Considerations for Use of AAV Vectors in Gene Therapy

What Are Viral Vectors?

Viral vectors are commonly used for delivery of gene therapy and are selected based on their unique properties[1]

Adeno-associated viruses (AAVs) are ideal vectors for in vivo gene transfer due to their:

Safety profile

- DNA integration into host genome is extremely rare, posing little risk of insertional mutagenesis[2,3]
- Unable to replicate on their own[4]

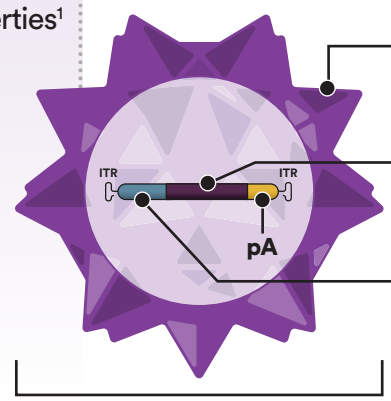
Stability

- Able to withstand broad temperature and pH changes[4]

Transduction efficiency

- Deliver transgenes to a wide variety of target tissues through the use of different serotypes[2-4]

Essential Components of a Gene Therapy Viral Vector



Recombinant AAV vector

Vector capsid
Important determinant of safety experience and transduction efficiency[5,6]

Transgene
Important determinant of functional impact[5,7]

Promoter
Important determinant of expression levels and tissue specificity[5,8]

AAV is the most widely used viral vector for in vivo gene therapy[1]

AAV serotypes have been identified and categorized into clades based on functional and serologic characteristics[9-11]

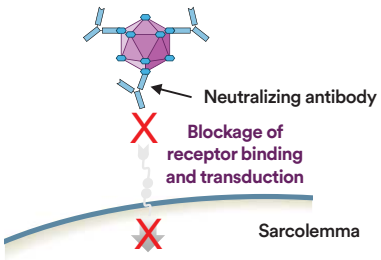
Safety profiles of AAV-based therapies vary due to the use of different vector serotypes with varying properties and tissue affinities[10,11]

Antibody-Mediated Immunologic Responses to AAV Capsids Are Driven by Neutralizing and Non-Neutralizing Antibodies[12]

Total Binding Abs

Neutralizing Abs

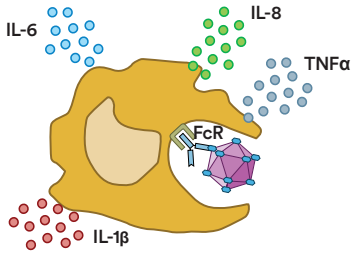
Transduction



Prevents virus from entering cells

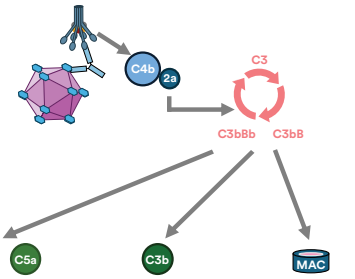
Non-Neutralizing Abs

Opsonization



Clearance and release of inflammatory cytokines

Complement Activation



Inflammatory anaphylatoxins | Opsonization | Cell lysis

Recombinant Adeno-Associated Virus Rhesus Isolate Serotype 74 (rAAVrh74) Has Been Studied as a Vector for Neuromuscular Diseases, such as DMD and LGMDs[11]

A Phase II Study Assessed the Percentage of Patients With DMD With Pre-Existing Immunity to rAAVrh74[11]

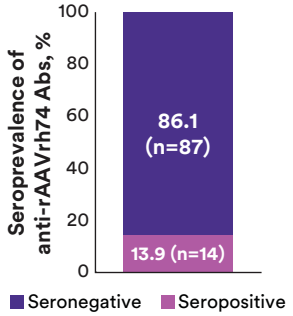
Overview: Patients from across the United States with DMD who have not previously received gene therapy, ages: ≥4 and <18 years

Objective: Determine seroprevalence[a] using a comprehensive enzyme-linked immunosorbent assay for TAbs

Primary endpoint: Percentage of patients with elevated (≥1:400) total antibody titers to rAAVrh74

[a]Seropositivity was defined as having an elevated titer (≥1:400 total antibody titer to rAAVrh74).[11]

Results (N=101)



