The safety and efficacy of pre-treatment with imlifidase prior to adeno-associated virus (AAV)-based gene therapy in non-human primates with pre-existing anti-AAVrh74 antibodies

Rachael A. Potter,[1]* Sohrab Khan,[1] John Snedeker,[1] Kaitlin Adegboye,[1] Alex Haile,[1] Behnam Sayanjali,[1] Nicole Pukos,[1] Kyle Cochran,[1] Jen Ahner,[1] Ting Su,[1] Nathalie Uzcátegui,[2] Yvonne Stenberg,[2] Catja Freiburghaus,[2] Lena Winstedt,[2] Louise R. Rodino-Klapac[1]

[1] Sarepta Therapeutics, Inc., Cambridge, MA, USA; [2] Hansa Biopharma, Lund, Sweden

*Presenter

Presented at the 26th Annual Meeting of the American Society of Gene + Cell Therapy (ASGCT) Los Angeles, CA, USA | May 16–20, 2023

Acknowledgments and disclosures

Acknowledgments

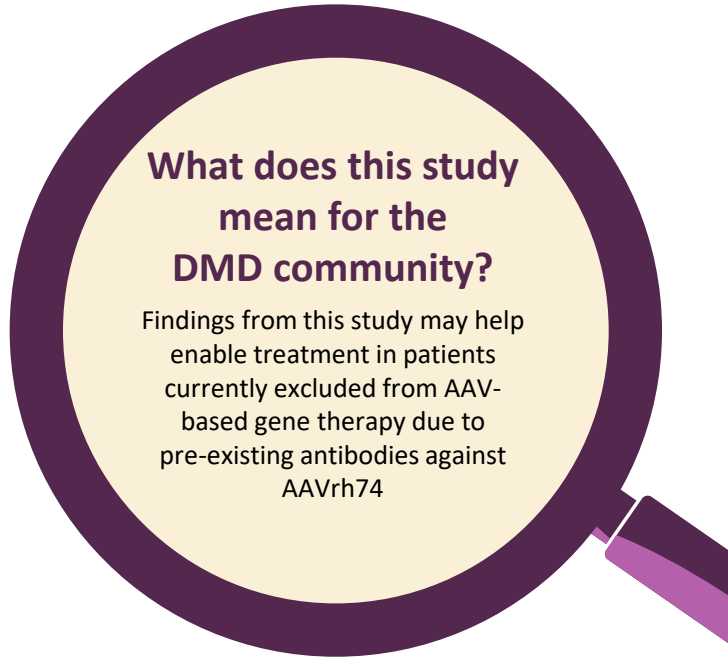
- Medical writing and editorial support were provided by David Kabanda, MBChB, on behalf of Nucleus Global, in accordance with Good Publication Practice (GPP) 2022 guidelines (https://www.ismpp.org/gpp-2022) and were funded by Sarepta Therapeutics Inc., Cambridge, MA, USA

Disclosures

- RAP, SK, JS, KA, AH, BS, NP, KC, JA and TS are employees of Sarepta Therapeutics and may have stock options
- NU, YS, CF and LW are employees of Hansa Biopharma and may have stock options
- LRRK is an employee of Sarepta Therapeutics, has received grant support from Sarepta Therapeutics and the Parent Project Muscular Dystrophy, as well as financial consideration from Sarepta Therapeutics and Myonexus Therapeutics (now acquired by Sarepta Therapeutics). In addition, she is a co-inventor of AAVrh74.MHCK7.micro-dys technology
- This research was funded by Sarepta Therapeutics

Study overview

- Duchenne muscular dystrophy (DMD) is an X-linked, neuromuscular disease caused by mutations in the *DMD* gene that prevent the production of functional dystrophin protein[1,2]
- Despite generally low levels of pre-existing immunity to AAVrh74 in humans, anti-AAV antibodies do occur and can impact the safety and efficacy of gene therapies and preclude their use in otherwise eligible patients
- We assessed the ability of imlifidase, a unique endopeptidase that cleaves IgG, to lower anti-AAVrh74 antibodies as a potential means of overcoming or reducing pre-existing immunity in non-human primates

