Long-term survival and cardiac efficacy of delandistrogene moxeparvovec gene therapy in the Duchenne muscular dystrophy rat model

S Baine,[1] C Wier,[1] L Lemmerman,[1] G Cooper-Olson,[1] A Kempton,[1] A Haile,[1] J Endres,[1] A Fedoce,[1] E Nesbit,[1] LR Rodino-Klapac,[1] RA Potter[1]*

[1]Sarepta Therapeutics, Inc., Cambridge, MA, USA
*Presenting on behalf of the authors (email address: medinfo@sarepta.com)

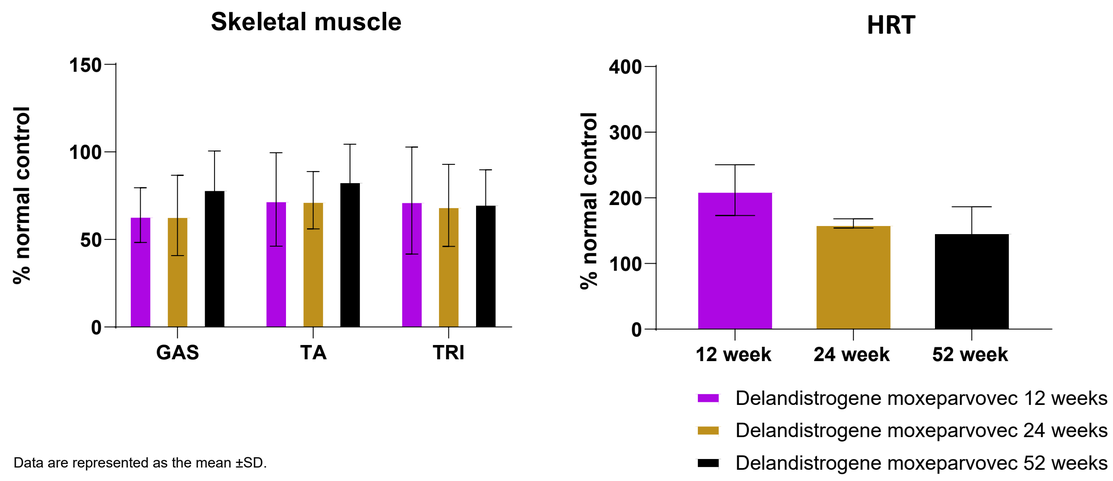
SUPPLEMENTARY MATERIALS

SUPPLEMENTARY METHODS

- *In vitro*, individual cardiomyocyte function was assessed using sarcomere shortening and CaT 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's solution containing 5 µM Fura-2AM for 30–35 minutes at room temperature. Intracellular CaT and sarcomere shortening measurements were induced by electrical field stimulation between 0.2 Hz and 4 Hz.

