Evaluation of Safety Parameters and Dystrophin Expression by Sequential Administration of Exon-Skipping and Gene Therapy in a DMD^{mdx} Mouse Model

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Key Finding

Safety and dystrophin expression after sequential administration were consistent with individual treatment, suggesting that continuous exon-skipping therapy may be administered prior to AAV GT

Background

- Promising treatment approaches have emerged for Duchenne muscular dystrophy (DMD), including exon skipping and adeno-associated virus-based vector gene therapy (AAV GT), which restore functional dystrophin by distinct mechanisms[1,2]
- Exon skipping with phosphorodiamidate morpholino oligomers (PMOs) restores the *DMD* gene open reading frame, enabling translation of shortened functional dystrophin protein
  - In the US, 4 PMOs are approved for patients with DMD; PMO clinical studies indicate that continuous exon-skipping therapy provides dystrophin restoration, preserves muscle, and slows disease progression[3–9]
  - Peptide phosphorodiamidate morpholino oligomers (PPMOs) are a next-generation chemistry platform in which a cell-penetrating peptide is conjugated to the PMO backbone, with the goal of increasing cellular uptake, exon skipping, and dystrophin production
- Delandistrogene moxeparvovec is a recombinant AAV (rAAV)-based gene therapy, designed to compensate for the absence of functional dystrophin in DMD by delivering a transgene encoding delandistrogene moxeparvovec micro-dystrophin, an engineered dystrophin protein that retains key functional domains of the wild-type protein[10–12]
  - Delandistrogene moxeparvovec is approved in the United States, UAE, and Qatar for the treatment of ambulatory pediatric patients aged 4 through 5 years with DMD with a confirmed mutation in the DMD gene[13–15,a,b]
- Here, the safety of sequential administration of RC-1001 (an exon 23–skipping PPMO) and AAV GT (a mouse codon–optimized version of delandistrogene moxeparvovec) and its impact on dystrophin expression were investigated in DMD^{mdx} mice

[a]Delandistrogene moxeparvovec is contraindicated in patients with any deletion in exon 8 and/or exon 9 in the *DMD* gene. [b]As of January 2024.

Objective

To investigate safety parameters and dystrophin expression following sequential PPMO and AAV GT administration in the mdx mouse model of DMD

Methods

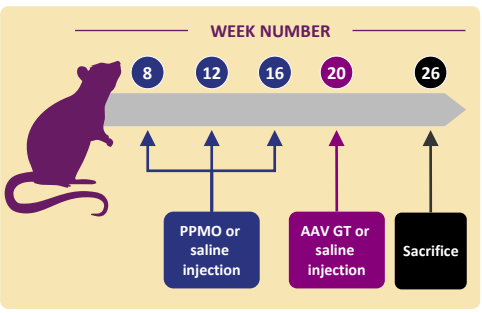
Study design

- DMD^{mdx} mice (C57BL/10ScSn-DMD^{mdx}/J strain), a well-established model in nonclinical DMD research in which a nonsense mutation in exon 23 of the *DMD* gene causes dystrophin production deficiency, were used[16]
- Mice received 3 doses of PPMO (RC-1001) or placebo (saline) at 8, 12, and 16 weeks of age (**F1**)
- At week 20, mice received a single clinical dose of AAV GT (AAVrh74.MHCK7.Mouse-µDys2.0 construct) or saline
- All animals were euthanized at week 26

Outcomes

- Serum chemistries: alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, creatinine, blood urea nitrogen (BUN)
- Dystrophin expression: western blot (WB), immunofluorescence (IF)
- Mortality
- Histopathology

F1 *Study Design*



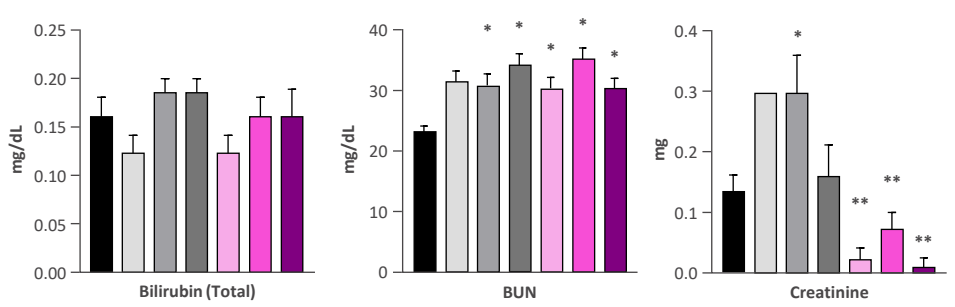
Group		Size	3 PPMO or saline injections at 8, 12, and 16 weeks	AAV GT or saline injection at 20 weeks[a]
	WT	n=8	Saline	Saline
	mdx	n=8	Saline	Saline
	mdx	n=8	10 mg/kg	Saline
	mdx	n=8	40 mg/kg	Saline
	mdx	n=8	Saline	1.33e14 vg/kg
	mdx	n=8	10 mg/kg	1.33e14 vg/kg
	mdx	n=8	40 mg/kg	1.33e14 vg/kg

[a]Lower doses (4.43e13 vg/kg) were studied but not included here.

