Abstract #236

Vector shedding in patients with DMD treated with delandistrogene moxeparvovec and seroconversion from shed vector in naïve mice

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***Presenting on behalf of the authors (email address: medinfo@sarepta.com)**

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Acknowledgments and disclosures

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- These data are an encore of data first presented by Dr. Jyoti Malhotra at the 27th International Annual Congress of the World Muscle Society (WMS) 2022

Disclosures

- ESS, JM, SL, XZ, DA, SW, LE, RAP 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

Objectives and overview

To evaluate:

- The PK of vector shedding following administration of delandistrogene moxeparvovec (SRP-9001) to patients
- Seroconversion of naïve mice after mucosal administration of vector, to simulate the consequences of exposure to shed vector

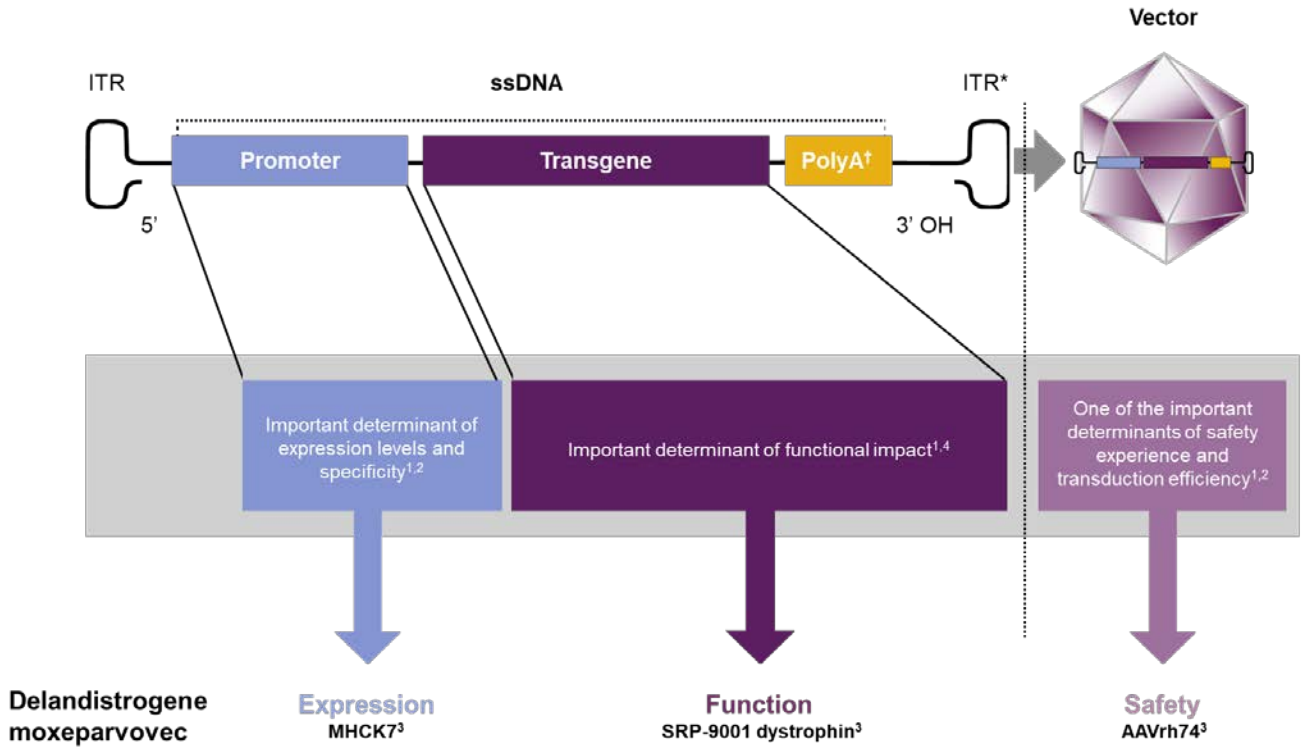
What does this study mean for the DMD community?

- 99% of vector was shed in patients by Week 4 post-treatment
- Naïve mice remained seronegative following topical ocular delivery of even the highest titers of shed vector
- These data suggest a low risk of seroconversion from exposure to shed vector

DMD, Duchenne muscular dystrophy; PK, pharmacokinetics.

