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Evaluating pharmacology and efficacy of delandistrogene moxeparvovec in DMD^{mdx} rats

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Objective

To evaluate the efficacy and myocardial safety of delandistrogene moxeparvovec (SRP-9001) in DMD^{mdx} rats.

What does this study mean for the DMD community?

These findings confirmed the expected SRP-9001 dystrophin protein expression in cardiac muscle, and demonstrated the efficacy and myocardial safety of delandistrogene moxeparvovec.

CONCLUSIONS

- **Data from 12 weeks and 24 weeks post-systemic delandistrogene moxeparvovec delivery demonstrated no evidence of cardiac toxicity.**
- **DMD^{mdx} rats treated with delandistrogene moxeparvovec exhibited improved histopathology and reduced fibrosis.**
- **This study demonstrated the efficacy and myocardial safety of delandistrogene moxeparvovec in an animal model of DMD that exhibits cardiac dysfunction.**

BACKGROUND

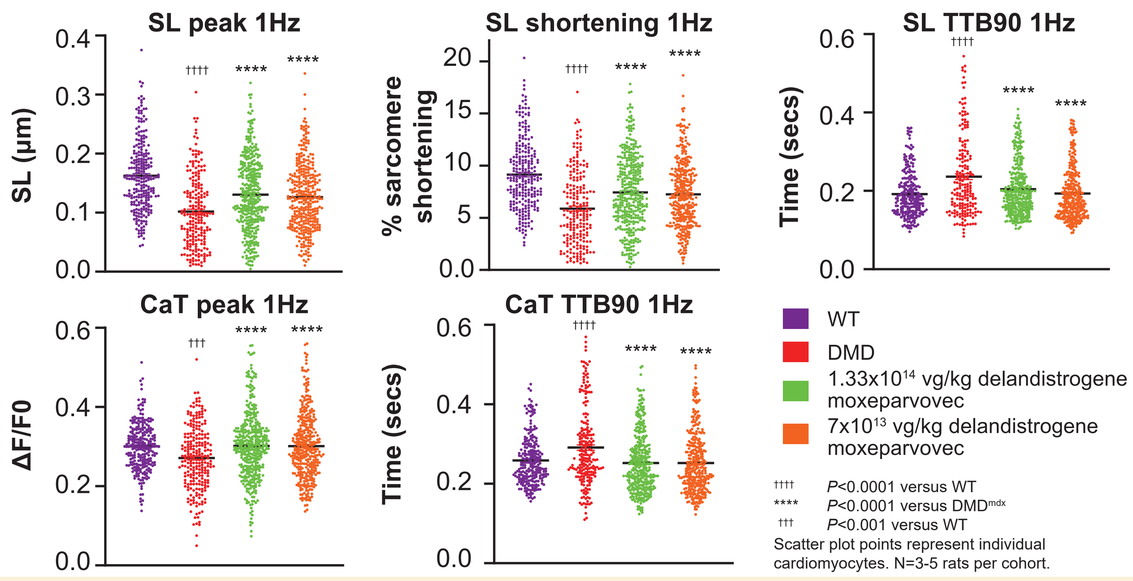
- Gene transfer therapy is a promising treatment for patients with DMD.
- Delandistrogene moxeparvovec is an investigational gene transfer therapy developed for targeted skeletal and cardiac muscle expression of SRP-9001 dystrophin—a shortened, functional dystrophin protein.
- Systemic delivery of delandistrogene moxeparvovec in the DMD^{mdx} mouse model led to improvements in dystrophic histopathology and function of skeletal muscle, with no toxicity observed.[1]
- DMD^{mdx} mice do not develop early dilated cardiomyopathy, as seen in patients.[2] To evaluate the efficacy and safety of delandistrogene moxeparvovec in the heart, DMD^{mdx} rats present a valuable alternative animal model of DMD, as they demonstrate cardiac dysfunction that recapitulates cardiac dysfunction in patients with DMD.

