# Micro-dystrophin gene therapy delivery and therapeutic plasma exchange (TPE) in non-human primates (NHP)

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- SRP-9001 is an investigational therapy and has not been reviewed or approved by the FDA
- ELP, RAP, DG, SL, EP, and LRK are employees of Sarepta Therapeutics and may have stock options. LRK is a co-inventor of rAAVrh74.MHCK7.micro-dys technology, which is exclusively licensed to Sarepta Therapeutics. AM was an employee of Sarepta Therapeutics at the time of this study
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FDA, Food and Drug Administration; GPPE, Good Publication Practice; ISMPP, International Society for Medical Publication Professionals.

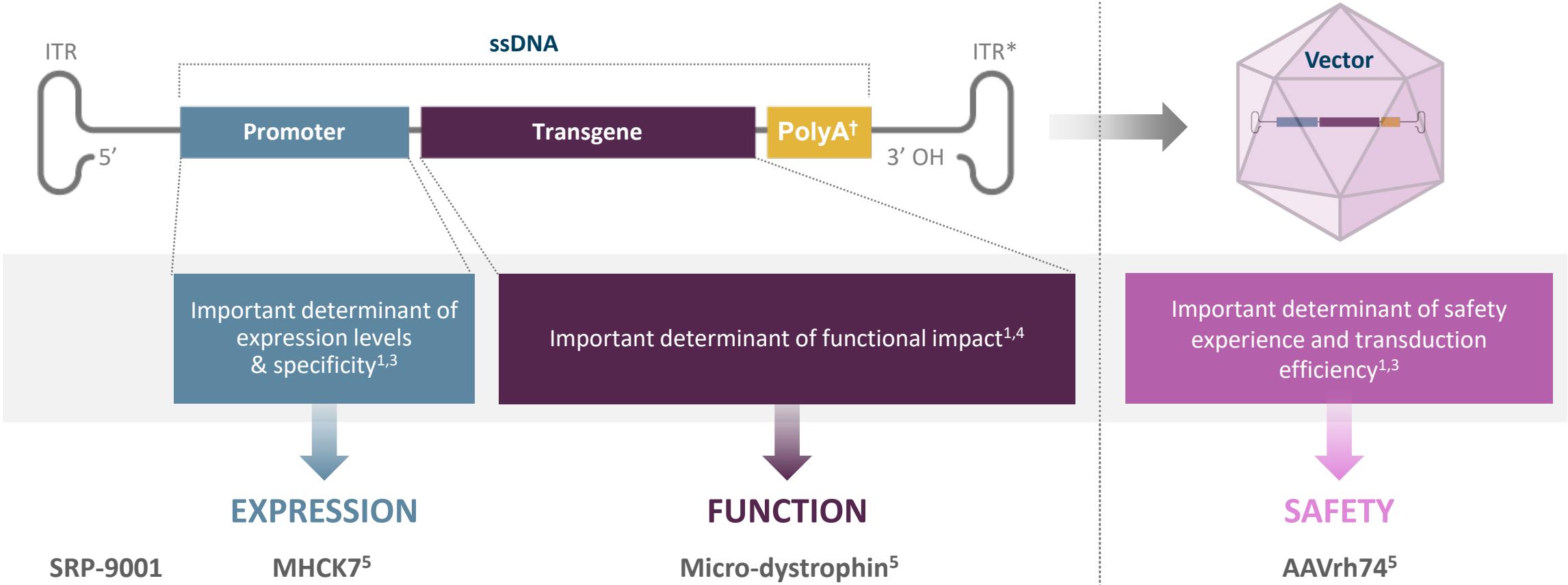
# Introduction

- Duchenne muscular dystrophy (DMD) is a rare, X-linked, and fatal neuromuscular disease caused by mutations in the *DMD* gene that disrupt the production of functional dystrophin protein[1,2]
- Gene transfer therapy using systemic AAV delivery is being extensively investigated for the treatment of monogenic diseases, including DMD[3]
- A significant challenge to gene transfer therapy is pre-existing immunity to AAV vectors, which can result in immune-mediated destruction of transduced cells and limit therapeutic efficacy[3,4]
- **Clinical development of gene transfer therapy is advancing rapidly; it is therefore imperative to evaluate strategies to optimize safety and efficacy, as well as for dosing individuals with pre-existing antibodies against the vectors used for delivery[3]**

AAV, adeno-associated virus; DMD, Duchenne muscular dystrophy.

