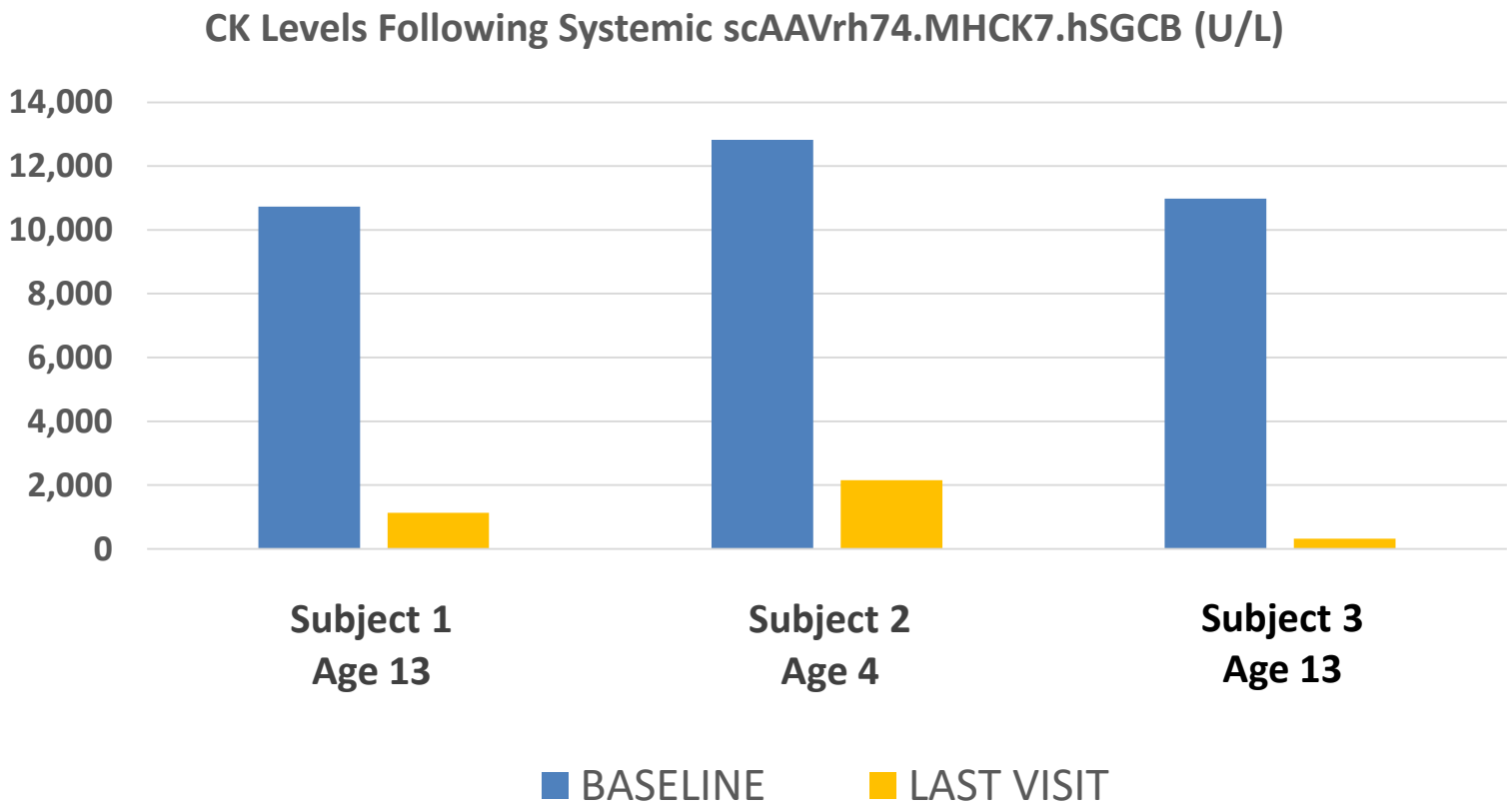
Colocalization (Merge)



NCT03652259; Data on file from Patient 3, Sarepta Therapeutics Inc

90% Mean Reduction of Creatine Kinase (CK) Levels Observed with Systemic β-sarcoglycan Gene Transfer

- Patients in Study 1 were on a rigorous steroid regimen through at least one day before to at least one month post dose
- Drops in CK have not been observed following only GC treatment. Example:
  - In Duchenne muscular dystrophy, GCs are standard of care
  - Marked drops in CK are not observed and levels remain elevated
- Following SCGB gene transfer, CK levels fell dramatically