METHODS

- We performed systemic, intravenous delivery of delandistrogene moxeparvovec in 21- to 35-day-old Sprague–Dawley *DMD*-mutated, dystrophin-null (DMD^{mdx}) rats.[3,4]
- Rats received a dose (1.33x10^{14} or 7.00x10^{13} vg/kg) of delandistrogene moxeparvovec or 0.9% sterile saline unless otherwise specified.
- Analyses of expression, biodistribution, physiology and activity were conducted.
- Ambulation and vertical activity were recorded via the Photobeam Activity System—Open Field.[5]
- Echocardiograms, serum troponin I analysis, and histological analyses of fibrosis were used to evaluate cardiac disease.
- Individual cardiomyocyte function was assessed using sarcomere shortening and calcium transient analyses.
- Cardiomyocytes were enzymatically isolated using Liberase TH; calcium was reintroduced step-wise to 1.8mM. Myocytes were incubated in a low Ca2+ Tyrode solution containing 5µM Fura-2AM for 30-35 minutes at room temperature. Intracellular Ca2+ transient and sarcomere shortening measurements were induced by electrical field stimulation between 0.2Hz-4Hz. Cardiomyocyte and calcium release measurements occurred on 12-week-old (+/- 1 week) rats.
- Endpoints were measured at 12 and 24 weeks.
- Twelve-week sample sizes were n=10 (delandistrogene moxeparvovec) and n=8 (saline), and 24-week sample sizes were n=6 (delandistrogene moxeparvovec) and n=5 (saline), unless otherwise specified.

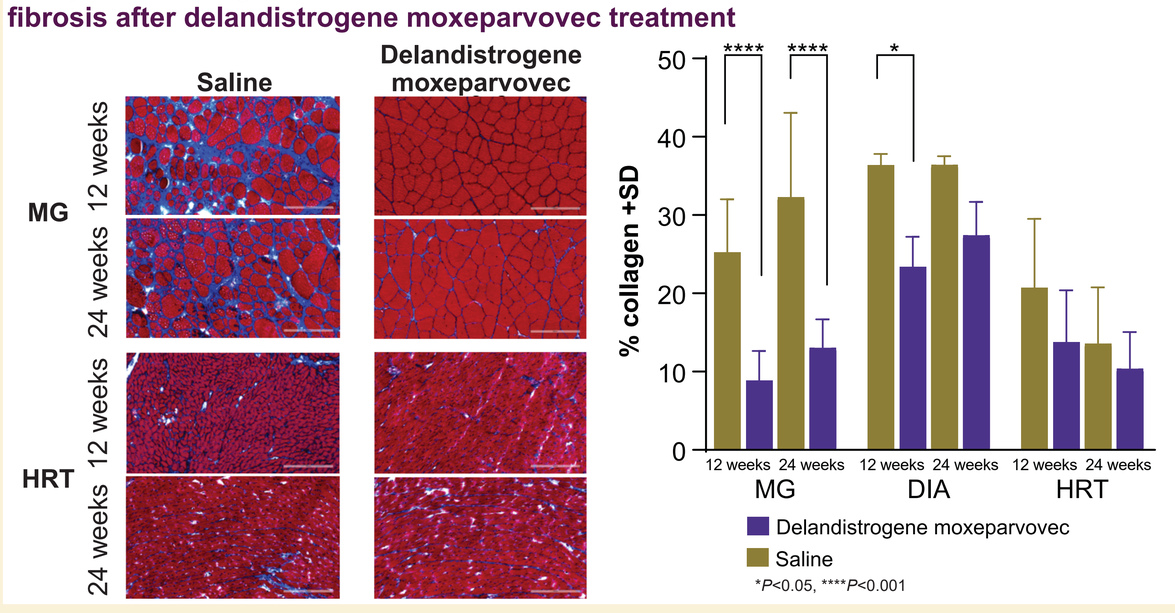
RESULTS

Delandistrogene moxeparvovec restores cardiomyocyte contractile function and calcium kinetics in DMD^{mdx} rats



