Presented at WMS | 20-24 September 2021

Microdystrophin gene transfer therapy and therapeutic plasma exchange in nonhuman primates

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Objectives

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

- Hypothesis: The duration/regimen of steroids may influence gene transfer therapy safety and transduction efficiency.

Part 2: analyze the safety and efficacy of TPE as a potential pretreatment for individuals with preexisting immunity.

- Hypothesis: Performing TPE before redosing with gene transfer therapy will reduce AAVrh74 antibody titers, allowing for safer administration in seropositive individuals.

CONCLUSIONS

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

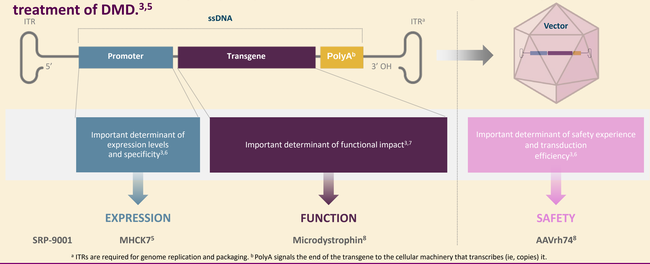
- Anti-AAVrh74 total antibody response to AAVrh74 was similar among all NHP cohorts, with no evidence of abnormal immunologic responses.
- A few NHPs from cohorts 1-4 experienced transient liver enzyme elevations.
  - This is an expected AE with gene therapy treatment.
  - Levels returned to normal in all cohorts.
- NHPs from cohort 5 (treated with triple immunosuppression regimen) developed hives 2-3 weeks post vector injection.
  - Two of these NHPs started vomiting 15 weeks post vector injection.
  - AAV titers in cohort 5 never decreased, despite continued sirolimus and an additional rituximab dose at 17 weeks.
- There were no observed differences in transduction or protein expression with the immunosuppressive regimens tested.

Part 2: analyze the safety and efficacy of TPE as a potential pretreatment for individuals with preexisting immunity

- The TPE procedure was well tolerated, with no abnormal clinical or immunologic 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.
- Further studies are needed to evaluate the safety and efficacy of gene therapy dosing with preexisting immunity to AAVrh74.
- 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.

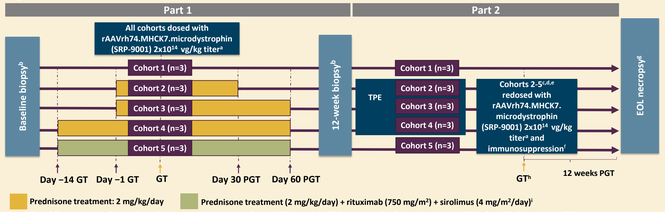
BACKGROUND

- 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 preexisting 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 dose individuals with preexisting antibodies against the vectors used for delivery.[3]**
- **rAAVrh74.MHCK7.micro-dystrophin (SRP-9001) is a novel AAV-based gene therapy for the treatment of DMD.[3,5]**



[a] ITRs are required for genome replication and packaging. [b] PolyA signals the end of the transgene to the cellular machinery that transcribes (ie, copies) it.

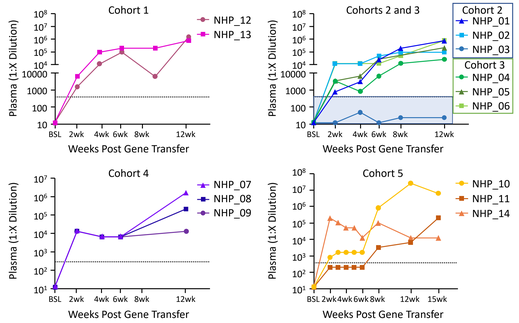
STUDY DESIGN



[a] Supercoiled qPCR titer method. [b] Biopsy collected from gastrocnemius muscle. [c] One NHP did not undergo TPE due to lack of antibody response to AAVrh74. [d] One NHP did not undergo TPE due to poor vascular access. [e] Cohort 5 did not undergo TPE due to incompatibility with previous treatment with rituximab. [f] All NHPs received prednisone (2 mg/kg/day) from 1 day before to 30 days after redosing with SRP-9001. [g] EOL necropsy collected from gastrocnemius, heart, and diaphragm. [h] Immediately post-TPE, the NHPs were disconnected from the apheresis unit and systemically redosed with rAAVrh74.MHCK7.microdystrophin (SRP-9001). [f] Sirolimus was continued 3 days prior out past the second dose, rituximab 14 days prior, 7 days prior, and day of dosing.

