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Poster 87

Analysis of vector shedding following treatment with delandistrogene moxeparvovec, an investigational rAAVrh74-based gene therapy for DMD

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Objective

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.

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.

BACKGROUND

- Delandistrogene moxeparvovec is an investigational rAAVrh74-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]

METHODS

- We evaluated the extent and magnitude of shedding and clearance from delandistrogene moxeparvovec using interim data from ENDEAVOR (Study 103; SRP-9001-103; NCT04626674), 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.

RESULTS

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

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

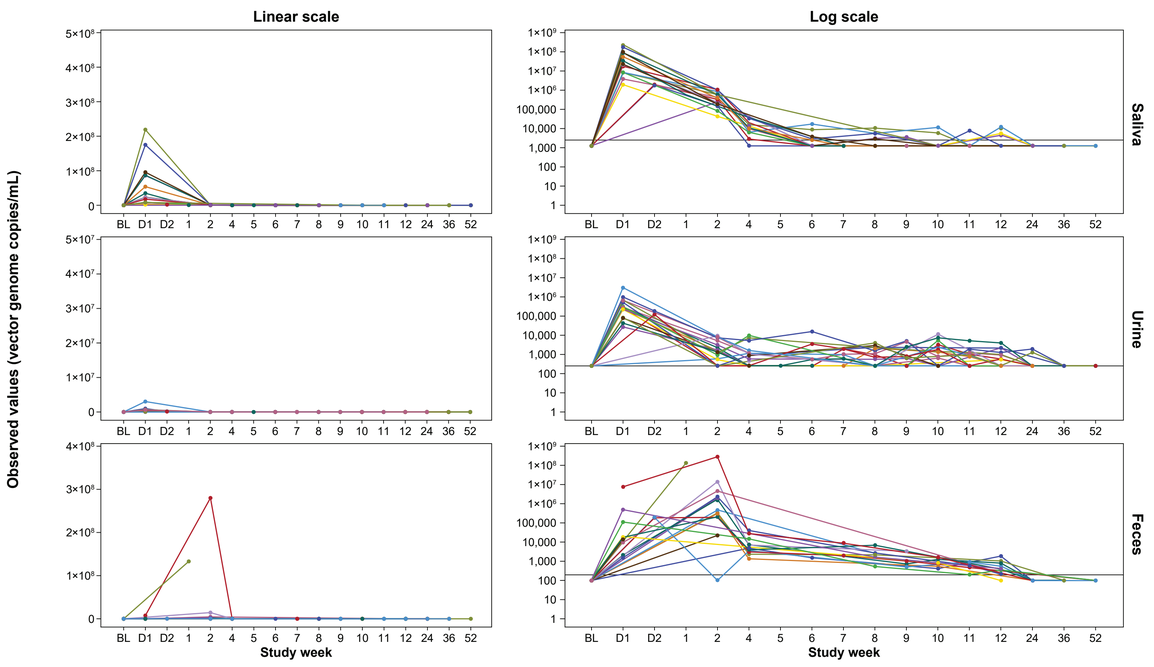
*These records were not excluded from the analysis but were set to LLOQ (M3 method) for population PK model development. For the number of BLOD samples and number of samples included in the analysis, the total number of samples and percentage of total samples are provided. †Values based on a modeling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

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%.

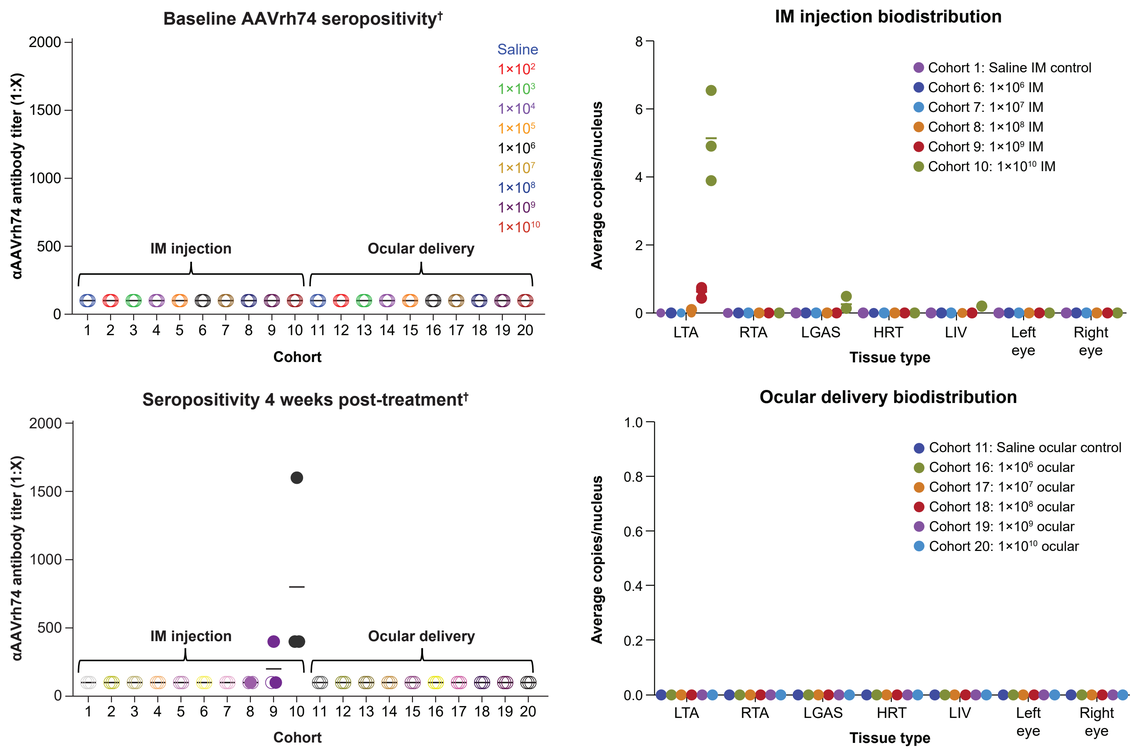
Sample	Mean peak concentration	Mean Week 4 concentration	Percentage decrease from peak to Week 4
Saliva	$5.6x10^7$ vgc/mL (n=15; Day 1)	$1.4x10^4$ vgc/mL (n=12)	99.97%
Urine	$4.8x10^5$ vgc/mL (n=17; Day 1)	$1.7x10^3$ vgc/mL (n=18)	99.64%
Feces	$2.4x10^7$ vgc/µg total DNA (n=13; Week 2)	$1.1x10^4$ vgc/µg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

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.

Seropositivity to AAVrh74 and biodistribution of eGFP in ocular and intramuscular delivery in mice (n=3 for all cohorts)*



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

- 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.
- 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.

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ABBREVIATIONS

AAVrh74, adeno-associated virus rhesus isolate serotype 74; BL, baseline; BLOD, below the limit of detection; CMV, cytomegalovirus promoter; D, days; ddPCR, droplet digital polymerase chain reaction; DMD, Duchenne muscular dystrophy; eGFP, enhanced green fluorescent protein; ELISA, enzyme-linked immunosorbent assay; HRT, heart; IM, intramuscular; LGAS, left gastrocnemius; LIV, liver; LLOQ, lower limit of quantitation; LOD, limit of detection; LTA, left tibialis anterior; PK, pharmacokinetics; rAAVrh74, recombinant AAV rhesus isolate serotype 74; RTA, right tibialis anterior; vg, vector genome; vgc, vector genome copies.

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