1. Hoffman EP, et al. *Cell*. 1987;51(6):919-928; 2. Koenig M, et al. *Cell*. 1987;50(3):509-517; 3. Asher DR, et al. *Expert Opin Biol Ther*. 2020;20(3):263-274; 4. Manno CS, et al. *Nat Med*. 2006; 12(3):324-347.

# rAAVrh74.MHCK7.micro-dystrophin (SRP-9001) is a novel AAV-based gene therapy for the treatment of DMD[1,2]




*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. 1. Asher DR, et al. *Expert Opin Biol Ther*. 2020;20(3):263-74. 2. US National Library of Medicine. Help Me Understand Genetics: Gene Therapy; 2013. Available at: https://ghr.nlm.nih.gov/primer/therapy/genetherapy. Last accessed: April 2021. 3. Zheng C and Baum BJ. *Methods Mol Biol*. 2008;434:205-19. 4. Chandler RJ and Venditti CP. *Transl Sci Rare Dis*. 2016;1(1):73-89. 5. Mendell JR, et al. *JAMA Neurol*. 2020;77(9):1-10. AAV, adeno-associated virus; DMD, Duchenne muscular dystrophy; ITR, inverted terminal repeat; OH, hydroxyl; polyA A, polyadenylation; rAAVrh74, recombinant AAV rhesus isolate serotype 74; ssDNA, single-stranded DNA.

# Objective

## PART 1 OBJECTIVE

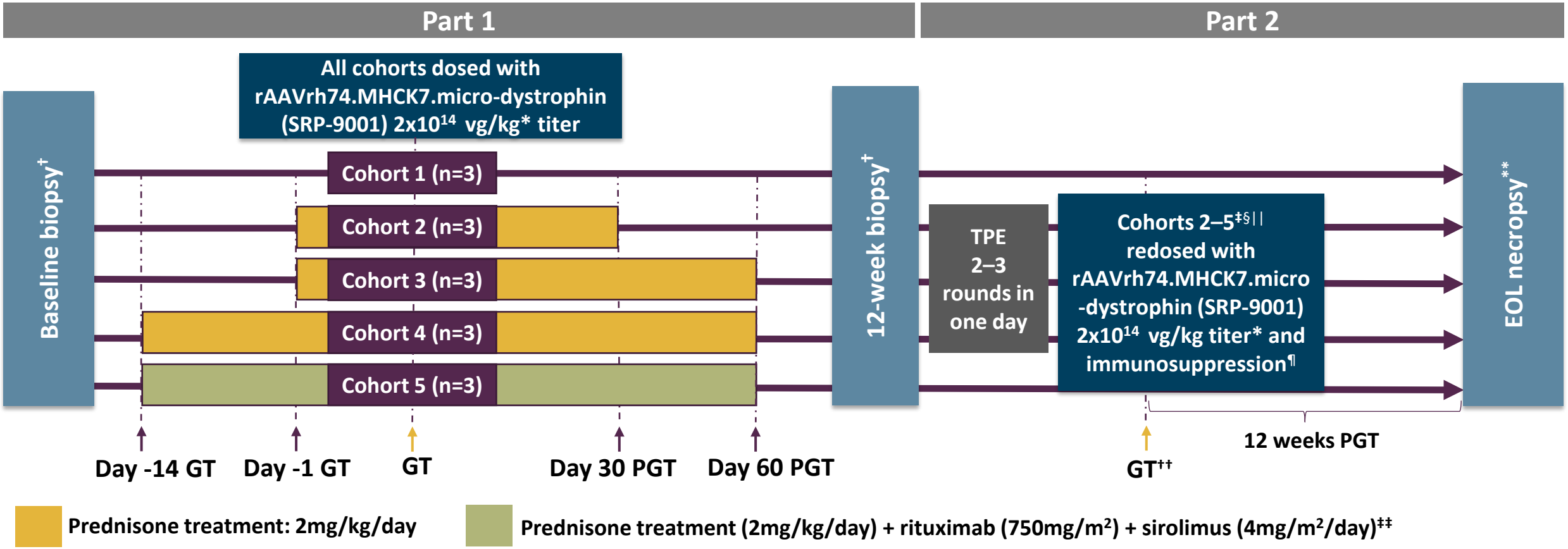
- Investigate the impact of various immunosuppression strategies on the safety and efficacy of gene transfer therapy
- **Hypothesis:** The duration/regimen of steroids leads to higher vector genome copy numbers