Background

- 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[1–4]
- Although rAAV vectors are incapable of replication and cannot "infect" others as a virus can, vector shedding via excretions and secretions is expected following the administration of a vector-based gene therapy product. This raises the theoretical possibility of exposure and consequent seroconversion of untreated individuals, such as patients' family members and caregivers[5]
- The actual seroconversion risk posed by shed vector is unknown, but as a precaution, families and caregivers may go to great lengths to reduce potential exposure (e.g. by sequestering siblings from the treated patient)[5]



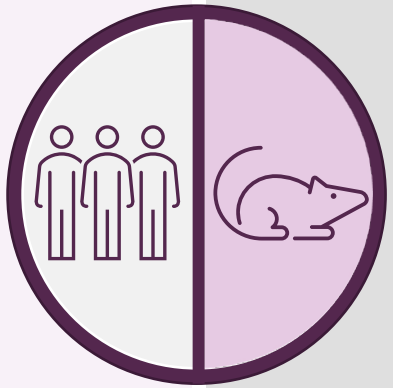
*ITRs are required for genome replication and packaging. †PolyA signals the end of the transgene to the cellular machinery that transcribes (i.e. copies) it.

AAVrh74, adeno-associated virus rhesus isolate serotype 74; DMD, Duchenne muscular dystrophy; ITR, inverted terminal repeat; MHCK, myosin-heavy-chain kinase; OH, hydroxyl; PolyA, polyadenylation; rAAV, recombinant adeno-associated virus; ssDNA, single-stranded DNA.

1. Asher DR, et al. *Expert Opin Biol Ther*. 2020; 20:263–274; 2. Zheng C and Baum BJ. *Methods Mol Biol*. 2008; 434:205–219; 3. Mendell JR, et al. *JAMA Neurol*. 2020; 77:1122–1131; 4. Chandler RJ and Venditti CP. *Transl Sci Rare Dis*. 2016; 1:73–89;
5. Brown AM, et al. *Appl Biosaf*. 2020; 25:184–193.

Methods

- We evaluated the extent and magnitude of shedding and clearance of delandistrogene moxeparvovec using interim data from ENDEAVOR (Study 103; NCT04626674),[1] a Phase 1 study assessing the safety and expression of intended commercial process delandistrogene moxeparvovec material in patients with DMD
  - Delandistrogene moxeparvovec vector exposure in saliva, urine and feces was quantified by ddPCR in ENDEAVOR (n=20) to characterize vector shedding (proportion of observations BLOD) in participants
  - This method uses a vector-specific primer probe set for sequences of the MHCK7 promoter (within the SRP-9001 dystrophin gene cassette)



- In a non-clinical study, we tested naïve mice to determine the risk of AAVrh74 seroconversion following mucosal vector exposure, with doses based on exposure levels (vector genome copies) demonstrated in non-clinical and clinical studies
  - Mice were exposed to AAVrh74.CMV.eGFP via topical ocular delivery. An intramuscular route of delivery was also utilized as a positive control
  - Seropositivity: Antibody levels were measured by AAVrh74 ELISA at baseline and 4 weeks post-delivery. A 1:100 dilution was the most concentrated serum dilution assessed. An absorbance ratio >2.0 is considered seropositive. The most diluted serum level at which seropositivity was detected is its titer (1:X). Animals were assessed for baseline seropositivity to ensure that none had pre-existing antibodies against AAVrh74
  - Biodistribution: The tissues collected at terminal sacrifice were used to isolate DNA and determine the biodistribution of vector genomes using ddPCR

AAVrh74, adeno-associated virus rhesus isolate serotype 74; BLOD, below the limit of detection; CMV, cytomegalovirus promoter; ddPCR, droplet digital polymerase chain reaction; DMD, Duchenne muscular dystrophy; eGFP, enhanced green fluorescent protein; ELISA, enzyme-linked immunosorbent assay; MHCK, myosin-heavy-chain kinase.

1. ClinicalTrials.gov. NCT04626674 (Accessed April 2023).

Results

Evaluation of vector exposure in various biomaterials after administration of delandistrogene moxeparvovec in participants from ENDEAVOR (Cohort 1)

Biomaterial	Number of samples	Number of BLOD samples*	Number of samples included in analysis	Cut-off used in data analysis, days	Time of last observation above LOD, days†
Saliva (n=18)	132	67 (50.8%)	115 (87.1%)	100	84.17
Urine (n=20)	172	76 (44.2%)	154 (89.5%)	200	175.96
Feces (n=10)	58	10 (17.2%)	54 (93.1%)	200	90.92

*These records were not excluded from the analysis but were set to LLOQ (M3 method) for population PK model development. For the number of BLOD samples and number of samples included in the analysis, the total number of samples and percentage of total samples are provided. †Values based on a modeling prediction.

BLOD, below the limit of detection; LLOQ, lower limit of quantitation; LOD, limit of detection; PK, pharmacokinetics.

