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

Ellyn L. Peterson,[1] Rachael A. Potter,[1] Danielle Griffin,[1] Sarah Lewis,[1] Eric Pozsgai,[1] Aaron Meadows,[2] and Louise R. Rodino-Klapac[1]

[1.] Sarepta Therapeutics, Inc., Cambridge, MA, USA;

[2.] Wexner Medical Center, The Ohio State University in Columbus, OH, USA.

Presented at the 2021 MDA Virtual Clinical & Scientific Conference | March 15–18, 2021

Acknowledgments & disclosures

- This study was funded by Sarepta Therapeutics, Inc.
- 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 AAVrh74.MHCK7.micro-dys technology, which is exclusively licensed to Sarepta Therapeutics. AM was an employee of Sarepta Therapeutics at the time of this study
- Medical writing and editorial support was provided by Ana Rondelli, PhD of MediTech Media, UK, in accordance with Good Publication Practice (GPP3) guidelines (http://www.ismpp.org/gpp3)

FDA, Food and Drug Administration; GPPE, Good Publication Practice; ISMPP, International Society for Medical Publication Professionals.

Introduction

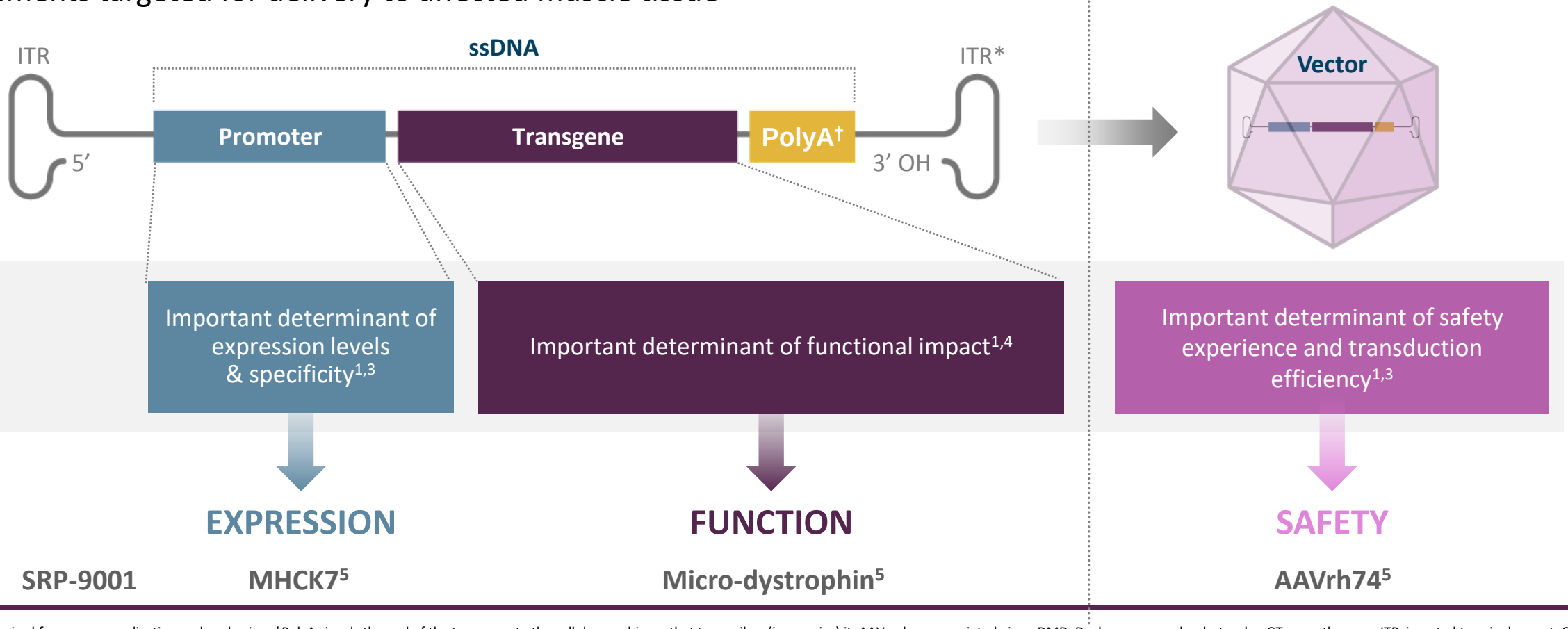
- Duchenne muscular dystrophy (DMD) is a rare, X-linked, and fatal neuromuscular disease caused by mutations in the *DMD* gene that disrupt the production of functional dystrophin protein[1,2]
- Gene transfer therapy using systemic AAV delivery is being extensively investigated for the treatment of monogenic 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 E P, et al. *Cell*. 1987;51(6):919-928; 2. Koenig M, et al. *Cell*. 1987;50(3):509-517; 3. Asher D R, et al. *Expert Opin Biol Ther*. 2020;20(3):263-274; 4. Manno C S, 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]