## PART 2 OBJECTIVE

- Analyze the safety and efficacy of TPE as a potential pre-treatment for individuals with pre-existing immunity
- **Hypothesis:** Performing TPE before redosing will reduce antibody titers towards AAVrh74

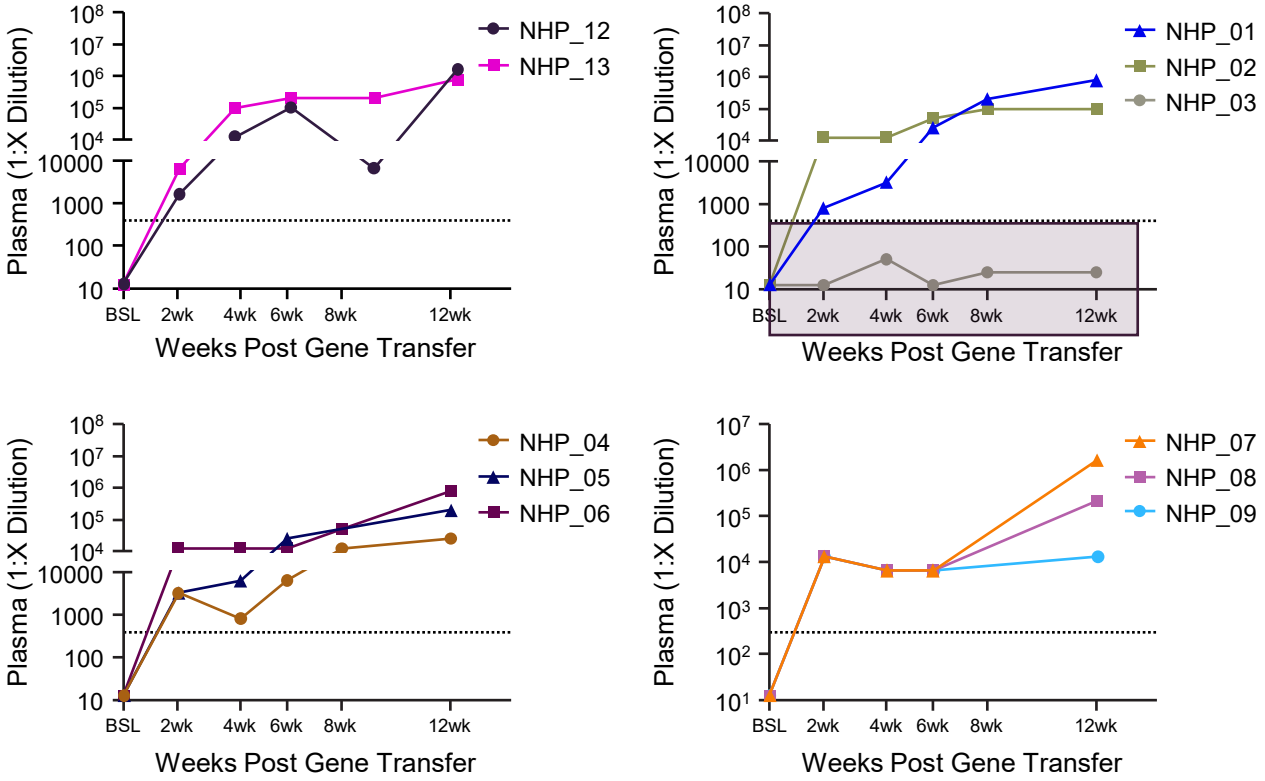
AAVrh74, adeno-associated virus rhesus isolate serotype 74; TPE, therapeutic plasma exchange.