Results
Mean vector genome DNA at peak compared with Week 4

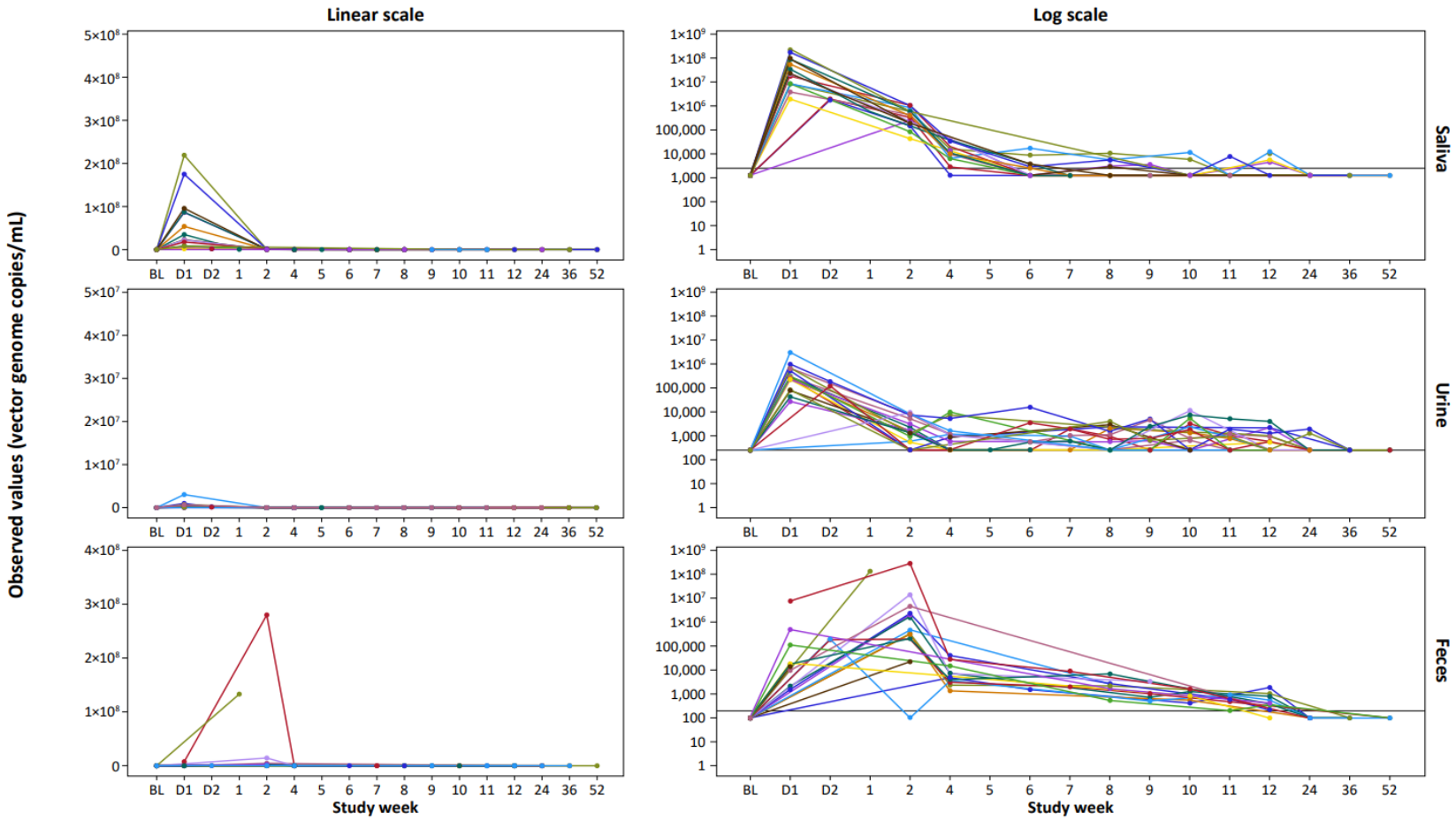
- The vector DNA concentration peaked roughly at Day 1 in saliva and urine, and at Week 2 in feces. The mean concentration in all samples declined significantly by Week 4
- The percentage decrease from peak (Day 1 for saliva and urine; Week 2 for feces) to Week 4 was greater than 99%

Sample	Mean peak concentration	Mean Week 4 concentration	Percentage decrease from peak to Week 4
Saliva	$5.6x10^7$ vgc/mL (n=15; Day 1)	$1.4x10^4$ vgc/mL (n=12)	99.97%
Urine	$4.8x10^5$ vgc/mL (n=17; Day 1)	$1.7x10^3$ vgc/mL (n=18)	99.64%
Feces	$2.4x10^7$ vgc/µg total DNA (n=13; Week 2)	$1.1x10^4$ vgc/µg total DNA (n=11)	99.99%

vgc, vector genome copies.

