Adeno-associated Virus Serotype rh74 Prevalence in Muscular Dystrophy Population

#724

Danielle A. Griffin[1,3], Rachael A. Potter[1,3], Eric R. Pozsgai[1,3], Ellyn L. Peterson[1,3] and Louise R. Rodino-Klapac[1,2,3]
[1]Center for Gene Therapy, The Research Institute at Nationwide Children's Hospital, Columbus, Ohio, USA, [2]Department of Pediatrics and Neurology, The Ohio State University, Columbus, Ohio, USA, [3]Sarepta Therapeutics, Cambridge, Massachusetts, USA.

BACKGROUND

- Pre-existing antibodies against adeno-associated virus (AAV) are a major challenge impacting efficacy of *in vivo* gene therapy, as AAV is the most common vehicle used for *in vivo* gene transfer.[1-3]
- To avoid the potential for blocking of the viral vector, current AAV gene therapy delivery practices require subjects to be free of antibodies against specific AAV serotypes before treatment.
- During candidate selection for our Duchenne muscular dystrophy (DMD) and limb girdle muscular dystrophy (LGMD) type 2E, 2C, 2D, 2B gene therapy clinical trials (NCT03652259, NCT01976091, NCT02710500, NCT03769116), patients are screened for pre-existing antibodies against AAVrh74 using a validated enzyme-linked immunosorbent assay (ELISA).
- Subjects with an endpoint absorbance ratio ≥2.00 detected at a serum dilution of >1:400 are considered seropositive and therefore do not meet inclusion criteria for our clinical trials.

OBJECTIVE

This report presents our methodology for detection of pre-existing AAVrh74 antibodies and prevalence in the LGMD and DMD populations screened at our site during clinical trial enrollment.

ELISA

PATIENT POPULATION

- A total of 95 DMD and LGMD (26 DMD, 13 LGMD2E, 19 LGMD2D, 34 LGMD2B and 3 LGMD2C) subjects were screened at Nationwide Children's Hospital (Columbus, Ohio) in this study.

SERUM SAMPLE COLLECTION AND SAMPLE PREPARATION

- Informed consent for participation and sample collection was obtained by the Principal Investigator in compliance with 21CFR50 and the International Conference on Harmonisation guidelines before entering the trials and signed by parents and subjects (for ages <17 years).
- Blood was collected in a BD red top clot activator tube. Blood was spun and serum was collected and stored at -80°C until analysis.

AAV PRODUCTION AND PURIFICATION

- AAVrh74 is a clade E serotype that was isolated from lymph nodes of rhesus monkeys and shares 93% amino acid identity to AAV8.[4,5]
- Recombinant AAVrh74 (rAAVrh74) was made in the GMP production facility at Nationwide Children's Hospital Manufacturing Facility by triple transfection as previously described.[6,7]
- For these assays, empty rAAVrh74 capsids could be used.

ELISA METHODOLOGY

- An indirect ELISA method was used to detect the presence of anti-AAVrh74 antibodies in serum samples (**Figure 1**).

ELISA continued

Figure 1. Step-by-step procedure for detection of anti-AAVrh74 in serum samples

Antigen Coating: On an Immunol 4HBX 96 well plate, add 100 µL AAV capsid (2×10^9 DNase-resistant particles/well)* (antigen-coated wells). In separate wells, add 100 µL of carbonate buffer **only** solution/well to serve as non-coated (background) control. Capsid protein (full or empty) can also be quantified using a standard protein assay to coat with known amount of protein (ng)

↓ Incubate O/N at 4°C

Blocking: Remove coating solution and add 100 µL of blocking solution** per well

↓ Incubate 1 hr at 37°C

Primary Antibody Incubation: Remove blocking solution. Add 200 µL total volume per well of 1:25 dilution to test sample wells, then serially dilute within plate to 100 µL per well. Control wells receive 100 µL total volume

Serum sample: serially diluted (1:25 → 1:3200) | Positive Control*** | Negative Control A**** | Negative Control B[ǂ]

↓ Incubate x 1 hr at RT

Wash: Discard primary antibody and wash plate 5X with 200 µL 0.05% PBS-Tween (without Ca^{2+} and Mg^{2+})

↓ After last wash, blot dry plate with paper towel

Secondary Antibody Incubation: Add 100 µL of secondary goat anti-human HRP antibody per well at 1:10,000 diluted in blocking solution

↓ Incubate x 30 min at RT

Wash: Discard secondary antibody and wash plate 5X with 200 µL 0.05% PBS-Tween (without Ca^{2+} and Mg^{2+})