86% of patients (mean age: 9.1 years) with DMD in this dataset would potentially meet the antibody eligibility criterion for entry into a clinical trial for rAAVrh74-based gene therapy[11]

Low seroprevalence of antibodies against rAAVrh74 supports the broad applicability of rAAVrh74-based research for gene therapy[11]

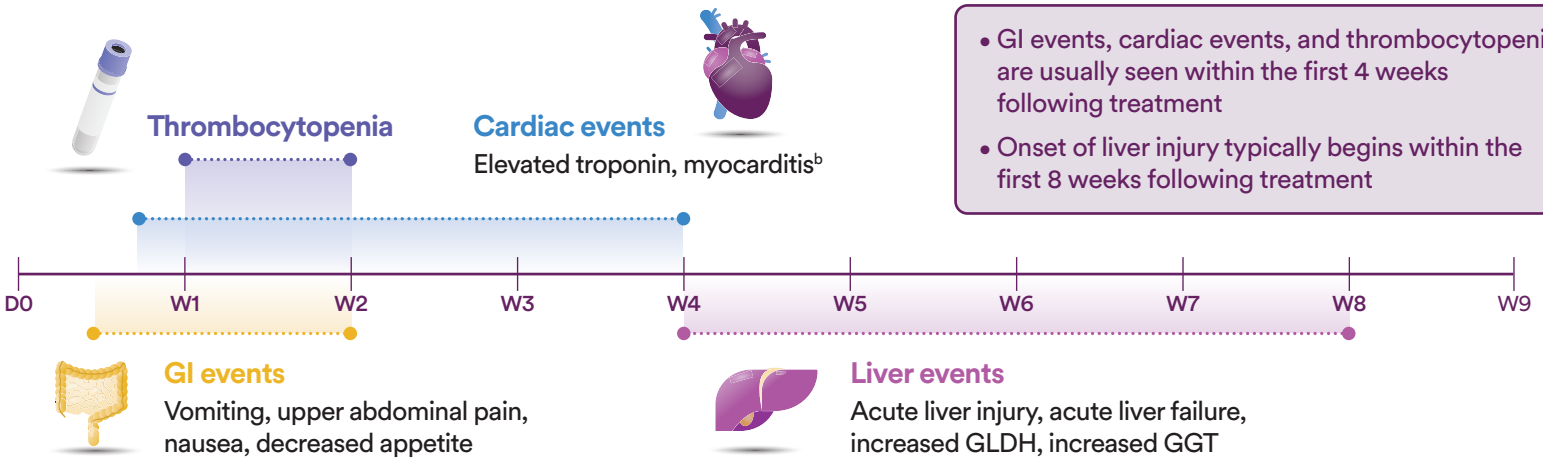
Safety and Rationale for Use of rAAVrh74 Vector in Gene Therapy

rAAVrh74 Key Properties

- Relatively **low rate of pre-existing immunity** in humans[11,13]
- **Broad distribution in cardiac and skeletal muscles** as seen in preclinical studies[11]
- **No toxicity** observed in preclinical testing of NHPs or mice, even at high doses[14-18]
- **>1,000 patient exposures** in clinical trials and commercial settings, with follow-up post dosing extending up to **5 years**[19-21]
- No observed serious adverse events in clinical trials attributed to complement activation[11]

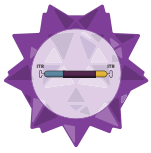
Ongoing Timeline of Adverse Events (AEs) Following Gene Therapy Using rAAVrh74[21-28]

- GI events, cardiac events, and thrombocytopenia are usually seen within the first 4 weeks following treatment
- Onset of liver injury typically begins within the first 8 weeks following treatment



The AEs presented are not a complete list. Safety data is not absolute and continues to evolve or change with ongoing clinical investigations.