- The investigational SRP-9001 gene transfer therapy aims to deliver a micro-dystrophin transgene to produce a functional protein in skeletal (including diaphragm) and cardiac tissue, using vector (rAAVrh74) and promoter (MHCK7) elements targeted for delivery to affected muscle tissue[1]



*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. AAV, adeno-associated virus; DMD, Duchenne muscular dystrophy; GT, gene therapy; ITR, inverted terminal repeat; OH, hydroxyl; PolyA, polyadenylation; rAAVrh74, recombinant AAV rhesus isolate serotype 74; ssDNA, single-stranded DNA.

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: February 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.

Objective

- The objectives of this NHP study were to:

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

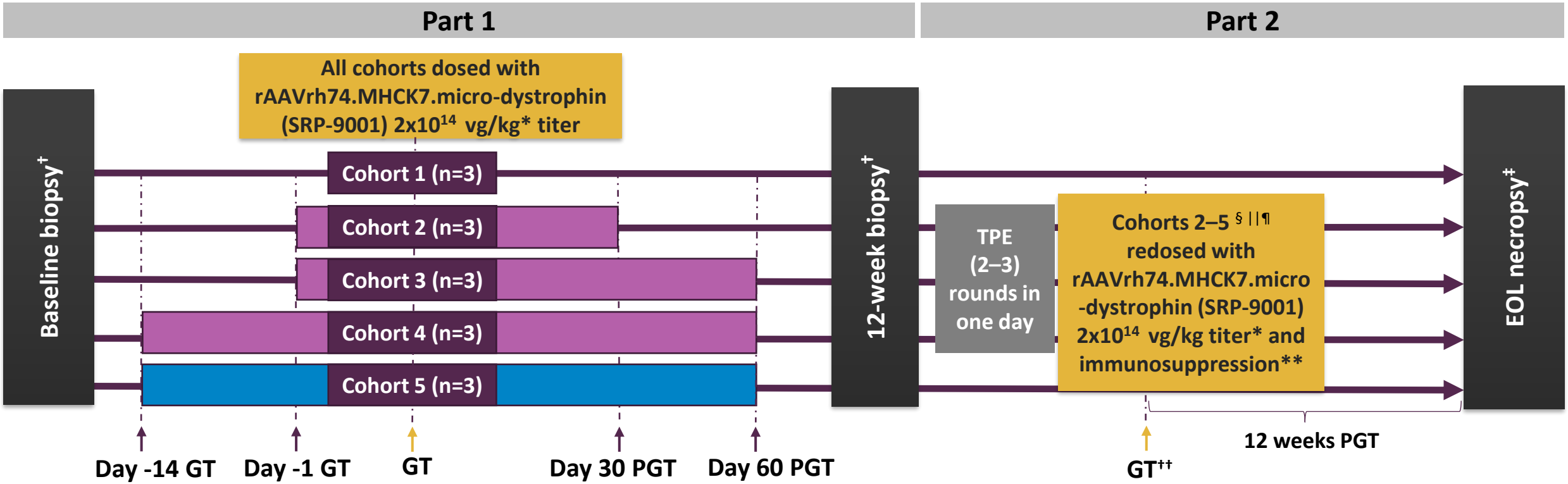
Hypothesis: The duration/regimen of steroids leads to higher vector genome copy numbers

Part 2: 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; NHP, non-human primate; TPE, therapeutic plasma exchange.

Study design



*Supercoiled qPCR titer method. †Biopsy collected from gastrocnemius muscle. ‡EOL necropsy collected from gastrocnemius, heart, and diaphragm. §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.††Immediately post-TPE, the NHPs were disconnected from the apheresis unit and systemically redosed with AAVrh74.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; TPE, therapeutic plasma exchange.