Results

Quantification of delandistrogene moxeparvovec vector shedding over time for Cohort 1 of the ENDEAVOR study population

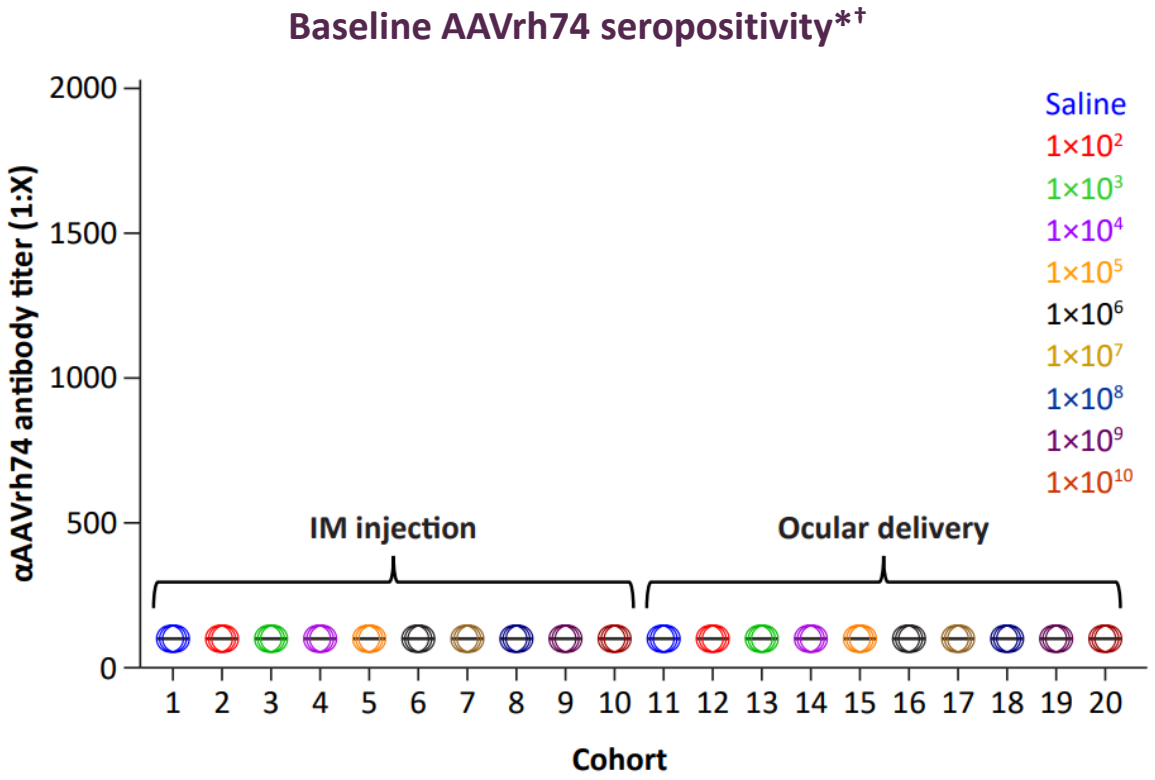


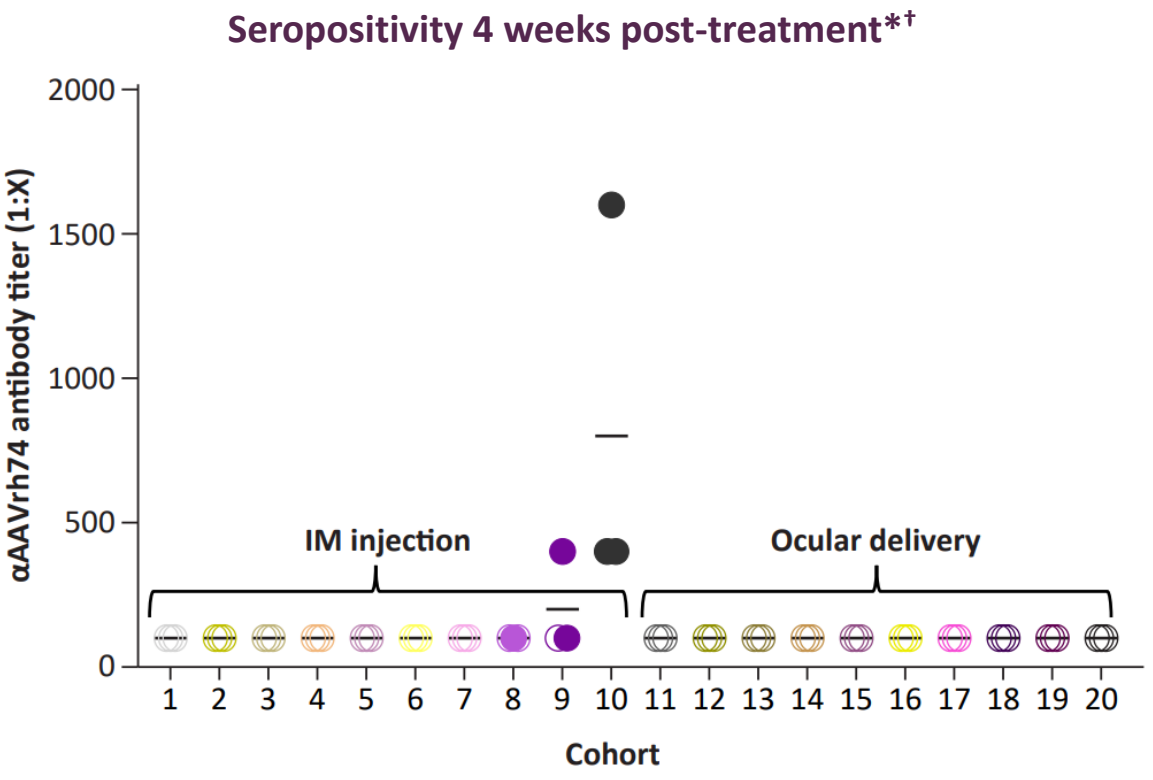
- The horizontal line is the reference line for LOD: 2,500 vgc/mL for saliva samples, 500 vgc/mL for urine samples and 200 vgc/µg total DNA for feces samples
- There was considerable variability in shed vector on Day 1 following treatment, while a high proportion of observations in the terminal phase were BLOD
- A delayed rate of kinetics of vector shed was observed in feces relative to saliva and urine samples due to the innate differences between the biomaterials. The local peak seen in vector shedding in feces declined by >99% from Weeks 2–4
- Results from biodistribution and vector shedding data obtained in non-clinical studies were consistent with clinical shedding results and support the clinical shedding analysis