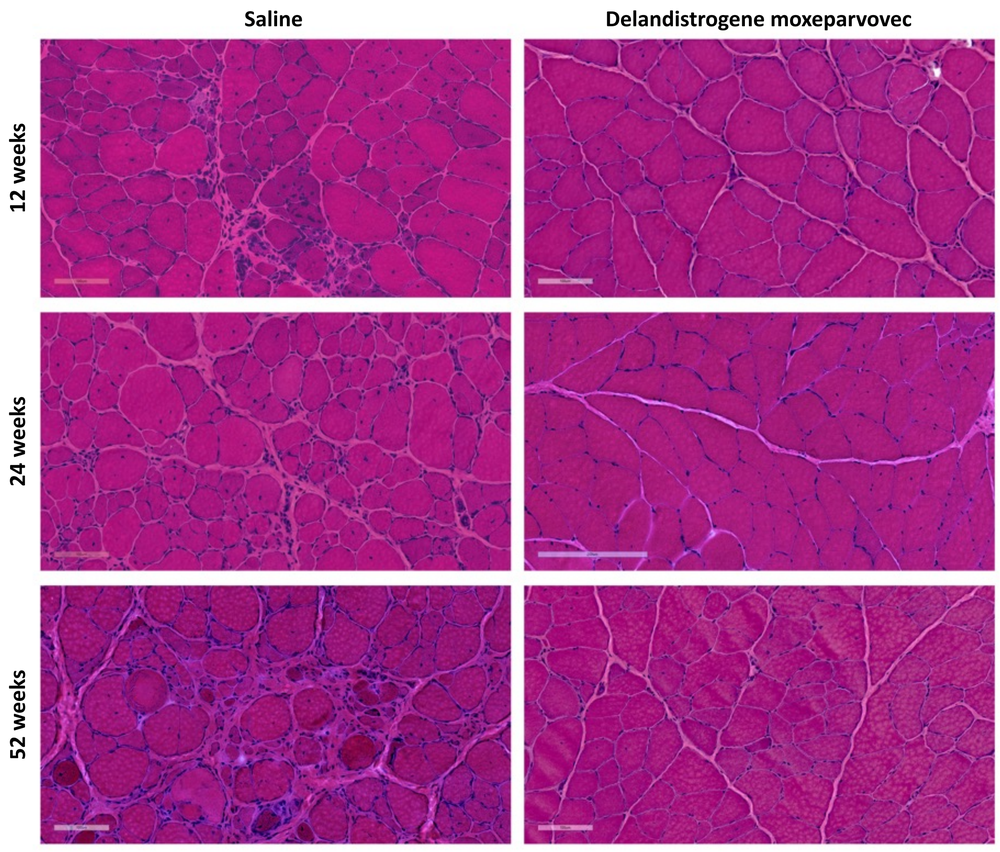
SUPPLEMENTARY RESULTS

Western blot and quantification of delandistrogene moxeparvovec micro-dystrophin expression in muscle

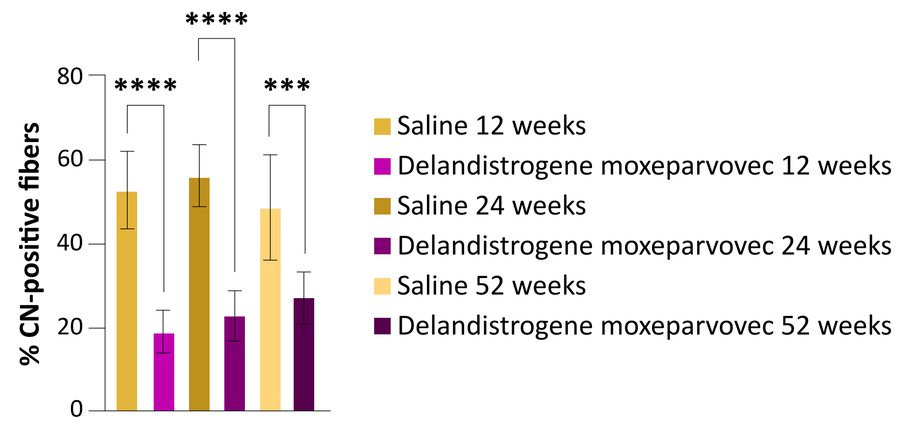


Data are represented as the mean ±SD.

H&E demonstrated improved muscle histology (decreased CN) in the gastrocnemius; following treatment with delandistrogene moxeparvovec in DMD^{MDX} rats

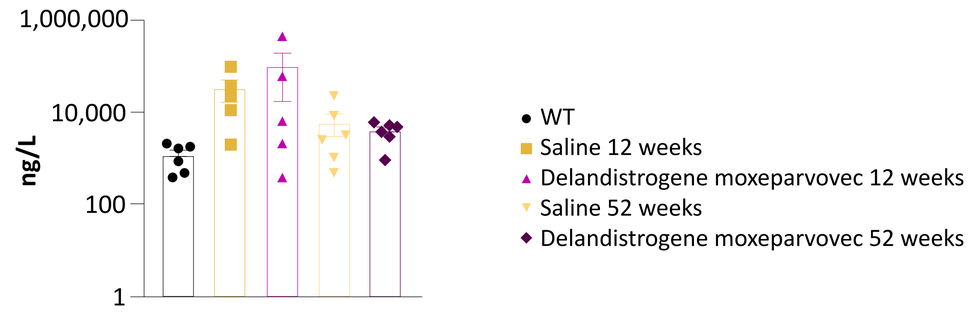


Scale bar, 400 µm. 10× objective.

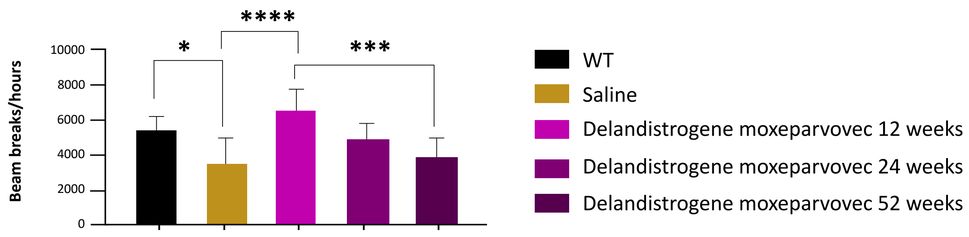


Data are represented as the mean ±SD.***P<0.001; ****P<0.0001.