Part 1 results: safety profile and transduction efficiency

Safety profile (serum chemistry and immunology)

- Anti-AAVrh74 total antibody response to AAVrh74 was similar across cohorts with no evidence of abnormal observations, except for:
  - One NHP from Cohort 2 (NHP_03) that did not mount an antibody response to AAVrh74
  - NHPs from Cohort 5, which despite being treated with a triple immunosuppressive regimen demonstrated a similar antibody response to AAVrh74 to that observed with Cohorts 1–4
- AEs experienced by NHPs from Cohorts 1–4 included transient elevated ALT and AST liver enzymes
  - Two NHPs from Cohort 1 (NHP_12, NHP_13), one NHP from Cohort 3 (NHP_06) and one from Cohort 4 (NHP_07) showed elevated ALT and AST liver enzymes at 12 weeks post-gene transfer therapy

Transduction efficiency (vector genome copies/µg DNA)

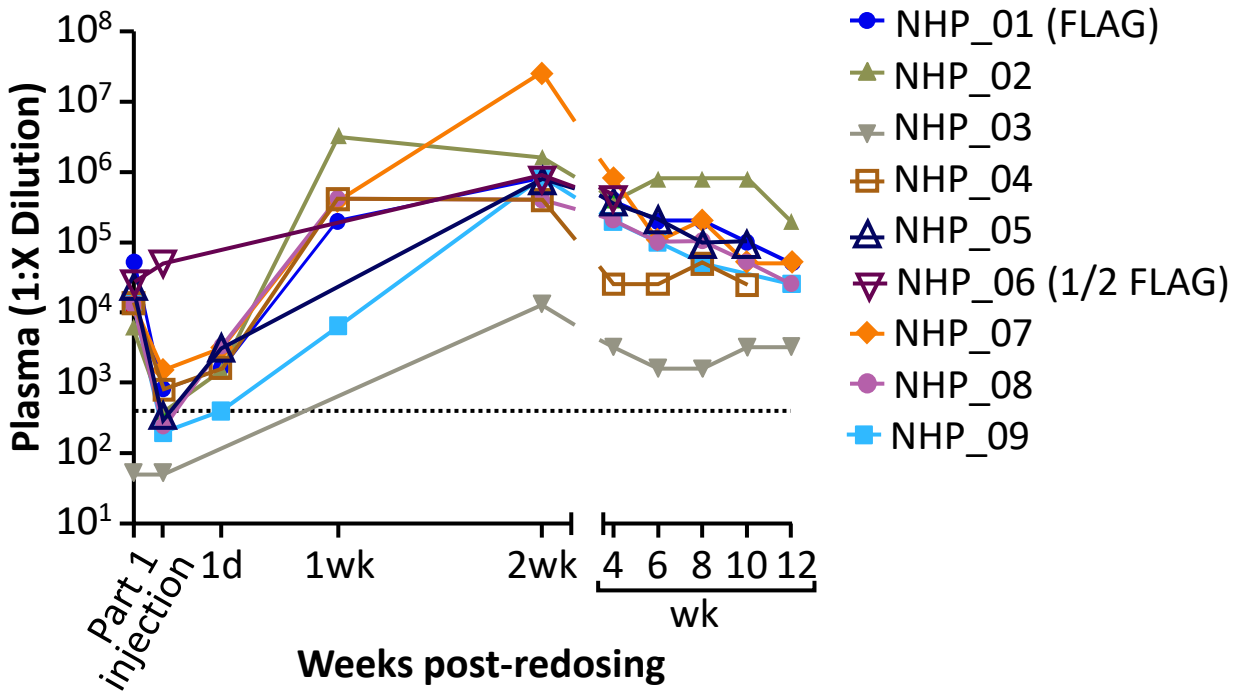
- No statistically significant difference in vector genome copies was observed between NHP Cohorts 1–5 at 12 weeks post-gene transfer therapy ($P>0.05$)

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

Part 2 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 AAVrh74.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; 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* 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, with pale, and shallow breathing; these all resolved after administration of diphenhydramine and dexamethasone