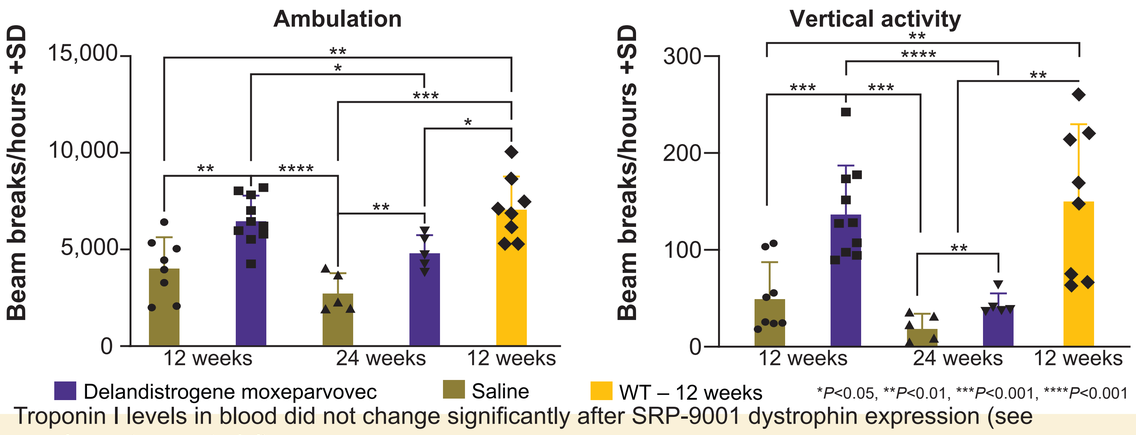
†††† *P*<0.0001 versus WT
**** *P*<0.0001 versus DMD^{mdx}
††† *P*<0.001 versus WT
Scatter plot points represent individual cardiomyocytes. N=3-5 rats per cohort.

Analyses of collagen deposition in skeletal and cardiac muscle showed reduction in fibrosis after delandistrogene moxeparvovec treatment



*P<0.05, ****P<0.001

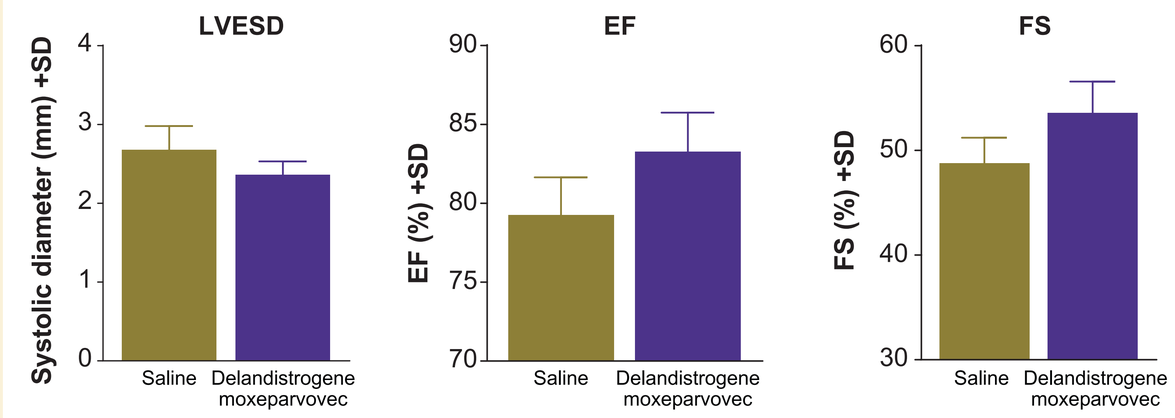
Delandistrogene moxeparvovec increased ambulation and vertical activity, and improvements were maintained at 24 weeks



*P<0.05, **P<0.01, ***P<0.001, ****P<0.001

- Troponin I levels in blood did not change significantly after SRP-9001 dystrophin expression (see supplementary material).