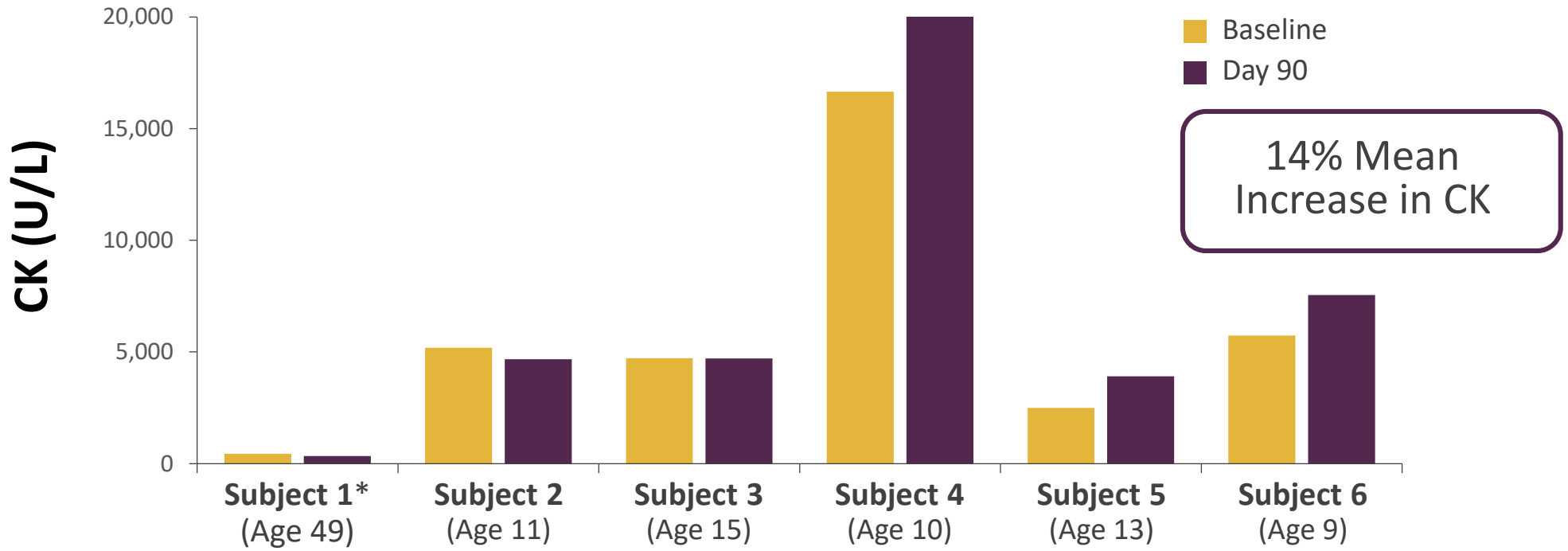


90% Mean Reduction in CK

NCT03652259; Data on file, Sarepta Therapeutics Inc

Steroids Had No Impact on CK Levels in A Prior, Lower-Dose, Non-systemic LGMD2D (α-sarcoglycan) Study

CK Levels Following ILP α-sarcoglycan Delivery



LGMD2E and LGMD2D studies share the same steroid protocol

*Non-ambulant patient; ClinicalTrials.gov Identifier: NCT03652259. Data on File, Sarepta Therapeutics Inc.

Safety and Tolerability of scAAVrh74.MHCK7.SGCB

- **Patients 1,2: 90 days follow up, patient 3: 60 days follow up**
- **Two patients had elevated liver enzymes, one of which was designated a serious adverse event (SAE), as the patient had associated transient increase in bilirubin**
  - Both events occurred when the patients were tapered off oral steroids
  - Elevated liver enzymes returned to baseline and symptoms resolved within days following supplemental steroid treatment
- **No other clinically significant laboratory findings**
  - No decreases in platelet counts observed
- **Two patients had transient mild nausea generally within the first week coincident with increased steroid dosing**
  - Did not correlate with liver enzyme elevations or any other abnormality

Conclusions

- **scAAVrh74.MHCK7.SGCB is an adeno-associated virus (AAV)-mediated gene therapy designed to deliver functional β-sarcoglycan to muscle cells**
- **Systemic treatment of 3 LGMD2E patients with scAAVrh74.MHCK7.SGCB led to robust and consistent expression of β-sarcoglycan in muscle fibers 60 days post gene transfer**
  - Co-localization of β-sarcoglycan with α-sarcoglycan suggests that scAAVrh74.MHCK7.SGCB restores the sarcoglycan complex
- **Substantial (90%) reductions in CK were observed in all 3 patients**
- **These findings support our approach to rational approach to systemic AAVrh74-mediated gene transfer and is our second gene therapy to demonstrate robust transgene expression in patients with a form of muscular dystrophy (AAVrh74.MHCK7.Micro-dystrophin in DMD)**

Thank you