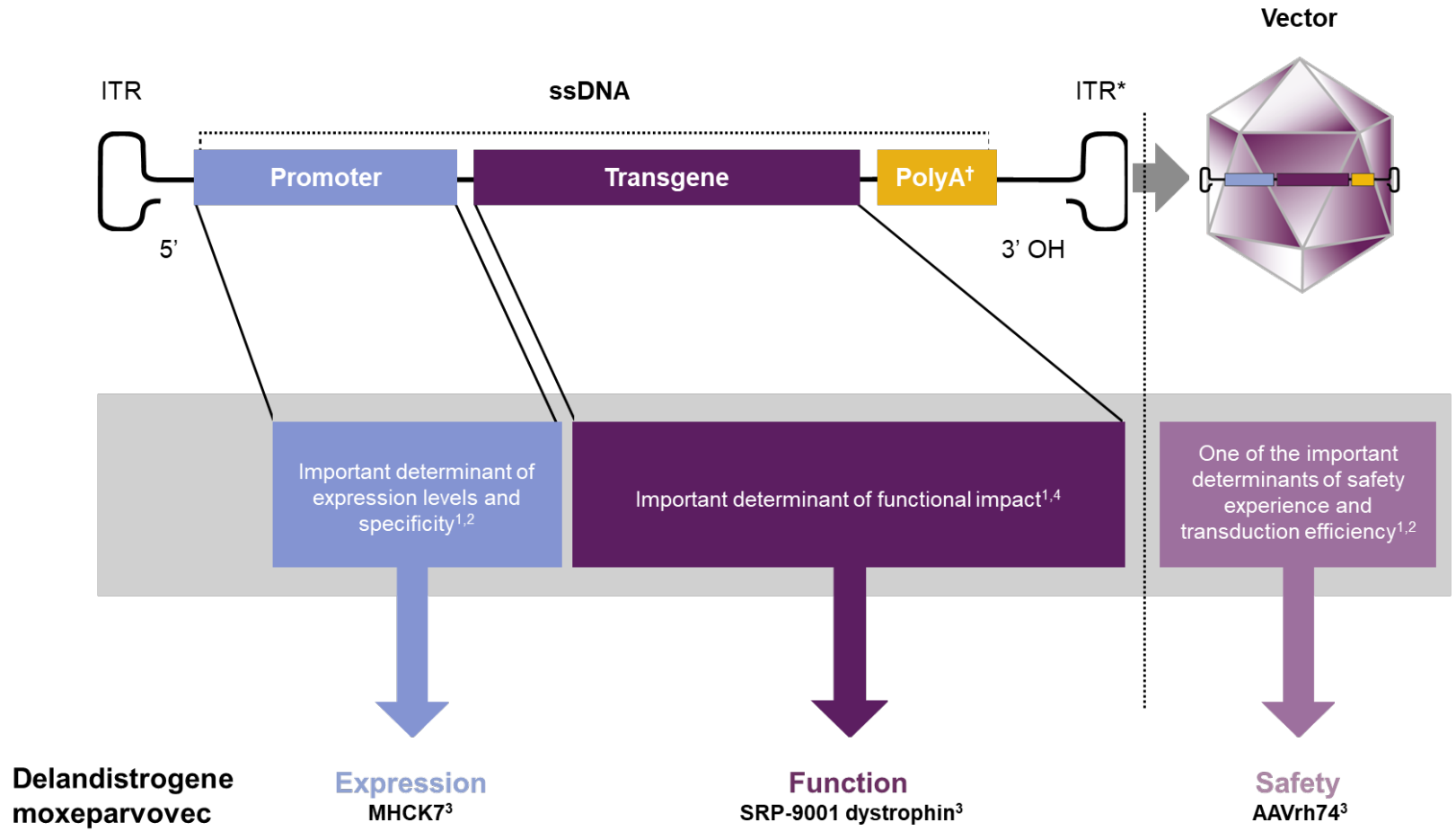


AAV, adeno-associated virus; AAVrh74, AAV rhesus isolate serotype 74; IgG, immunoglobulin G.

1. Bushby K, et al. *Lancet Neurol*. 2010; 9:77–93; 2. Mendell JR, et al. *Ann Neurol*. 2013; 74:637–647.

Background: delandistrogene moxeparvovec

- Delandistrogene moxeparvovec (SRP-9001) is an investigational rAAV vector-based gene therapy, designed to compensate for missing dystrophin in DMD by delivering a transgene encoding SRP-9001 dystrophin, an engineered dystrophin protein that retains key functional domains of the wild-type protein[1–4]



* 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; AAVrh74, AAV rhesus isolate serotype 74; DMD, Duchenne muscular dystrophy; ITR, inverted terminal repeat; OH, hydroxyl; PolyA, polyadenylation; rAAV, recombinant AAV; ssDNA, single-stranded DNA.