Results

- No abnormal liver or renal serum chemistries, as shown with bilirubin and BUN (**F2**)
- Creatinine elevations observed are within the normal range
- ALT and AST are impacted by muscle injury due to disease, and therefore are not shown, as conclusions cannot be made concerning the impact of treatment on these serum chemistries
- No treatment-related cage-side observations or morbidity
- No treatment-related abnormal histopathology following analysis of multiple tissues by a board-certified veterinary pathologist

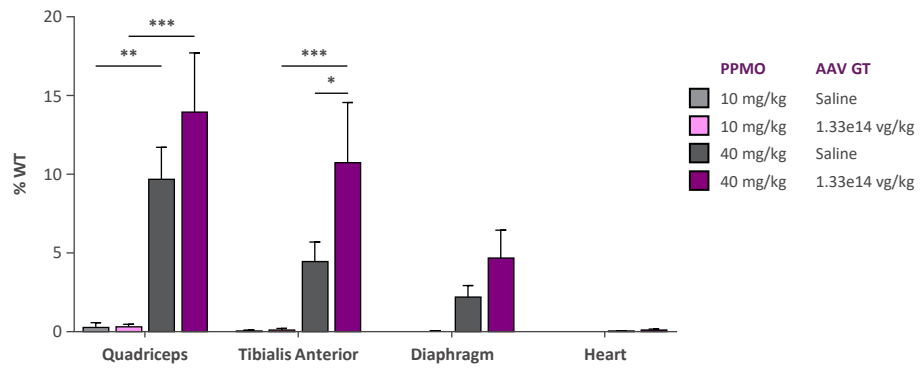
F2 *Serum Chemistries at 26 Weeks*



No safety events were observed after sequential administration of PPMO and AAV GT up to 26 weeks in DMD^{mdx} mice

Bars represent mean ± SEM. *P<0.05 vs WT saline + saline controls; **P<0.05 vs DMD^{mdx} saline + saline controls. Significant statistical differences between other groups are not shown.

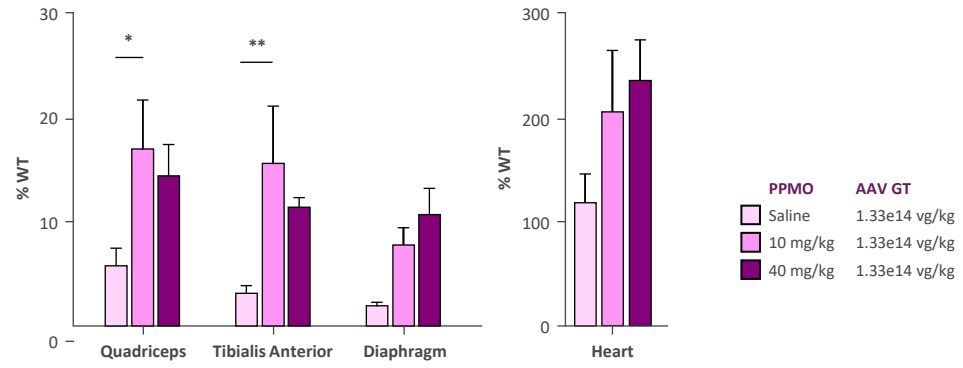
F3 *Exon-Skipped Dystrophin Expression by WB at 26 Weeks (10 Weeks After Last PPMO Injection)*



Bars represent mean ± SEM. *P<0.05; **P<0.001; ***P<0.0001. Significant statistical differences between all groups are not shown. Protein expression is represented as % WT mouse tibialis anterior expression. DYS1 antibody (Leica Biosystems) was used as the primary antibody for protein detection.

Exon-skipped dystrophin expression was observed with sequential treatment with AAV GT in DMD^{mdx} mice (F3)

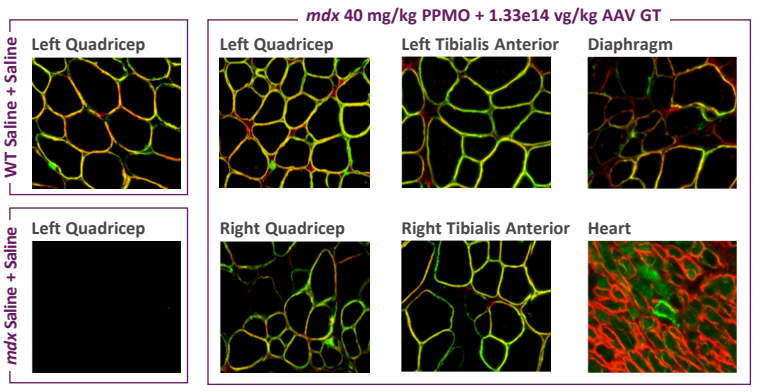
F4 *AAV GT Micro-dystrophin Protein Expression by WB at 26 Weeks*



Bars represent mean ± SEM. *P<0.05; **P<0.01. Protein expression is represented as % WT mouse tibialis anterior expression. MANEX1A (4C7) antibody (DSHB) was used as the primary antibody for protein detection.