Cardiac function at 24 weeks after delandistrogene moxeparvovec gene transfer



- H&E, quantification of SRP-9001 dystrophin-positive fibres, SRP-9001 dystrophin transgene distribution and troponin I data are presented in the supplementary material.

REFERENCES

1. Potter RA, et al. *Hum Gene Ther*. 2021; 32:375–389;
2. Wasala NB, et al. *Hum Mol Genet*. 2013; 22:2634–2641;
3. Kobayashi YM, et al. *Nature*. 2008; 456:511–515;
4. Beastrom N, et al. *Am J Pathol*. 2011; 179:2464–2474;
5. Photobeam Activity System - Open Field. San Diego Instruments; San Diego, CA.

ABBREVIATIONS

ΔF/F0, peak heights of the Ca2+ transients; CaT, Ca2+ transients; DIA, diaphragm; DMD, Duchenne muscular dystrophy; EF, ejection fraction; FS, fractional shortening; H&E, haematoxylin and eosin; HRT, heart; LVESD, left ventricular end systolic diameter; mdx, muscular dystrophy X-linked; MG, medial gastrocnemius; SD, standard deviation; SL, sarcomere length; TTB90, time to baseline 90%; vg, vector genome; WT, wild type.

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RAP, CW, SB, GCO, JE, AK, LC, KA, AH, EP and LRRK are employees of Sarepta Therapeutics and may have stock options. LRRK has received grant support from Sarepta Therapeutics and the Parent Project Muscular Dystrophy, as well as financial consideration from Sarepta Therapeutics and Myonexus Therapeutics (now acquired by Sarepta Therapeutics). In addition, she is a co-inventor of AAVrh74.MHCK7.micro-dys technology. This research used DMD^{mdx} rats, which were generated and characterized in the following publication: T. Larcher, et al. Characterization of dystrophin deficient rats: a new model for Duchenne muscular dystrophy. *PLoS One*. 2014; 9:e110371.