BL, baseline; BLOD, below the limit of detection; LOD, limit of detection; vgc, vector genome copies.

Results

Seropositivity to AAVrh74 following ocular and IM delivery in mice (n=3 for all cohorts)





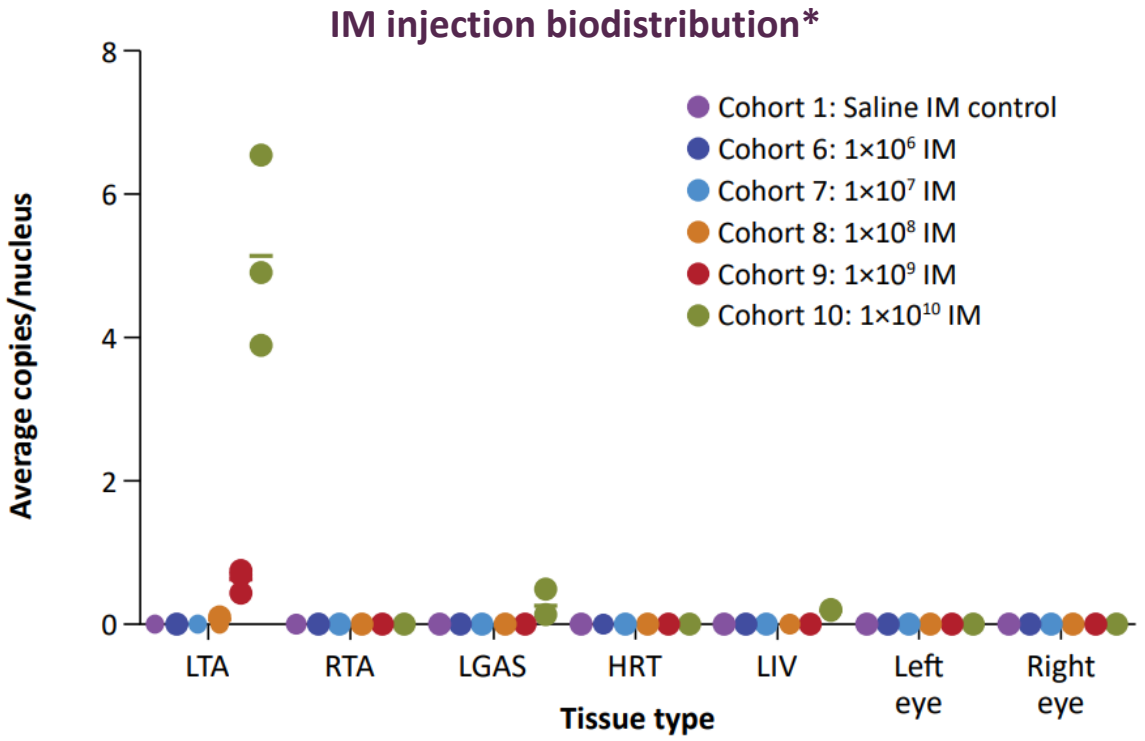
No mice in the study demonstrated seropositivity to AAVrh74 at baseline. After 4 weeks of treatment with AAVrh74.CMV.eGFP, serum was reassessed for seropositivity to AAVrh74