AAV GT micro-dystrophin expression was observed regardless of prior treatment with PPMO in DMD^{mdx} mice (F4)

F5 *IF Showing Sarcolemmal Localization at 26 Weeks*



[a]Exon-skipped dystrophin; [b]AAV GT micro-dystrophin; [c]exon-skipped dystrophin + AAV GT micro-dystrophin.
Images courtesy of the Histology Group at Genetic Therapies Center of Excellence (GTCOE), Columbus, OH. Composite images of anti-dystrophin antibody (H-5), Santa Cruz Biotechnology, catalog #sc-365954 and anti-dystrophin antibody, Abcam, catalog #ab15277 in red and green fluorescent tag.

Sarcolemmal localization of exon-skipped dystrophin and AAV GT micro-dystrophin at 26 weeks was observed with sequential treatment (F5)

Conclusions

Results from the DMD^{mdx} mouse model support the safety of sequential administration of PPMOs and AAV GT and demonstrate noninterfering dystrophin restoration consistent with that of each individual treatment (PPMO or AAV GT)

- No treatment-related adverse events were observed, including absence of abnormal histopathology
- Sequential treatment showed co-localization of exon-skipped dystrophin and AAV GT micro-dystrophin

These findings suggest that patients with DMD may be able to receive continuous exon-skipping therapy prior to AAV GT without the need for a washout period of exon-skipping therapy, thus allowing dystrophin restoration by distinct mechanisms

Abbreviations

AAVrh74=adeno-associated virus serotype rh74; ALT=alanine aminotransferase; AST=aspartate aminotransferase; BUN=blood urea nitrogen; DSHB=Developmental Studies Hybridoma Bank; GT=gene therapy; IF=immunofluorescence; MHCK=myosin heavy-chain muscle creatine kinase promoter; PMO=phosphorodiamidate morpholino oligomer; PPMO=peptide-conjugated phosphorodiamidate morpholino oligomer; WB=western blot; WT=wild type; µDys=mouse micro-dystrophin.

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References

1. Lim KR, et al. *Drug Des Devel Ther*. 2017;11:533-45. **2.** Mendell JR, et al. *Mol Ther Methods Clin Dev*. 2022;25:74-83. **3.** Sarepta Therapeutics, Inc. Data on file. **4.** Mendell JR, et al. *Neuromuscul Dis*. 2021;8:469-79. **5.** Khan N, et al. *J Neuromuscul Dis*. 2019;6:213-25. **6.** Charleston JS, et al. *Neurology*. 2018;90:e2146-54. **7.** Scaglioni D, et al. *Acta Neuropathol Commun*. 2021;9:7. **8.** Iff J, et al. Poster presented at WMS; October 11–15, 2022; Halifax, Canada. **9.** Masterson-Ricchetti K, et al. Poster presented at WMS; October 11–15, 2022; Halifax, Canada. **10.** Asher DR, et al. *Expert Opin Biol Ther*. 2020; 20:263-74. **11.** Zheng C, Baum BJ. *Methods Mol Biol*. 2008; 434:205-19. **12.** Mendell JR, et al. *JAMA Neurol*. 2020; 77:1122-31. **13.** US Food and Drug Administration. ELEVIDYS™ Highlights of prescribing information. https://www.fda.gov/media/169679/download. Published 2023 (Accessed September 2023). **14.** UAE Ministry of Health & Prevention. https://mohap.gov.ae/en/services/registered-medical-product-directory (Accessed September 2023). **15.** [Ad hoc announcement pursuant to Art. 53 LR] Roche announces EMBARK trial in Duchenne muscular dystrophy (DMD) did not reach primary endpoint, but shows positive efficacy outcomes on all timed functional key endpoints. GlobeNewswire. https://kommunikasjon.ntb.no/pressemelding/18015651/ad-hoc-announcement-pursuant-to-art-53-lr-roche-announces-embark-trial-in-duchenne-muscular-dystrophy-dmd-did-not-reach-primary-endpoint-but-shows-positive-efficacy-outcomes-on-all-timed-functional-key-endpoints?publisherId=4954260. Published October 10, 2023. [Accessed January 31, 2024]. **16.** McGreevy JW, et al. *Dis Model Mech*. 2015;8:195-213.

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