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Evaluating pharmacology and efficacy of delandistrogene moxeparvovec in young and aged 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 and 24 weeks following systemic administration of delandistrogene moxeparvovec demonstrated no evidence of cardiac toxicity, and there were no deaths attributed to treatment.
- 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

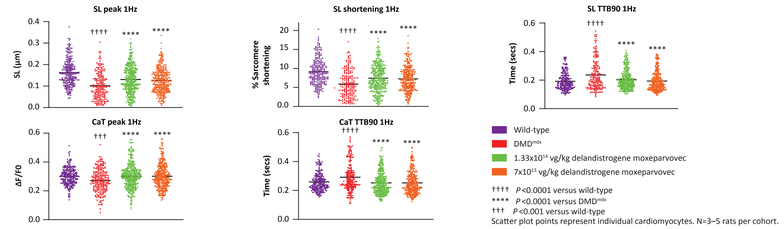
- Gene transfer therapy is a promising treatment strategy in development for patients with DMD.
- Delandistrogene moxeparvovec is an investigational rAAV vector-based gene therapy, designed to compensate for missing dystrophin in DMD by delivering a transgene encoding SRP-9001 dystrophin, an engineered dystrophin protein that retains key functional domains of the wild-type 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

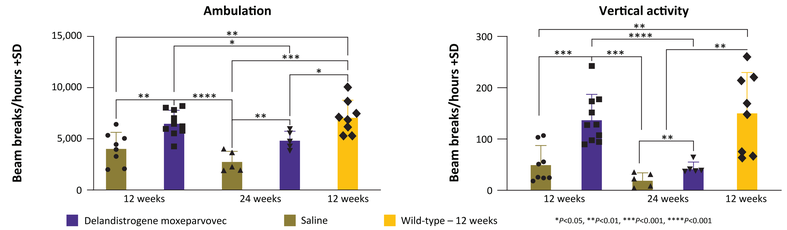
- We performed systemic, intravenous delivery of delandistrogene moxeparvovec in 21- to 35-day-old Sprague-Dawley *DMD*-mutated, dystrophin-null (DMD^{mdx}) rats.[3,4]
- Rats received a dose (1.33×10^{14} or 7.00×10^{13} vg/kg) of delandistrogene moxeparvovec or 0.9% sterile saline, unless otherwise 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 histologic analyses of fibrosis were used to evaluate cardiac disease.
- Individual cardiomyocyte function was assessed using sarcomere shortening and Ca^{2+} transient analyses. Cardiomyocytes were enzymatically isolated using Liberase TH; Ca^{2+} was reintroduced step-wise to 1.8 mM. Myocytes were incubated in a low-Ca^{2+} Tyrode solution containing 5 µM Fura-2AM for 30–35 minutes at room temperature. Intracellular Ca^{2+} transient and sarcomere shortening measurements were induced by electrical field stimulation between 0.2 Hz and 4 Hz. Cardiomyocyte and Ca^{2+} release was measured in 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 Ca^{2+} kinetics in DMD^{mdx} rats

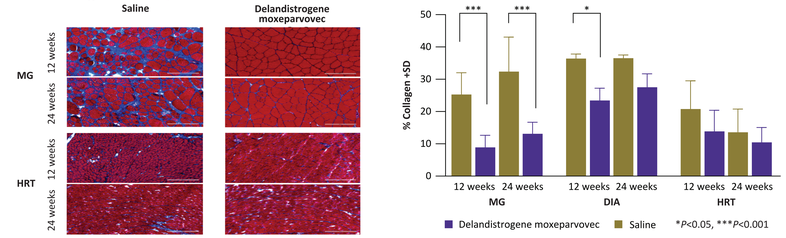


Improvements in ambulation and vertical activity were maintained at 24 weeks following treatment with delandistrogene moxeparvovec

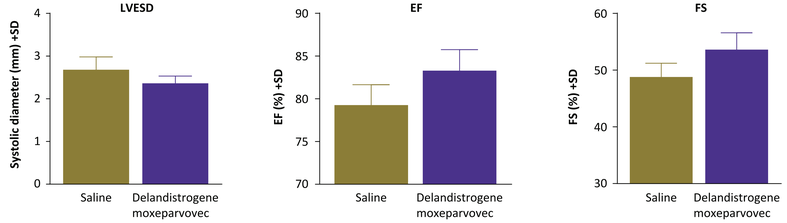


- Troponin I levels in blood did not change significantly following expression of SRP-9001 dystrophin (see Supplementary Materials).