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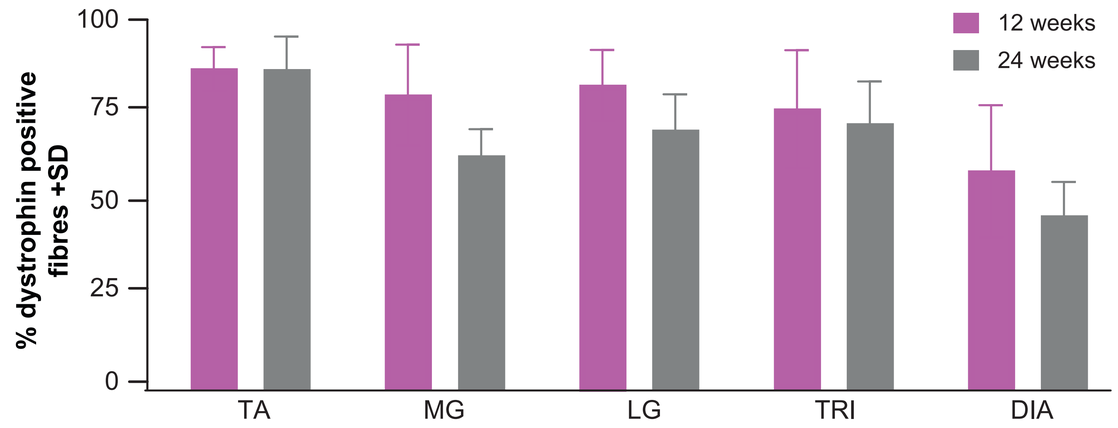
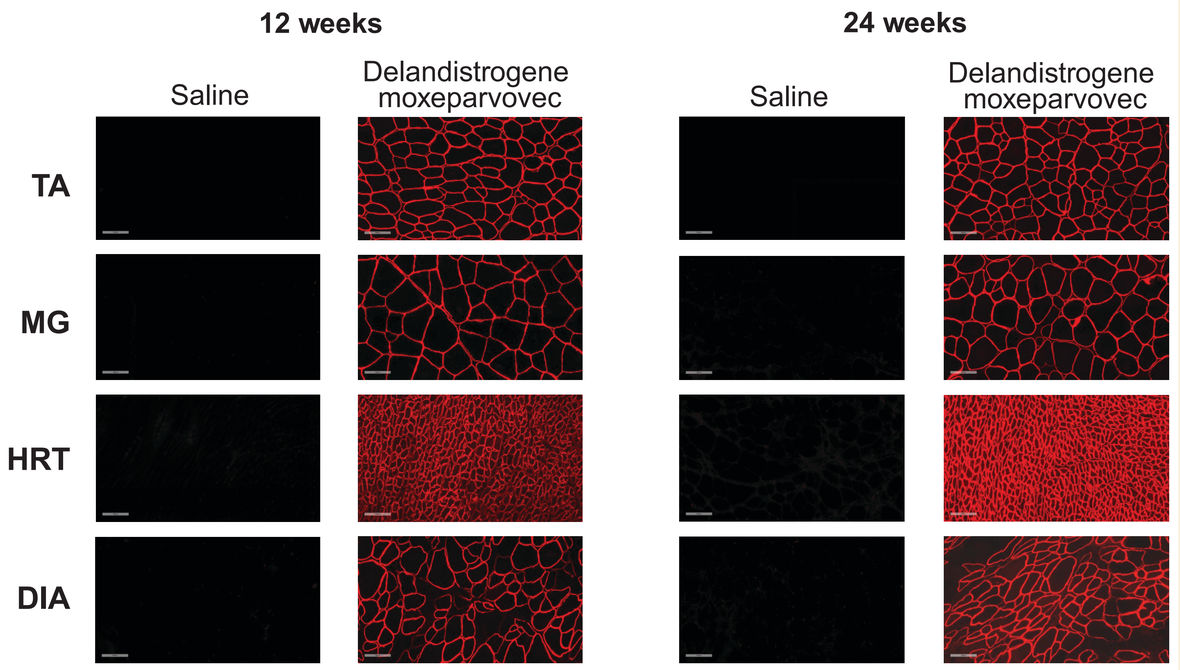
SUPPLEMENTARY MATERIALS

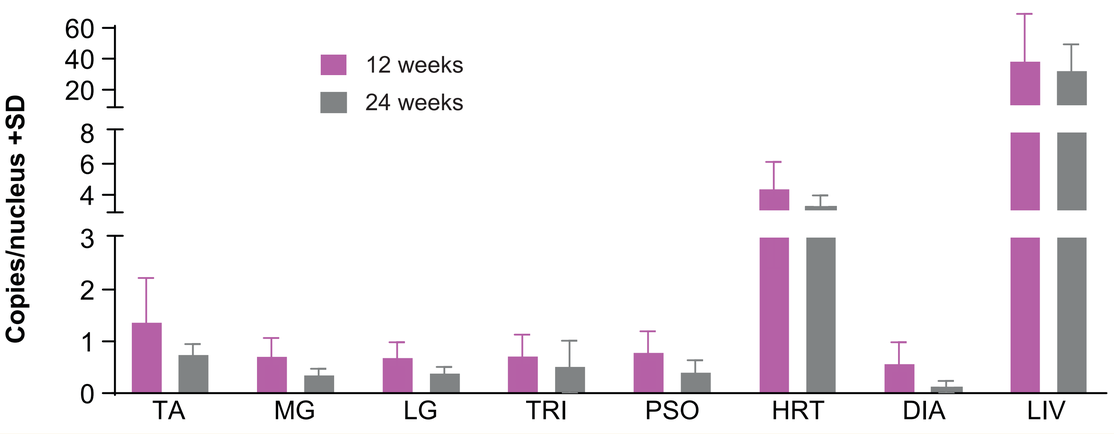
Quantification of SRP-9001 dystrophin-positive fibres showed no significant differences between 12 and 24 weeks within the same tissue types