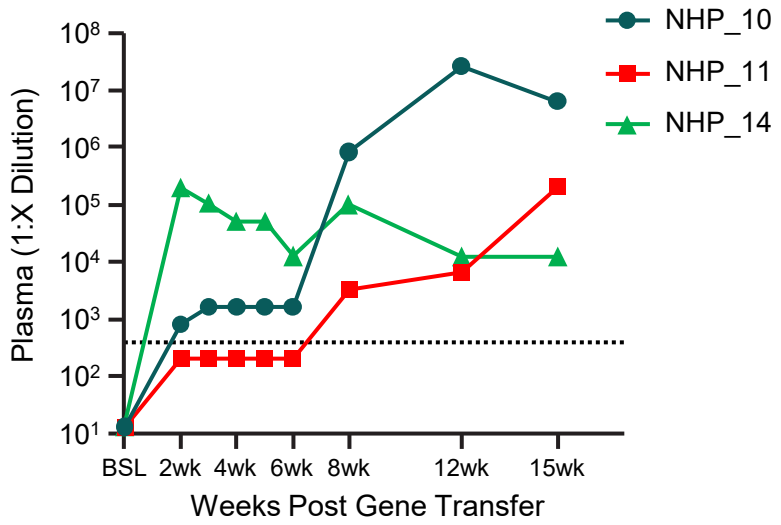
# Study design




*Supercoiled qPCR titer method. †Biopsy collected from gastrocnemius muscle. ‡One NHP did not undergo TPE due to lack of antibody response to AAVrh74. §One NHP did not undergo TPE due to poor vascular access. ||Cohort 5 did not undergo TPE due to incompatibility with previous treatment with rituximab. ¶All NHPs received prednisone (2mg/kg/day) from 1 day-pre to 30 days post-redosing with SRP-9001. **EOL necropsy collected from gastrocnemius, heart, and diaphragm. ††Immediately post-TPE, the NHPs were disconnected from the apheresis unit and systemically redosed with rAAVrh74.MHCK7.micro-dystrophin (SRP-9001). ‡‡Siroliumus was started 3 days prior to the first GT dose and was continued until three weeks after the second dose. NHPs received one dose of Rituximab 14 days and 7 days prior to the first GT dose, as well as one dose on the day of GT dosing and one dose prior to redose.. AAVrh74, adeno-associated virus rhesus isolate serotype 74; EOL, end of life; GT, gene transfer; NHP, non-human primate; PGT, post-gene therapy; qPCR, quantitative polymerase chain reaction; TPE, therapeutic plasma exchange; vg/kg, vector genomes per kilogram bodyweight.

# Part 1 results: AAVrh74 Antibody response: Part 1




Cohorts 1-4: no abnormal antibody response was observed, except in NHP_03, which did not mount a robust antibody response (despite IV infusion)

Cohort 5: Triple immunosuppression regimen did not suppress 2 of 3 NHPs; 1 NHP exhibited slight immunosuppression for 6 weeks

AAVrh74, adeno-associated virus rhesus isolate serotype 74; IV, intravenous; NHP, non-human primate; wk, week.

