P23

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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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Objectives

To investigate safety parameters and dystrophin expression following sequential peptide-conjugated phosphorodiamidate morpholino oligomer (PPMO) and adeno-associated virus gene therapy (AAV GT) administration in the mdx mouse model of Duchenne muscular dystrophy (DMD)

Key Findings

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 DMD, including exon skipping and AAV-based vector gene therapy, 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]
  - 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 and UAE for the treatment of ambulatory pediatric patients aged 4 through 5 years with DMD with a confirmed mutation in the DMD gene[13,14,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 August 2023.

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

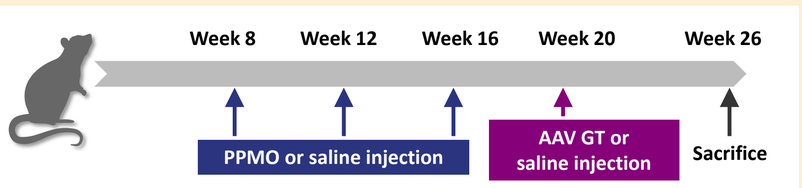
METHODS

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[15]
- Mice received 3 doses of PPMO (RC-1001) or placebo (saline) at 8, 12, and 16 weeks of age
- 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



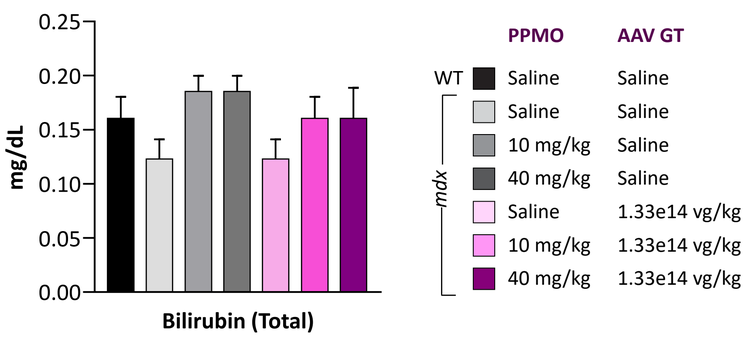
Group	Size	3 PPMO or saline injections at 8, 12, and 16 weeks	AAV GT or saline injection at 20 weeks[a]
WT	n=8	Saline	Saline
mdx	n=8	Saline	Saline
mdx	n=8	10 mg/kg	Saline
mdx	n=8	40 mg/kg	Saline
mdx	n=8	Saline	1.33e14 vg/kg
mdx	n=8	10 mg/kg	1.33e14 vg/kg
mdx	n=8	40 mg/kg	1.33e14 vg/kg

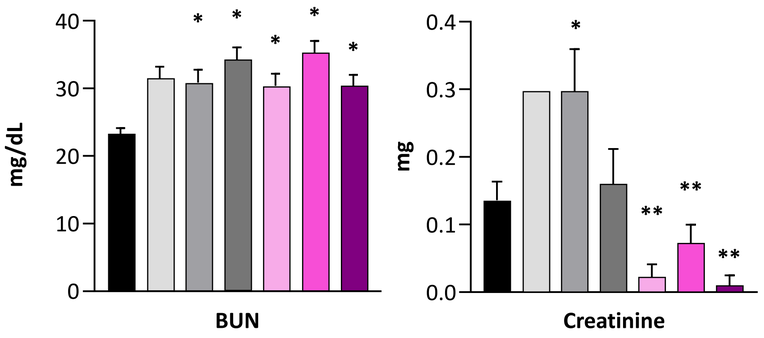
[a]Lower doses (4.43e13 vg/kg) were studied but not included here.