RESULTS

Part 1: AAVrh74 antibody response



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 (data not shown).

Part 1: 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 was given orally for 5-10 days.
- After rituximab infusion 15 weeks post vector injection, 2 patients in cohort 5 began vomiting and experienced elevated heart rates. Vomiting subsided and all other parameters returned to normal ranges when the dose was lowered. Primates recovered from anesthesia without further AEs.

Part 1: safety profile and transduction efficiency (continued)

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 NHPs in cohort 5 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>.05).

Part 2: safety and efficacy analysis of TPE as a potential pretreatment for individuals with preexisting immunity

- 7 NHPs from cohorts 2-4 underwent 2-3 consecutive cycles of TPE, resulting in reduced levels of circulating antibodies against AAVrh74.
  - Immediately after TPE, NHPs were successfully redosed with rAAVrh74.MHCK7.micro-dystrophin.
  - Cohort 5 was redosed without TPE.
- In 4 NHPs from cohorts 2-4 (NHP_02, NHP_05, NHP_08, and NHP_09), antibody titers of ≤1:400 were achieved.

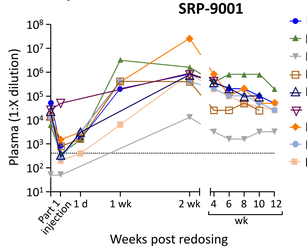
Part 2: total antibody titers against AAVrh74 in NHP prior to TPE and following TPE (before redosing with SRP-9001)

NHP (cohort)	Titer after part 1[a]	Titer after TPE[b]	TPE cycles, n
NHP_01 (2)	1:51200	1:800	2.5
NHP_02 (2)	1:6400	1:400	3
NHP_03 (2)	1:50	NA[c]	NA
NHP_04 (3)	1:12800	1:800	3
NHP_05 (3)	1:25600	1:400	3
NHP_06 (3)	1:25600	NA[d]	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

[a]12 weeks post initial gene transfer. [b] Prior to redose injection of rAAVrh74.MHCK7.microdystrophin. [c] NHP_03 was redosed without prior TPE due to lack of antibody response to AAVrh74. [d] NHP_06 only underwent 0.5 cycles of TPE due to small size and poor vascular access.

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

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



Dotted line represents inclusion criteria for total AAVrh74 antibody titer levels (threshold of 1:400 against AAVrh74).

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.

Part 2: safety profile and immune response to AAVrh74 before and after TPE

Safety profile (serum chemistry and immunology)

The TPE procedure was generally well tolerated.

- There were no abnormal immunologic observations as assessed by IFN-γ SFC levels against AAVrh74 and microdystrophin 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), and NHP_08 and NHP_09 (cohort 4).
  - These were resolved with continued daily administration of prednisone.

NHPs from cohort 5 did not receive TPE due to incompatibility with previous treatment[a] 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.

[a]Cohort 5 did not undergo TPE due to incompatibility with previous rituximab treatment; 2 NHPs (NHP_10, NHP_11) were redosed.

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ABBREVIATIONS

AAV, adeno-associated virus; AAVrh74, adeno-associated virus rhesus isolate serotype 74; AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DMD, Duchenne muscular dystrophy; EOL, end of life; FDA, Food and Drug Administration; GT, gene transfer; IFN-γ SFC, interferon gamma spot-forming cells; ITR, inverted terminal repeat; IV, intravenous; MHCK, myosin heavy chain kinase; NA, not available; NHP, nonhuman primate; OH, hydroxyl; PGT, post gene therapy; polyA A, polyadenylation; qPCR, quantitative polymerase chain reaction; rAAVrh74, recombinant AAV rhesus isolate serotype 74; ssDNA, single-stranded DNA; TPE, therapeutic plasma exchange; vg/kg, vector genomes per kilogram bodyweight.

ACKNOWLEDGMENTS AND 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 coinventor of rAAVrh74.MHCK7.micro-dystrophin 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 Jen Ciarochi, PhD, of MediTech Media, in accordance with Good Publication Practice (GPP3) guidelines (http://www.ismpp.org/gpp3).