# Part 1 results: safety profile and transduction efficiency

## SAFETY PROFILE (SERUM CHEMISTRY AND IMMUNOLOGY)

**AEs in NHPs from Cohorts 1–4 included transient elevated ALT and AST liver enzymes**

- NHPs from Cohort 5 (treated with a triple immunosuppressive regimen) developed hives 2-3 weeks post vector injection
  - Benadryl given orally 5-10 days
  - After the final rituximab infusion, 2 subjects in Cohort 5 began vomiting and experienced elevated heart rates. Vomiting subsidized and all other parameters returned to normal ranges when the dose was lowered. Primates recovered from anesthesia without further adverse events

## IMMUNOSUPPRESSION EFFECT IN ANTIBODY RESPONSE AND TRANSDUCTION EFFICIENCY

**Anti-AAVrh74 total antibody response was similar across cohorts**

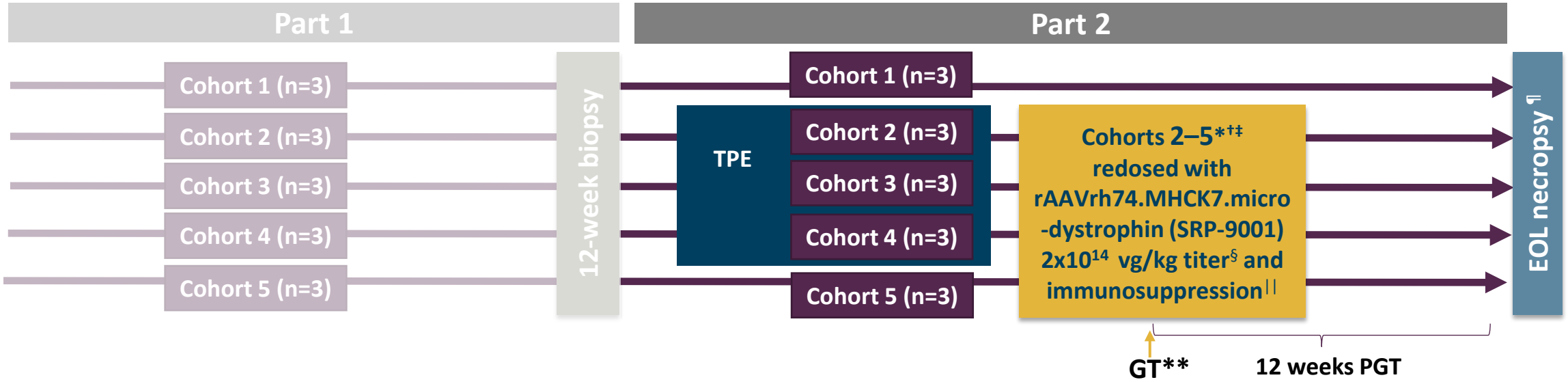
- No abnormal observations aside from one NHP in Cohort 2 that did not mount an antibody response

**The antibody response to AAVrh74 in Cohort 5 NHPs was similar to that of NHPs in Cohorts 1–4**

**The difference in vector genome copies among NHP Cohorts 1–5 was not statistically significant at 12 weeks post-gene transfer therapy (P>0.05)**

AAVrh74, adeno-associated virus rhesus isolate serotype 74; AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase; NHP, non-human primate.

# Part 2: analyze the safety and efficacy of TPE as a potential pre-treatment for individuals with pre-existing immunity




Seven NHPs from Cohorts **2-4** underwent 2–3 consecutive cycles of TPE, resulting in reduced levels of circulating antibodies against AAVrh74