Immune response to AAVrh74 pre- and post-TPE

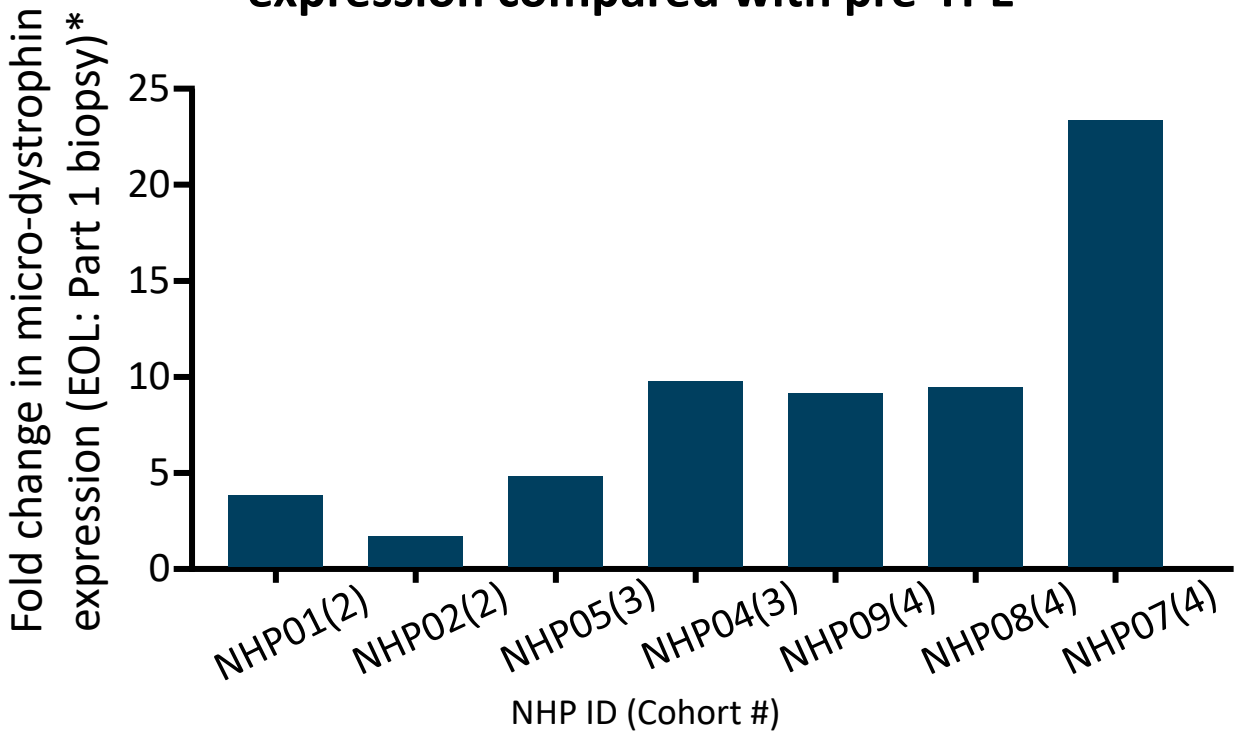
- Seven NHPs underwent 2–3 consecutive cycles of TPE, resulting in reduced levels of circulating antibodies against AAVrh74
  - Immediately following TPE, NHPs were successfully redosed with rAAVrh74.MHCK7.micro-dystrophin
  - In two NHPs from Cohort 4 (NHP_08 and NHP_09) antibody titers of 1:200 were achieved

*Cohort 5 did not undergo TPE due to incompatibility with previous treatment with rituximab 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.

Part 2 results: increased expression of micro-dystrophin protein was observed in tissue samples from NHPs redosed with SRP-9001 post-TPE

Post-TPE fold change in micro-dystrophin expression compared with pre-TPE



Pre-TPE titer	**1:51200**	**1:6400**	**1:25600**	**1:12800**	**1:12800**	**1:12800**	**1:12800**
Post-TPE titer	**1:800**	**1:400**	**1:400**	**1:800**	**1:200**	**1:200**	**1:1600**

Micro-dystrophin protein expression: key results

- Micro-dystrophin expression was increased in all NHPs redosed with rAAVrh74.MHCK7.micro-dystrophin post-TPE compared with expression pre-TPE from biopsy at Week 12 (Part 1)

Increased micro-dystrophin expression in skeletal muscle (e.g., GN), heart and diaphragm was also observed (data not shown)

*Densitometry values of Western blot from the GN muscle of NHPs post-TPE at EOL (Part 2, 12 weeks post-second GT [redose]) normalised to pre-TPE biopsy at Week 12 post-initial GT (Part 1) from the seven NHPs that underwent TPE and were redosed.
AAVrh74, adeno-associated virus rhesus isolate serotype 74; EOL, end of life; GN, gastrocnemius; NHP, non-human primate; TPE, therapeutic plasma exchange.

Conclusions

Conclusions Part 1

- Anti AAVrh74 total antibody response to AAVrh74 was similar in all NHP cohorts with no evidence of abnormal immunological responses. A few NHPs from Cohorts 1–4 experienced transient liver enzyme elevations, which 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

Conclusions Part 2

- These results demonstrate that redosing with rAAVrh74.MHCK7.micro-dystrophin after TPE led to an increase in micro-dystrophin protein expression compared with pre-TPE/redosing expression values
- 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 were safely redosed
- Further studies are needed to evaluate the safety and efficacy of gene therapy dosing with pre-existing immunity to AAVrh74
- The presented data suggest TPE as a safe and efficacious strategy to consider for lowering AAVrh74 antibodies

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