Capsid-directed immune responses have been observed within several weeks after treatment in rAAVrh74 clinical trials



In clinical studies evaluating rAAVrh74-based gene therapy for patients with DMD, the most common treatment-related AEs, occurring in ≥15% of patients, were vomiting, nausea, decreased appetite, increased GLDH, and upper abdominal pain

Acute liver injury (ALI) is an identified risk following treatment with AAV-based gene therapies.[29] There have been reported fatal ALI cases that progressed to acute liver failure following treatment with an rAAVrh74 vector-based gene therapy.[29]

rAAVrh74 forms the foundation of Sarepta's investigational AAV-based gene transfer clinical programs[30]

[b]Myocarditis occurred in 2/156 patients (~1%) and resolved.[27]

Abbreviations: AAV, adeno-associated virus; Ab, antibody; AE, adverse event; ALI, acute liver injury; DMD, Duchenne muscular dystrophy; DNA, deoxyribonucleic acid; FcR, Fc receptor; GGT, gamma-glutamyl transferase; GI, gastrointestinal; GLDH, glutamate dehydrogenase; IL, interleukin; ITR, inverted terminal repeat; kg, kilogram; LGMD, limb-girdle muscular dystrophy; MAC, membrane attack complex; *mdx*, X-chromosome–linked muscular dystrophy; Myo, myotropic; NHP, nonhuman primate; pA, polyadenylation site; rAAVrh74, recombinant adeno-associated virus rhesus isolate serotype 74; rh74, rhesus isolate serotype 74; TAb, total binding antibody; TNF, tumor necrosis factor; vg, vector genome; VR, variable region.

References: 1. Zhao Z, et al. *Bioeng Transl Med*. 2021;7(1):e10258. 2. McClements ME, MacLaren RE. *Yale J Biol Med*. 2017;90(4):611-623. 3. Lau CH, Suh Y. *F1000Res*. 2017;6:2153. 4. Naso MF, et al. *BioDrugs*. 2017; 31(4):317-334. 5. Asher DR, et al. *Expert Opin Biol Ther*. 2020;20(3):263-274. 6. US National Library of Medicine. Accessed July 15, 2025. https://medlineplus.gov/genetics/understanding/therapy/procedures 7. Chandler RJ, Venditti CP. *Transl Sci Rare Dis*. 2016;1(1):73-89. 8. Zheng C, Baum BJ. *Methods Mol Biol*. 2008;434:205-219. 9. Drouin LM, Agbandje-McKenna M. *Future Virol*. 2013;8(12):1183-1199. 10. Colella P, et al. *Mol Ther Methods Clin Dev*. 2017;8:87-104. 11. Goedeker NL, et al. *Ther Adv Neurol Disord*. 2023;16:17562864221149781. 12. Mendell JR, et al. *Mol Ther Methods Clin Dev*. 2022;25:74-83. 13. Zygmunt DA, et al. *Hum Gene Ther*. 2017;28(9):737-746. 14. Potter RA, et al. *Hum Gene Ther*. 2021;32(7-8):375-389. 15. Chicoine LG, et al. *Mol Ther*. 2014;22(4):713-724. 16. Sondergaard PC, et al. *Ann Clin Transl Neurol*. 2015;2(3):256-270. 17. Seo Y-E, et al. *Mol Ther Methods Clin Dev*. 2023;28:284-299. 18. Asher DR, et al. *Expert Opin Biol Ther*. 2020;20(3):263-274. 19. Mendell JR, et al. Poster presented at: 29th Annual Congress of the World Muscle Society; October 8-12, 2024, Prague, Czechia. Poster 425P. 20. Sarepta Therapeutics. Data on file. 21. Mendell JR, et al. *Nat Med*. 2024;30(1):199-206. 22. Zaidman CM, et al. *J Neuromuscul Dis*. 2024;11(3):687-699. 23. Mendell JR, et al. *Pediatr Neurol*. 2024;153:11-18. 24. Mercuri E, et al. Poster presented at: 15th Congress of European Paediatric Neurology Society; June 20-24, 2023, Prague, Czechia. Poster 86. 25. Zaidman CM, et al. *Ann Neurol*. 2023;94(5):955-968. 26. Mendell JR, et al. *Muscle Nerve*. 2024;69(1):93-98. 27. Proud C, et al. Poster presented at: 29th Annual Congress of the World Muscle Society; October 8-12, 2024, Prague, Czechia. Poster 726LBP. 28. Delandistrogene moxeparvovec. Prescribing information. Sarepta Therapeutics, Inc; August 2024. 29. Shieh P, et al. Presented at: 30th Annual Congress of the World Muscle Society; October 7-11, 2025; Vienna, Austria. 30. Sarepta Therapeutics. Accessed December 19, 2025. https://www.sarepta.com/products-pipeline/pipeline

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