Cohort 5 was redosed without TPE

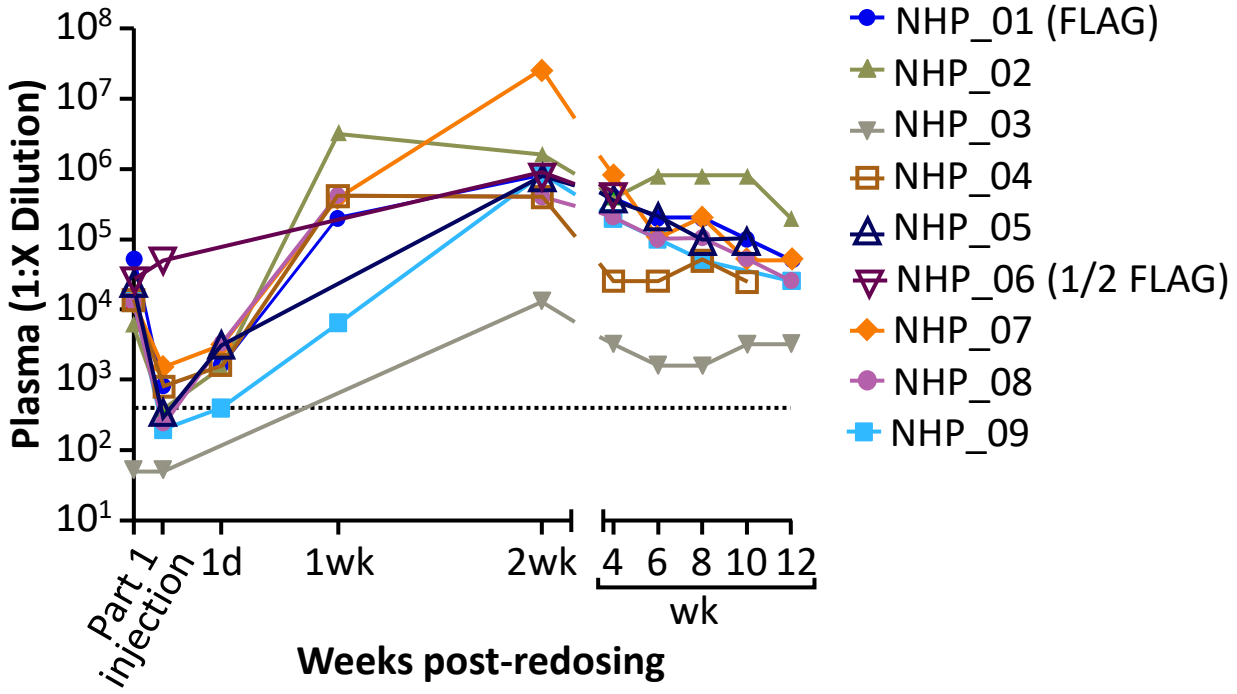
- Immediately following TPE, NHPs were successfully redosed with rAAVrh74.MHCK7.micro-dystrophin
- In four NHPs from Cohorts 2-4 (NHP_02, NHP_05, NHP_08 and NHP_09), antibody titers of ≤1:400 were achieved

*One NHP did not undergo TPE due to lack of antibody response to AAVrh74. †One NHP did not undergo TPE due to poor vascular access. ‡Cohort 5 did not undergo TPE due to incompatibility with previous treatment with rituximab. §Supercoiled qPCR titer method. ||All NHPs received prednisone (2mg/kg/day) from 1 day-pre to 30 days post-redosing with SRP-9001. ¶EOL necropsy collected from gastrocnemius, heart, and diaphragm. **Immediately post-TPE, the NHPs were disconnected from the apheresis unit and systemically redosed with rAAVrh74.MHCK7.micro-dystrophin (SRP-9001). AAVrh74, adeno-associated virus rhesus isolate serotype 74; EOL, end of life; GT, gene transfer; NHP, non-human primate; PGT, post-gene therapy; qPCR, quantitative polymerase chain reaction; TPE, therapeutic plasma exchange; vg/kg, vector genomes per kilogram bodyweight.

# Part 2 results: total antibody titers against AAVrh74 in NHP prior to TPE and following TPE (before redosing with SRP-9001)

| NHP (Cohort) | Titer after Part 1* | Titer after TPE† | Number of TPE cycles |
|---|---|---|---|
| NHP_01(2) | 1:51200 | 1:800 | 2.5 |
| NHP_02(2) | 1:6400 | 1:400 | 3 |
| NHP_03(2) | 1:50 | NA‡ | NA |
| NHP_04(3) | 1:12800 | 1:800 | 3 |
| NHP_05(3) | 1:25600 | 1:400 | 3 |
| NHP_06(3) | 1:25600 | NA§ | 0.5 |
| NHP_07(4) | 1:12800 | 1:1600 | 3 |
| NHP_08(4) | 1:12800 | 1:200 | 3 |
| NHP_09(4) | 1:12800 | 1:200 | 3 |