↓ After last wash, blot dry plate with paper towel

Development: Add 100 µL of 3,3',5,5'-tetramethylbenzidine (TMB)[ǂǂ] substrate to each well and store in the dark for 1-2 min at RT

↓

Termination: Stop reaction by adding 100 µL 1N sulfuric acid/well

↓

Colorimetric Reading: Immediately, scan plates at an absorbance of λ = 450 nm

*Antigen (AAV capsids were diluted in 0.2M carbonate buffer).
**Blocking solution was 5% non-fat dry milk, 1% goat serum, q.s. 100 mL Phosphate Buffered Saline (PBS) without Ca^{2+} and Mg^{2+}
***Positive control sample was serum from subjects confirmed to have pre-existing AAVrh74 antibodies. Samples was loaded at 1:400 dilution.
****Negative control sample was serum from subjects without pre-existing AAVrh74 antibodies.
[ǂ]No primary antibody was added and served as a negative antibody control.
[ǂǂ]TMB is light-sensitive.

ELISA ANALYSIS

- Raw absorbance values from a 450 nm read were exported to an excel file for computation.
- The optical density (OD) values of the duplicate AAV-coated and non-coated (background) wells were used to determine the absorbance ratio, as follows:

$$\text{Absorbance ratio} = \frac{\text{Average } OD_1 - \text{Average } OD_2}{\text{Average } OD_2}$$

OD_1 = optical density of antigen-coated wells
OD_2 = optical density of non-coated antigen wells

- This calculation was performed for every dilution (1:25-1:3200) and a ratio ≥2.00 was considered a positive antibody response.
- The endpoint rAAVrh74 antibody titers were determined by identifying the last serum dilution that yielded a ratio of ≥2.00.

CONCLUSIONS

- A standardized assay developed to screen for AAVrh74 seropositivity was shown to be an essential tool in screening patients for pre-existing antibodies to minimize safety concerns and improve therapeutic efficacy with rAAVrh74-based gene therapies.
- These findings support the selection of rAAVrh74 as a gene therapy vector and indicate promise that rAAVrh74-based gene therapy will not induce significant anti-capsid immune responses in the majority of patients with muscular dystrophies.

ELISA ANALYSIS continued

- A subject with a ratio of <2.00 at the serum dilution of >1:400 would meet inclusion criteria.
- Measures were taken to ensure inter-operator reliability and assay reliability. To confirm validity of the assay, several criteria had to be met (**Table 1**).

Table 1. Assay validity is confirmed based upon several acceptance criteria

Description of Criteria	Value
Absorbance ratio of positive control	≥2.00
Absorbance ratio of negative control A	<2.00
Absorbance ratio of negative control B (no primary antibody buffer only wells)	≤1.00
Absorbance ratio of non-coated rAAVrh74 wells	≤1.00
Difference in absorbance (optical density) values between duplicates	≤0.05

IDENTIFICATION OF THE CUTOFF FOR AAVrh74 SEROPOSITIVE SAMPLES

- Based on a previous study[8] showing that antibody titers at 1:800 promoted loss of transgene expression, the cutoff used to define seropositivity was >1:400.

SEROPREVALENCE

- Of the total 95 patients with muscular dystrophies screened as of September 25, 2018, 83% were identified as seronegative for pre-existing antibodies to AAVrh74 and were eligible for enrollment into our DMD or LGMD gene therapy trials.

ACKNOWLEDGEMENTS & DISCLOSURES

No competing financial interests exist for the authors. This study was sponsored by Sarepta Therapeutics, Inc. and Nationwide Children's Hospital. The authors would like to thank Khampaseuth Thapa for her assistance. Editorial support was provided by Health & Wellness Partners, LLC, Upper Saddle River, New Jersey, funded by Sarepta Therapeutics, Inc.

NATIONWIDE CHILDREN'S
When your child needs a hospital, everything matters.℠

REFERENCES

1. Masat E, et al. *Discov Med*. 2013;15(85):379-389.
2. Calcedo R, Wilson JM. *Front Immunol*. 2013;4:341.
3. Basner-Tschakarjan E, Mingozzi F. *Front Immunol*. 2014;5:350.
4. Johnson PR, et al. *J Virol*. 2005;79(2):955-965.
5. Gao G, et al. *J Virol*. 2004;78(12):6381-6388.
6. Mendell JR, et al. *Hum Gene Ther*. 2019; Mar 6 [Epub ahead of print].
7. Pozsgai ER, et al. *Mol Ther*. 2017;25(4):855-869.
8. Chicoine LG, et al. *Mol Ther*. 2014;22(2):338-347.

FPO

Presented at the American Society of Gene and Cell Therapy (ASGCT) 22nd Annual Meeting | April 29-May 2, 2019 | Washington, DC