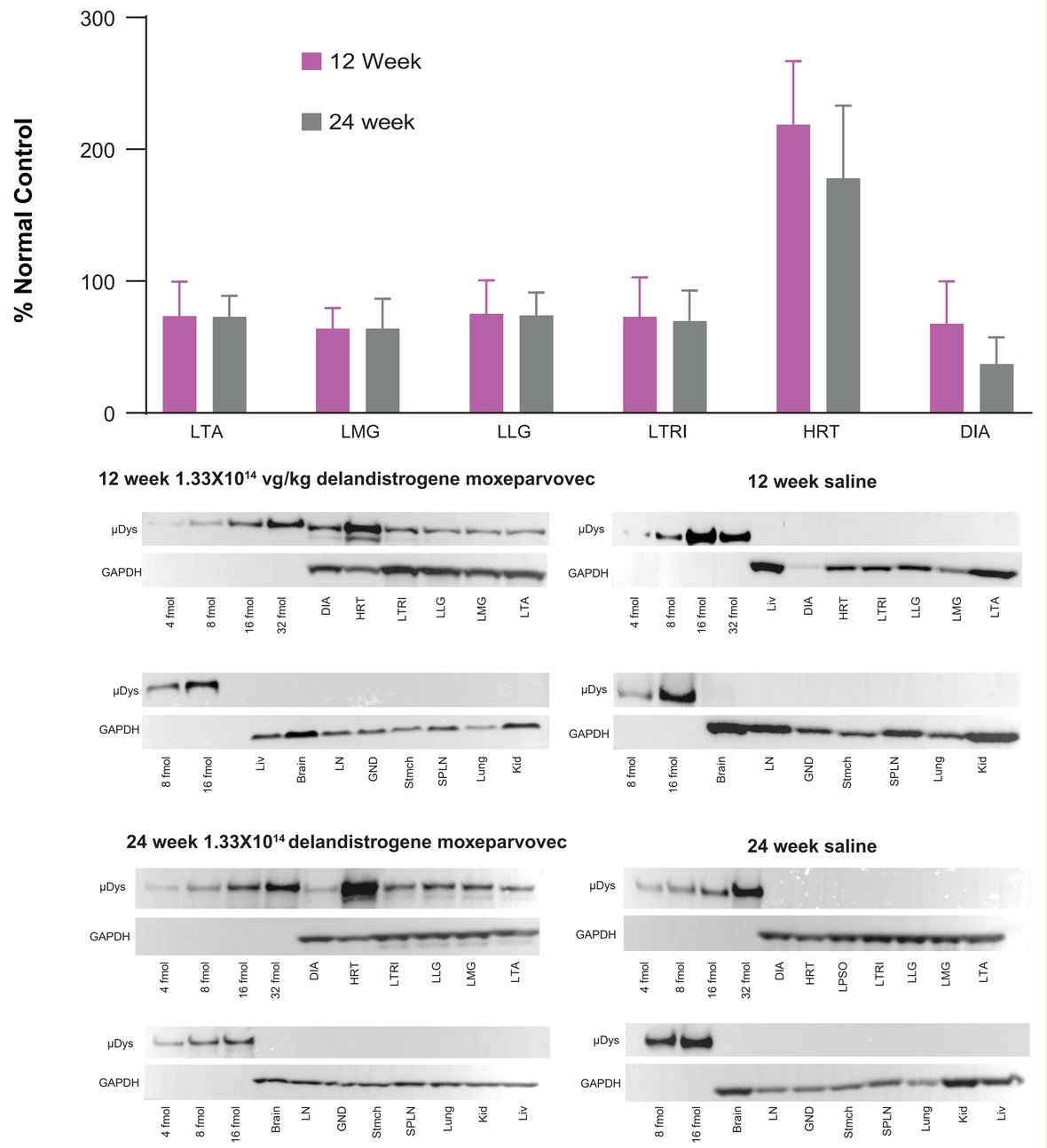
IF demonstrated SRP-9001 dystrophin localisation in muscle 12 and 24 weeks after treatment with delandistrogene moxeparvovec