1. Asher DR, et al. *Expert Opin Biol Ther*. 2020; 20:263–274; 2. Zheng C and Baum BJ. *Methods Mol Biol*. 2008; 434:205–219; 3. Mendell JR, et al. *JAMA Neurol*. 2020; 77:1122–1131; 4. Chandler RJ and Venditti CP. *Transl Sci Rare Dis*. 2016; 1:73–89.

Background: imlifidase

Figure 1. Imlifidase

Derived from *Streptococcus pyogenes*

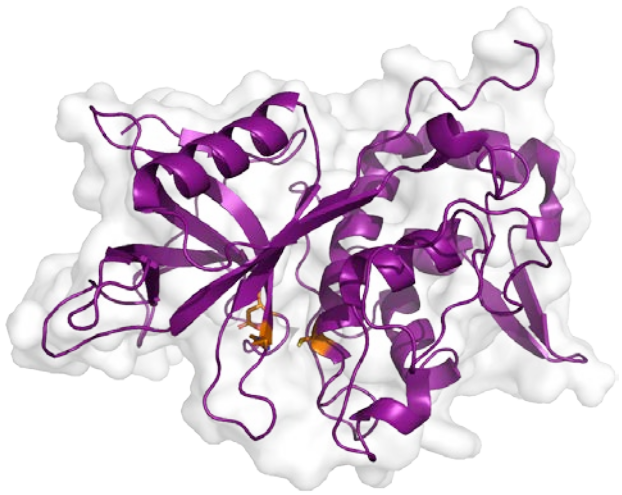


Figure 2. Imlifidase mechanism of action

An endopeptidase that specifically targets IgG and inhibits IgG-mediated immune response

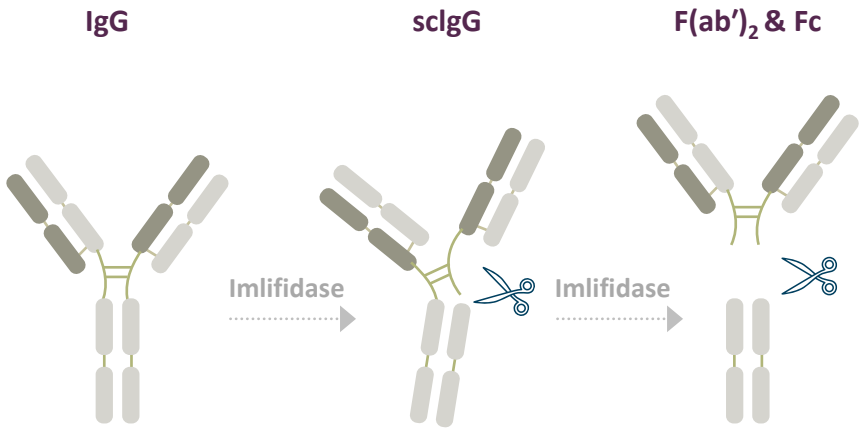
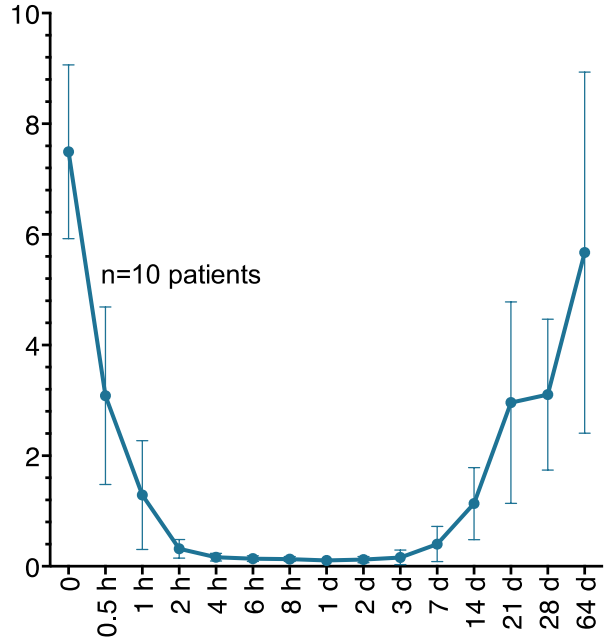


Figure 3. Imlifidase onset of action

Within hours after administration



d, days; F(ab')$_2$, fragment affinity-purified secondary antibodies; Fc, fragment crystallizable; h, hour; IgG, immunoglobulin G; sclgG, single cleaved immunoglobulin G.