**Antibody titer to AAVrh74 in NHPs following redosing with SRP-9001**




The number of TPE cycles that can be performed in NHPs is limited due to the lack of donor blood available
In humans, multiple rounds of TPE can be administered

*12 weeks post initial gene transfer. †Prior to redose injection of rAAVrh74.MHCK7.micro-dystrophin. ‡NHP_03 was redosed without prior TPE due to lack of antibody response to AAVrh74; §NHP_06 only underwent 0.5 cycles of TPE due to small size and poor vascular access; dotted line represents inclusion criteria for total AAVrh74 antibody titer levels (threshold of 1:400 against AAVrh74).
AAVrh74, adeno-associated virus rhesus isolate serotype 74; d, day; NHP, non-human primate; TPE, therapeutic plasma exchange; wk, week.

# Part 2 results: safety profile and immune response to AAVrh74 pre- and post-TPE

## SAFETY PROFILE (SERUM CHEMISTRY AND IMMUNOLOGY)

### The TPE procedure was generally well tolerated

- There were no abnormal immunological observations as assessed by IFN-γ SFC levels against AAVrh74 and micro-dystrophin peptides from peripheral blood mononuclear cells
- Redosing following TPE resulted in increased liver enzyme levels (ALT/AST) in the following NHPs: NHP_01 and NHP_02 (Cohort 2); NHP_04 (Cohort 3); NHP_08 and NHP_09 (Cohort 4)
  - These were resolved with continued prednisone daily administration

### NHPs from Cohort 5 did not receive TPE due to incompatibility with previous treatment* and had the total antibody titer to AAVrh74 higher than 1:51,200 before redosing

- NHPs redosed at high antibody titer (Cohort 5) experienced the following AEs: increased heart rate and ventilation rate, vomiting, rash near delivery site, paleness of the skin, and shallow breathing
  - These all resolved after administration of diphenhydramine and dexamethasone

*Cohort 5 did not undergo TPE due to incompatibility with previous rituximab treatment and two NHPs (NHP_10, NHP_11) were redosed.

AAVrh74, adeno-associated virus rhesus isolate serotype 74; ALT, alanine aminotransferase; AST, aspartate aminotransferase; IFN-γ SFC, interferon gamma spot-forming cells; NHP, non-human primate; TPE, therapeutic plasma exchange.

# Conclusions Part 1: Investigate the impact of various immunosuppression strategies on the safety and efficacy of gene transfer therapy

- Anti AAVrh74 total antibody response was similar among all NHP cohorts, with no evidence of abnormal immunological responses
- A few NHPs from Cohorts 1–4 experienced transient liver enzyme elevations
  - This is an expected AE with gene therapy treatment
  - Levels returned to normal in all cohorts
- There were no observed differences in transduction or protein expression with the immunosuppressive regimens tested

- NHPs from Cohort 5 (treated with triple immunosuppression regimen) developed hives 2-3 weeks post vector injection and had vomiting episodes
- AAV titers in Cohort 5 were not suppressed for the duration of the triple immunosuppression regimen

AAV, adeno-associated virus; AAVrh74, AAV rhesus isolate serotype 74; AE, adverse event; NHP, non-human primate; TPE, therapeutic plasma exchange.

# Conclusions Part 2: Analyze the safety and efficacy of TPE as a potential pre-treatment for individuals with pre-existing immunity

- The TPE procedure was well tolerated, with no abnormal clinical or immunological observations
- Levels of circulating antibodies to AAVrh74 were reduced after 2–3 consecutive rounds of TPE, and the NHPs that underwent TPE were safely redosed
- The presented data suggest TPE as a safe and efficacious strategy to consider for lowering AAVrh74 antibodies
- NHPs redosed with high titers experienced significant safety issues. When those titers were reduced with TPE, minimal safety issues were observed. **These results highlight the importance of reducing antibodies before dosing**
- Further studies are needed to evaluate the safety and efficacy of gene therapy dosing with pre-existing immunity to AAVrh74

AAVrh74, adeno-associated virus rhesus isolate serotype 74; AE, adverse event; NHP, non-human primate; TPE, therapeutic plasma exchange.