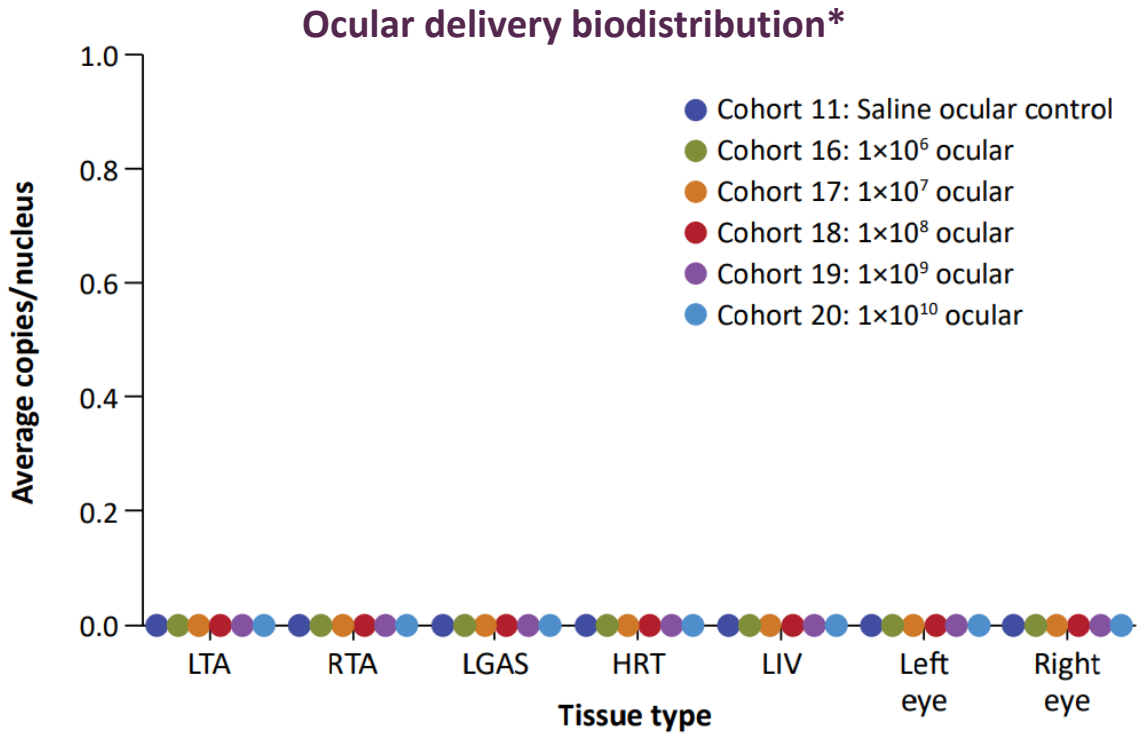
*Bar represents the mean. †Open circles denote seronegativity. Closed circles denote seropositivity.

AAVrh74, adeno-associated virus rhesus isolate serotype 74; CMV, cytomegalovirus promoter; eGFP, enhanced green fluorescent protein; IM, intramuscular.

Results

Biodistribution of eGFP following ocular and IM delivery in mice (n=3 for all cohorts)





High-dose IM-injected mice showed increasing eGFP DNA copies in LTA, LGAS and LIV

Mice with ocular delivery to the right eye showed no signs of eGFP DNA in any of their collected tissues, and showed no development of anti-AAVrh74 antibodies even at higher titer exposures

*Bar represents the mean.

AAVrh74, adeno-associated virus rhesus isolate serotype 74; eGFP, enhanced green fluorescent protein; HRT, heart; IM, intramuscular; LGAS, left gastrocnemius; LIV, liver; LTA, left tibialis anterior; RTA, right tibialis anterior.

Conclusions

In patients, peak vector shedding generally occurred in the first few days after infusion, then exponentially declined to insignificant levels by Week 4

Naïve mice remained seronegative following topical ocular exposure to the highest titers of vector shed in patients

- Only naïve mice exposed to this same titer intramuscularly, as a positive control, seroconverted

Results suggest that the AAVrh74 vector is not immunogenic when administered at relevant titers via a mucosal route, and that the risk of seroconversion following exposure to shed vector may be very low