Reduced fibrosis in skeletal and cardiac muscle in DMD^{mdx} rats following treatment with delandistrogene moxeparvovec



Cardiac function at 24 weeks following treatment with delandistrogene moxeparvovec



- H&E, quantification of SRP-9001 dystrophin-positive fibers, SRP-9001 dystrophin transgene distribution and troponin I data are presented in the Supplementary Materials.

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, USA.

ABBREVIATIONS

ΔF/F0, peak heights of the Ca^{2+} transients; CaT, Ca^{2+} transients; DIA, diaphragm; DMD, Duchenne muscular dystrophy; EF, ejection fraction; FS, fractional shortening; H&E, hematoxylin and eosin; HRT, heart; LVESD, left ventricular end systolic diameter; mdx, muscular dystrophy X-linked; MG, medial gastrocnemius; rAAV, recombinant adeno-associated virus; SD, standard deviation; SL, sarcomere length; TH, thermolysin high; TTB90, time to baseline 90%; vg, vector genome.

ACKNOWLEDGMENTS & DISCLOSURES

This research is funded by Sarepta Therapeutics, Inc., Cambridge, MA, USA. Writing and editorial assistance was provided by Ayesha Babar, MSc, of Nucleus Global in accordance with Good Publication Practice (GPP) 2022 guidelines (https://www.ismpp.org/gpp2022) and funded by Sarepta Therapeutics, Inc. RAP, CW, GCO, EW, ETA, AK, LC, KA, AH and EP are employees of Sarepta Therapeutics and may have stock options. LRRK is an employee of Sarepta Therapeutics, 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: Larcher T, et al. Characterization of dystrophin deficient rats: a new model for Duchenne muscular dystrophy. *PLoS One*. 2014; 9:e110371. These data are an encore of data first presented by Rachael A. Potter at the 27th International Annual Congress of the World Muscle Society (WMS) 2022.

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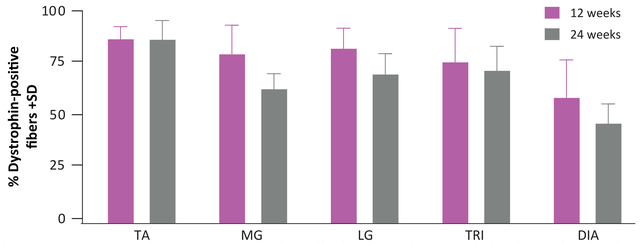
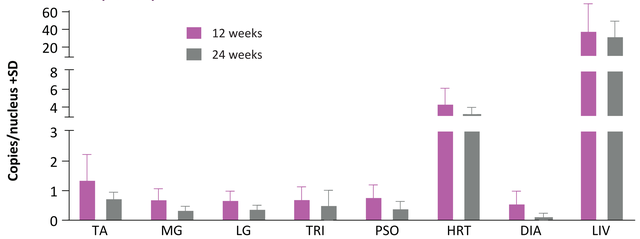
SUPPLEMENTARY MATERIALS

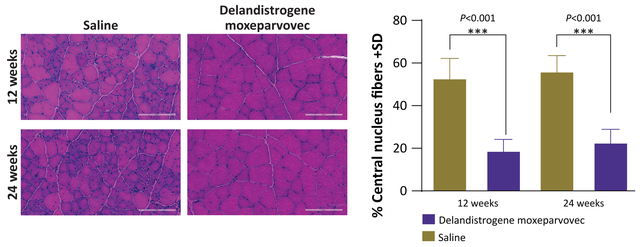
Quantification of SRP-9001 dystrophin-positive fibers showed no significant differences within the same tissue types between 12 and 24 weeks following treatment with delandistrogene moxeparvovec



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



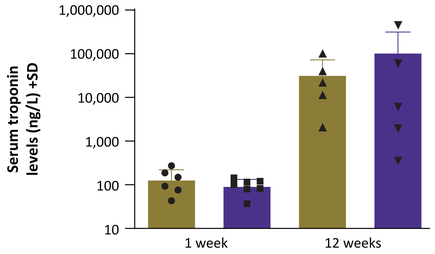
H&E demonstrated improved muscle histology (decreased central nucleation) in the gastrocnemius following treatment with delandistrogene moxeparvovec