Serum troponin I levels

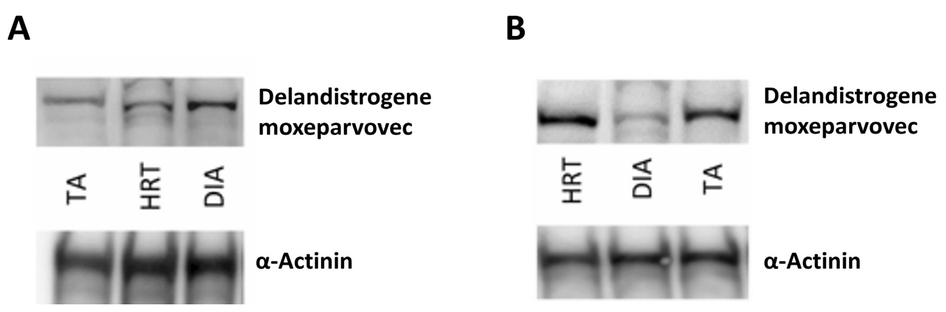


Improved ambulation of horizontal activity in delandistrogene moxeparvovec-treated DMD^{MDX} rats vs saline control.†



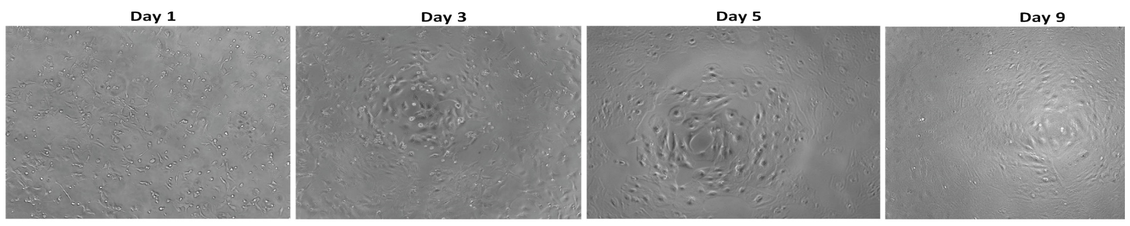
Data are represented as the mean ±SD. *P<0.05, ***P<0.001, ****P<0.0001. †Assay was performed shortly before endpoint at 12, 24- and 52-weeks following treatment with delandistrogene moxeparvovec

Representative western blots across HRT, diaphragm, and skeletal muscle of NHP (Part 2).* (A) Samples taken after single dose of delandistrogene moxeparvovec. (B) Samples taken after plasmapheresis and redosing with delandistrogene moxeparvovec or rAAVrh74.MHCK7.micro-dystrophin.FLAG treatment



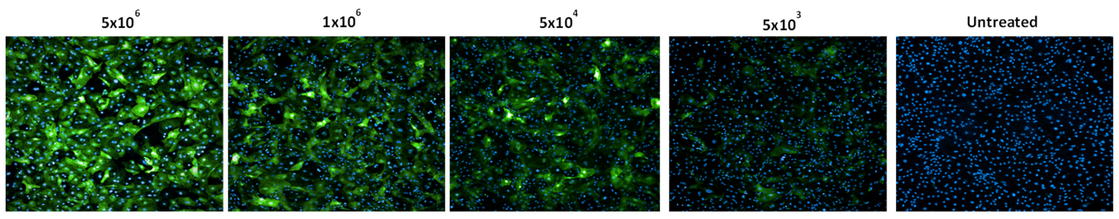
*In Part 2, NHP Cohorts 2–4 underwent plasmapheresis before redosing; Cohort 5 was redosed without plasmapheresis.

Representative phase contrast cardiomyocyte images show chronological morphology change from Day 1 to Day 5 (when cardiomyocytes were transduced) and Day 9 (fixed day)



10× objective.

Representative IF images of iCell cardiomyocyte cells expressing AAVrh74-MHCK7-GFP (green)



ABBREVIATIONS

CaT, Ca^{2+} transients; CN, central nucleation; DIA, diaphragm; DMD, Duchenne muscular dystrophy; GAS, gastrocnemius; H&E, hematoxylin and eosin; HRT, heart; IF, immunofluorescence; MDX, muscular dystrophy X linked; NHP, non-human primates; rAAVrh74, recombinant adeno-associated serotype 74; SD, standard deviation; TA, tibialis anterior; TH, thermolysin high; TRI, triceps; WT, wild type.

ACKNOWLEDGMENTS & DISCLOSURES

This research is funded by Sarepta Therapeutics, Inc., Cambridge, MA, USA. Medical writing and editorial support was provided by Leo Mahachi, PhD, on behalf of Nucleus Global, in accordance with Good Publication Practice (GPP) 2022 guidelines (https://www.ismpp.org/gpp-2022) and was funded by Sarepta Therapeutics Inc., Cambridge, MA, USA. SB, CW, LL, GCO, AK, AH, JE, AF, EN, and RAP are employees of Sarepta Therapeutics and may have stock options. LRR-K is an employee of Sarepta Therapeutics, has received grant support from Sarepta Therapeutics and 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.