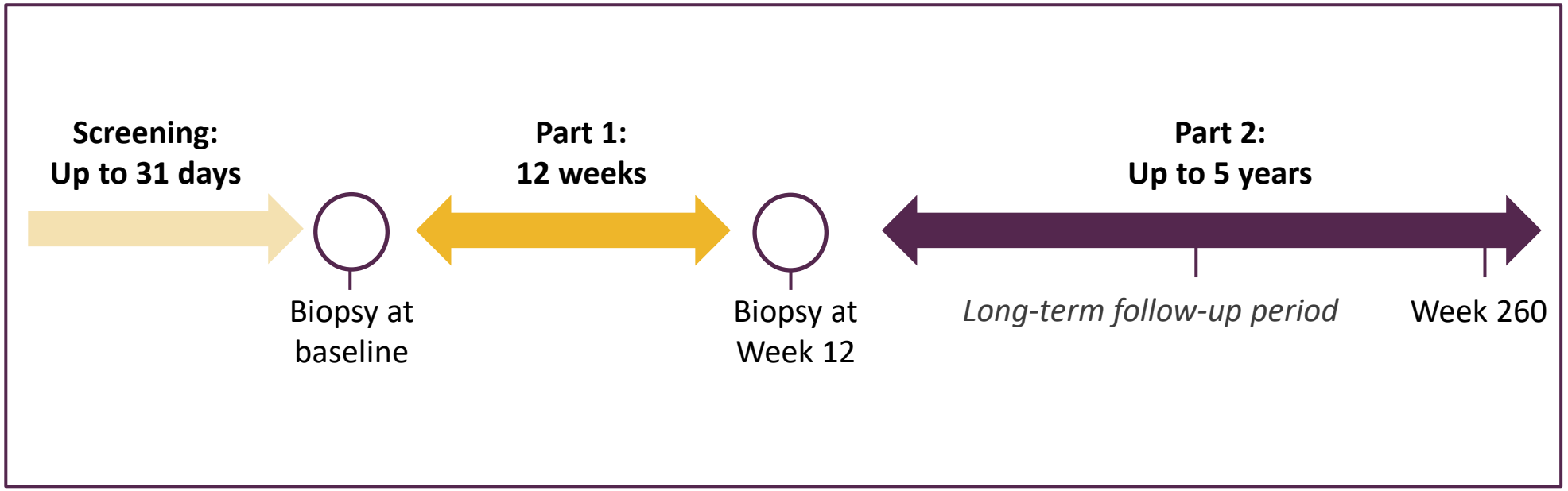
AAVrh74, adeno-associated virus rhesus isolate serotype 74.

Supplementary materials

Clinical assessment of delandistrogene moxeparvovec vector shedding in participants from ENDEAVOR

Study design: Single IV infusion dose of 1.33×10^{14} vg/kg* of intended commercial process delandistrogene moxeparvovec material

- **Cohort 1† (ambulatory, ≥4 to <8 years old)**
- **Cohort 2† (ambulatory, ≥8 to <18 years old)**
- **Cohort 3† (non-ambulatory)**
- **Cohort 4† (ambulatory, ≥3 to <4 years old)**
- **Cohort 5a† (ambulatory, ≥4 to <9 years old)**
- **Cohort 5b† (non-ambulatory)**



- ENDEAVOR is an ongoing, open-label, single-arm, single-dose, Phase 1b study with five cohorts and a two-part follow-up period conducted at four sites in the USA using the intended commercial process delandistrogene moxeparvovec material
- Samples from different clinical biomaterials at predefined time points over the course of the study (260 weeks) were collected from patients across the five cohorts

*Linear qPCR. †Only 1-year data for Cohort 1 are presented in this poster; 1-year data for other cohorts are not yet available; genetic mutation criteria varied by cohort.
IV, intravenous; qPCR, quantitative polymerase chain reaction; vg, vector genome.

Supplementary materials

Baseline clinical characteristics of Cohort 1 in ENDEAVOR

Characteristic	Total for Cohort 1 (N=20) Mean (SD)
Age, years*	5.8 (1.1)
Height, cm	108.8 (7.7)
Dosing weight, kg	21.2 (4.2)
Time since DMD diagnosis, years	2.4 (1.4)

*Age distribution: 11 (55.0%) patients in the age category 4–5 years and 9 (45.0%) patients in the age category 6–7 years.
DMD, Duchenne muscular dystrophy; SD, standard deviation.