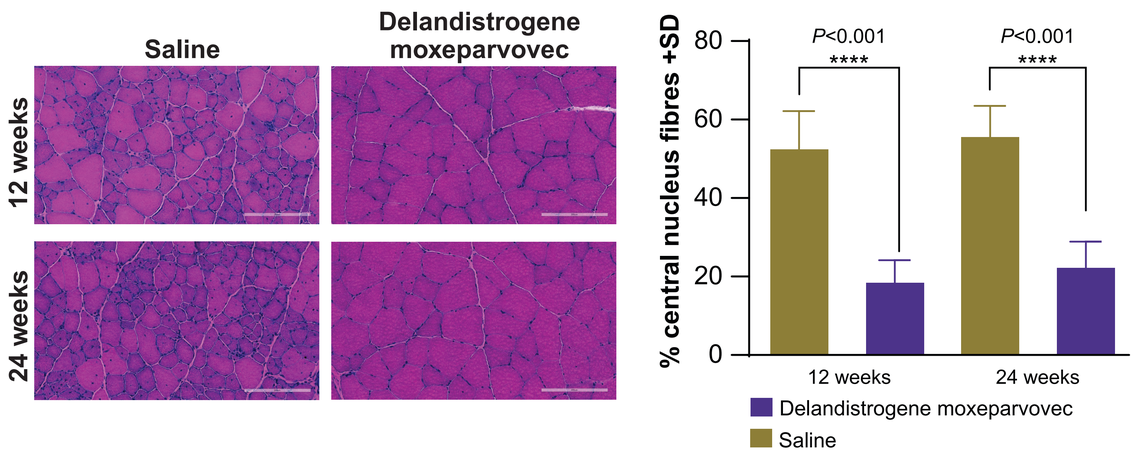
The SRP-9001 dystrophin transgene was broadly distributed across skeletal muscle, the diaphragm and the heart in DMD^{mdx} rats (ddPCR)



Western blot quantification of SRP-9001 dystrophin protein expression in DMD^{mdx} rats



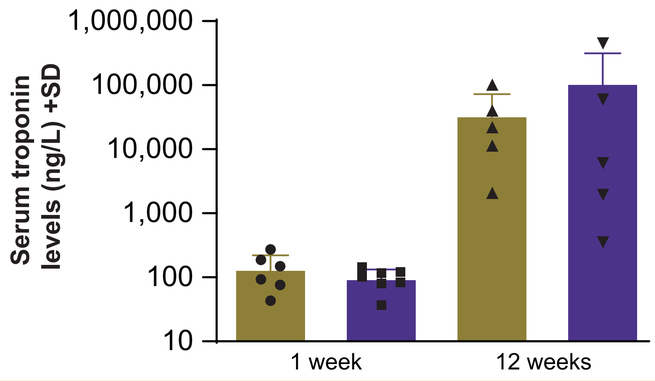
H&E demonstrated improved muscle histology (decreased central nucleation) in the gastrocnemius with delandistrogene moxeparvovec treatment



Troponin I levels in blood did not change significantly after SRP-9001 dystrophin expression

No significant difference compared to saline-treated DMD^{mdx} rats at 1 and 12 weeks post-dose.

- Delandistrogene moxeparvovec
- Saline



ABBREVIATIONS

ddPCR, droplet digital polymerase chain reaction; DIA, diaphragm; DMD, Duchenne muscular dystrophy; H&E, haematoxylin and eosin; HRT, heart; IF, immunofluorescence; LG, left gastrocnemius; LIV, liver; LLG, left lateral gastrocnemius; LMG, left medial gastrocnemius; LTA, left tibialis anterior; LTRI, left tricep; MDX, muscular dystrophy X-linked; MG, medial gastrocnemius; PSO, psoas; SD, standard deviation; TA, tibialis anterior; TRI, tricep.

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