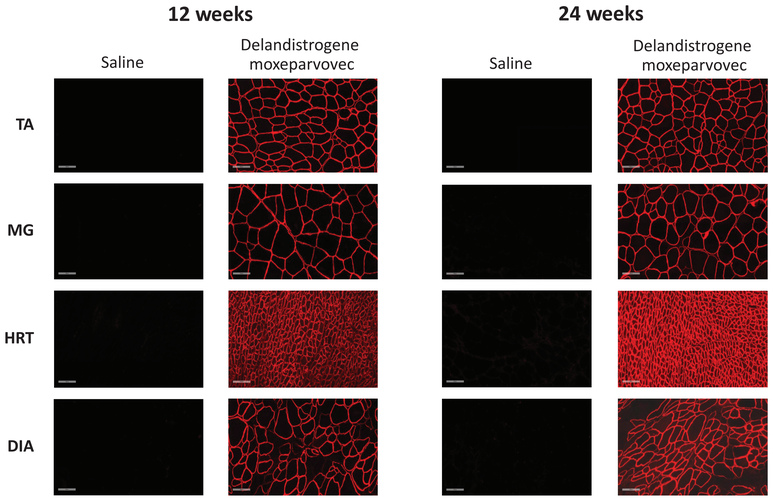
Troponin I levels in blood did not change significantly after SRP-9001 dystrophin expression

There was no significant difference compared with saline-treated DMD^{mdx} rats at 1 and 12 weeks post-dose.

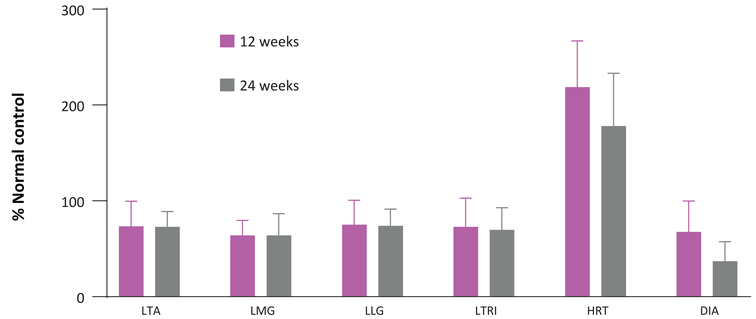
Delandistrogene moxeparvovec
Saline

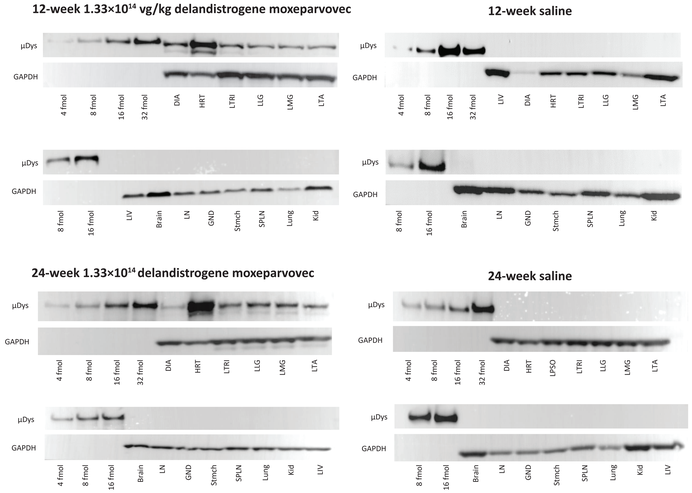


IF demonstrated SRP-9001 dystrophin localization in muscle at 12 and 24 weeks following treatment with delandistrogene moxeparvovec



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





ABBREVIATIONS

µDys, microdystrophin; ddPCR, droplet digital polymerase chain reaction; DIA, diaphragm; DMD, Duchenne muscular dystrophy; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GND, gonad; H&E, hematoxylin and eosin; HRT, heart; IF, immunofluorescence; Kid, kidney; LG, left gastrocnemius; LIV, liver; LLG, left lateral gastrocnemius; LMG, left medial gastrocnemius; LN, lymph node; LPSO, left psoas; LTA, left tibialis anterior; LTRI, left tricep; mdx, muscular dystrophy X-linked; MG, medial gastrocnemius; PSO, psoas; SD, standard deviation; SPLN, spleen; Stmch, stomach; TA, tibialis anterior; TRI, tricep.

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