Supplementary materials

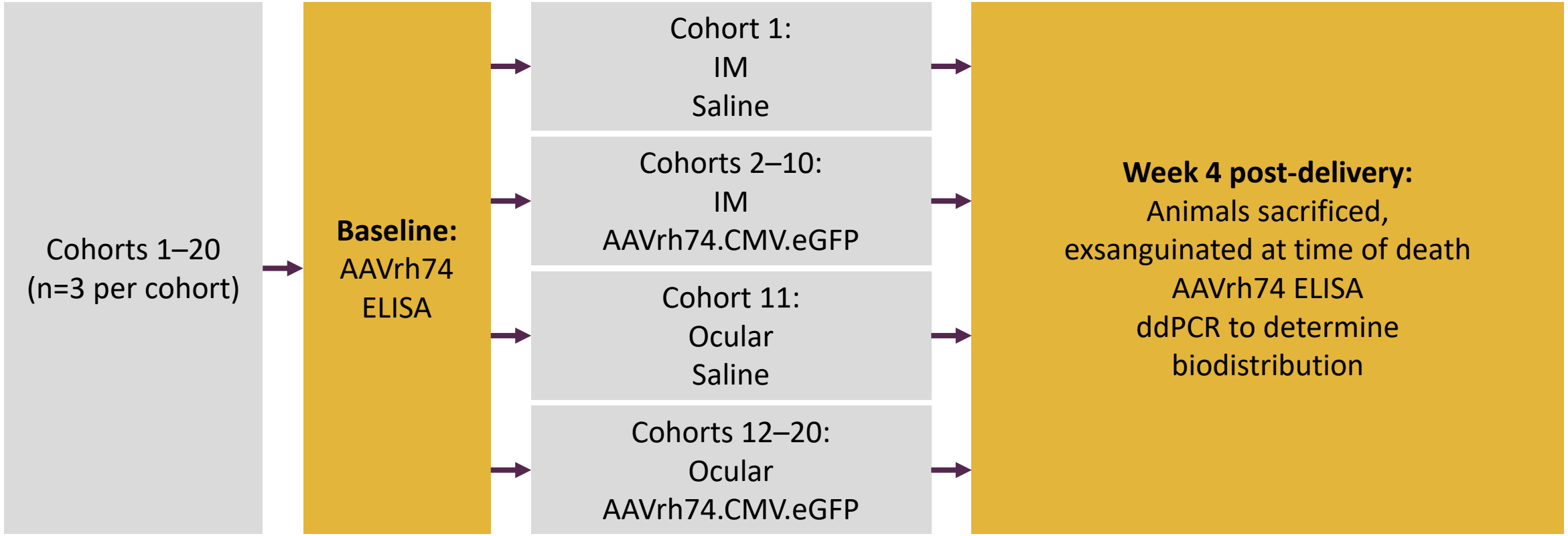
Preclinical experimental treatment groups

Strain	Cohort	Number of animals/sex	Test/Control article	Route*	Dose (vg)	Volume (µL)†
C57BL/6J	1	3/Male	Saline	IM	N/A	30
C57BL/6J	2	3/Male	AAVrh74.CMV.eGFP	IM	1.0×10^{2}	30
C57BL/6J	3	3/Male	AAVrh74.CMV.eGFP	IM	1.0×10^{3}	30
C57BL/6J	4	3/Male	AAVrh74.CMV.eGFP	IM	1.0×10^{4}	30
C57BL/6J	5	3/Male	AAVrh74.CMV.eGFP	IM	1.0×10^{5}	30
C57BL/6J	6	3/Male	AAVrh74.CMV.eGFP	IM	1.0×10^{6}	30
C57BL/6J	7	3/Male	AAVrh74.CMV.eGFP	IM	1.0×10^{7}	30
C57BL/6J	8	3/Male	AAVrh74.CMV.eGFP	IM	1.0×10^{8}	30
C57BL/6J	9	3/Male	AAVrh74.CMV.eGFP	IM	1.0×10^{9}	30
C57BL/6J	10	3/Male	AAVrh74.CMV.eGFP	IM	1.0×10^{10}	30

Strain	Cohort	Number of animals/sex	Test/Control article	Route*	Dose (vg)	Volume (µL)†
C57BL/6J	11	3/Male	Saline	Ocular	N/A	4
C57BL/6J	12	3/Male	AAVrh74.CMV.eGFP	Ocular	1.0×10^{2}	4
C57BL/6J	13	3/Male	AAVrh74.CMV.eGFP	Ocular	1.0×10^{3}	4
C57BL/6J	14	3/Male	AAVrh74.CMV.eGFP	Ocular	1.0×10^{4}	4
C57BL/6J	15	3/Male	AAVrh74.CMV.eGFP	Ocular	1.0×10^{5}	4
C57BL/6J	16	3/Male	AAVrh74.CMV.eGFP	Ocular	1.0×10^{6}	4
C57BL/6J	17	3/Male	AAVrh74.CMV.eGFP	Ocular	1.0×10^{7}	4
C57BL/6J	18	3/Male	AAVrh74.CMV.eGFP	Ocular	1.0×10^{8}	4
C57BL/6J	19	3/Male	AAVrh74.CMV.eGFP	Ocular	1.0×10^{9}	4
C57BL/6J	20	3/Male	AAVrh74.CMV.eGFP	Ocular	1.0×10^{10}	4

IM = intramuscular injection into the LTA muscle. *Test article delivery into the TA occurred on the left side, while ocular delivery occurred on the right side of the animal (i.e. left TA and right eye). †Doses were Q.S. up to total desired volume with saline. AAVrh74, adeno-associated virus rhesus isolate serotype 74; CMV, cytomegalovirus promoter; eGFP, enhanced green fluorescent protein; IM, intramuscular; LTA, left tibialis anterior; Q.S., quantum satis; TA, tibialis anterior; vg, vector genome.

Supplementary materials
Non-clinical study design



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