RESULTS

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

Serum Chemistries at 26 Weeks



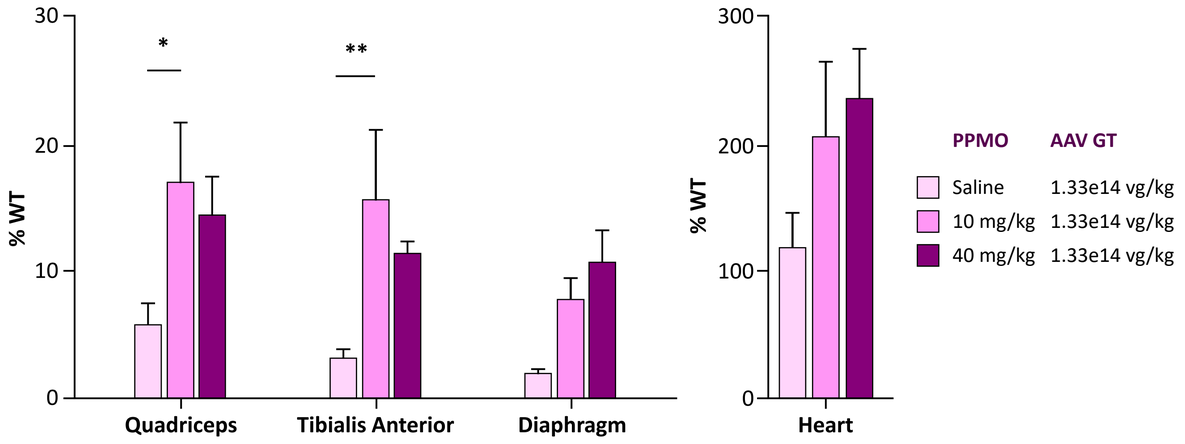


- No abnormal liver or renal serum chemistries, as shown with bilirubin and BUN
- Creatinine elevations observed are within the normal range
- ALT and AST are impacted by muscle injury due to disease, and therefore are not shown as conclusions cannot be made concerning the impact of treatment on these 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

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.

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

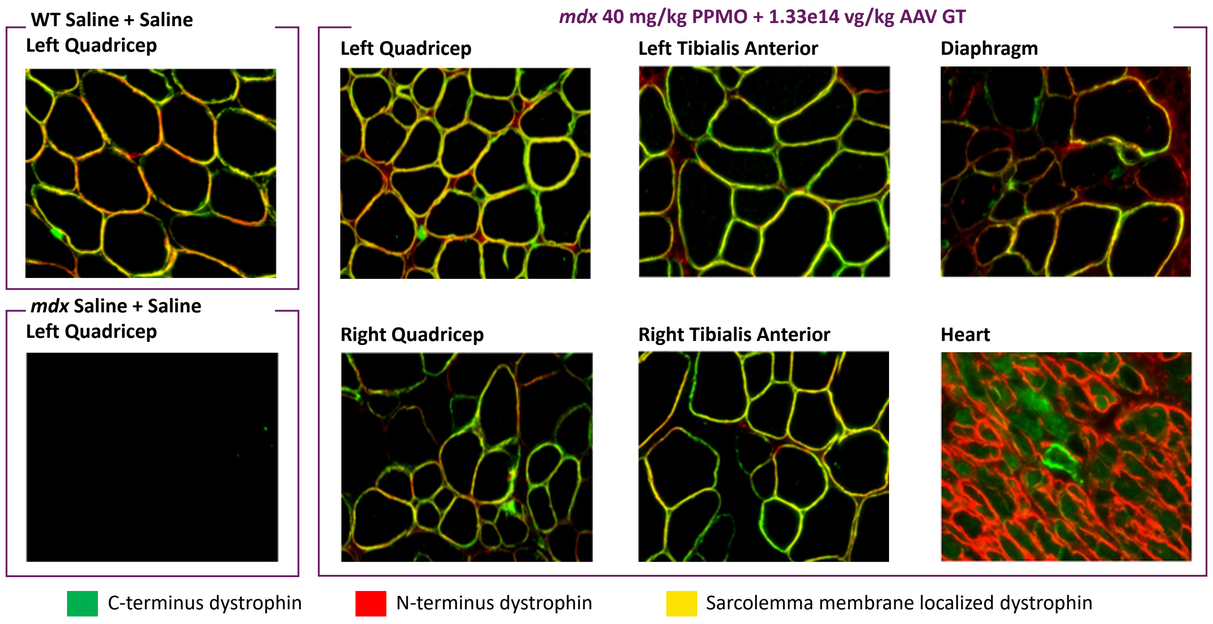
AAV GT Micro-Dystrophin Protein Expression by WB at 26 Weeks



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.

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

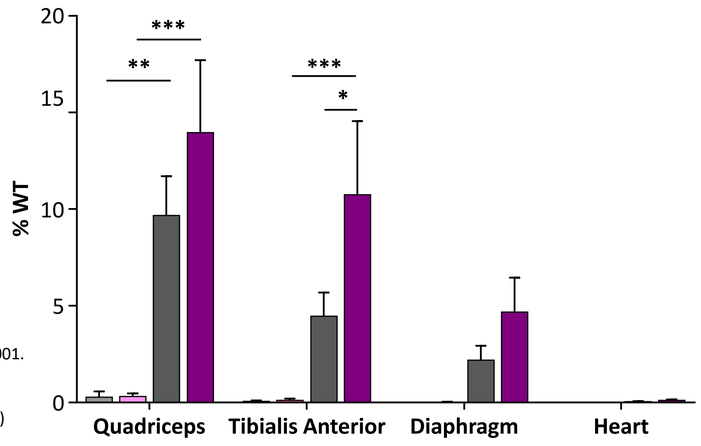
IF Showing Sarcolemmal Localization at 26 Weeks



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.

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

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.

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; PPMO=peptide-conjugated phosphorodiamidate morpholino oligomer; WB=western blot; WT=wild type; µDys=mouse micro-dystrophin.

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