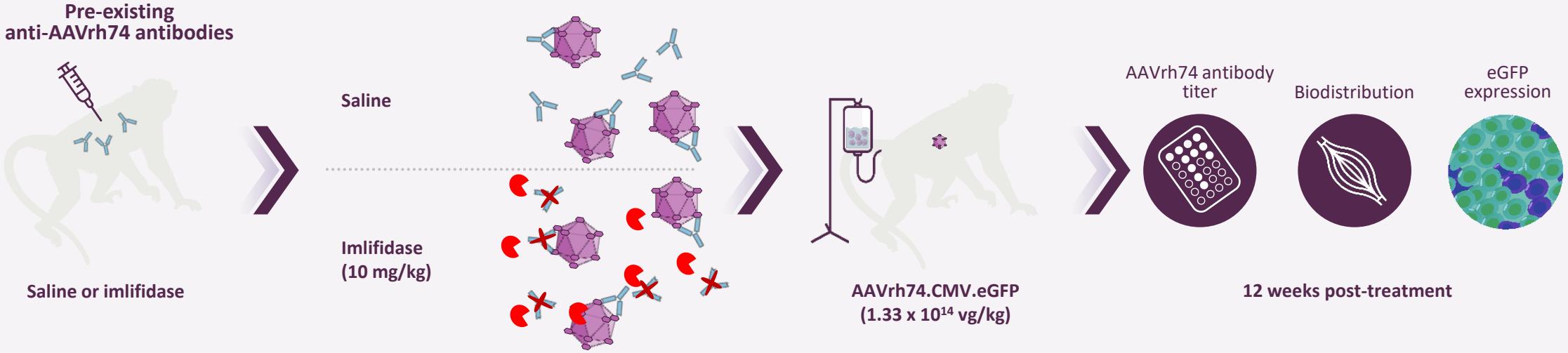
Figure 1. Imlifidase. Figure provided by J. Kapla, Hansa Biopharma; Figure 2. Imlifidase mechanism of action. Reprinted from Winstedt L, et al. *PLoS One*. 2015;10(7):e0132011. Creative Commons Attribution 4.0 International License (https://creativecommons.org/licenses/by/4.0/); Figure 3. Imlifidase onset of action. Adapted from Jordan SC, et al. *N Engl J Med*. 2017; 377:442–453.

Study design

OBJECTIVE

Assess the safety and efficacy of imlifidase pre-treatment prior to AAV-based gene therapy in non-human primates with pre-existing anti-AAVrh74 antibodies

STUDY OVERVIEW



AAV, adeno-associated virus; AAVrh74, AAV rhesus isolate serotype 74; CMV, cytomegalovirus; eGFP, enhanced green fluorescent protein; vg, viral genome.

1. Potter RA, et al. ASGCT. May 16–20, 2023.

AAVrh74.CMV.eGFP was administered as a single IV injection preceded by a single IV imlifidase injection

Group	Titer at screening*	Imlifidase or control article			AAVrh74.CMV.eGFP or control article		
		Dosing phase	Article given	Dose level	Dosing phase	Article given	Dose level
2: Saline control; AAVrh74 negative; n=3	≤1:400	Days 1/36	Saline control	1 ml/kg	Days 3/38	Saline control	5.47 ml/kg
3: Vector control; AAVrh74 negative; n=3	≤1:400	Day 1	Saline control	1 ml/kg	Day 3	AAVrh74.CMV.eGFP	1.33×10^{14} vg/kg/dose
4: Vector control; n=3	1:800–1:1600	Day 1	Saline control	1 ml/kg	Day 3	AAVrh74.CMV.eGFP	1.33×10^{14} vg/kg/dose
5: Imlifidase + vector; n=3	1:800–1:1600	Day 1	Imlifidase	10 mg/kg/dose	Day 3	AAVrh74.CMV.eGFP	1.33×10^{14} vg/kg/dose

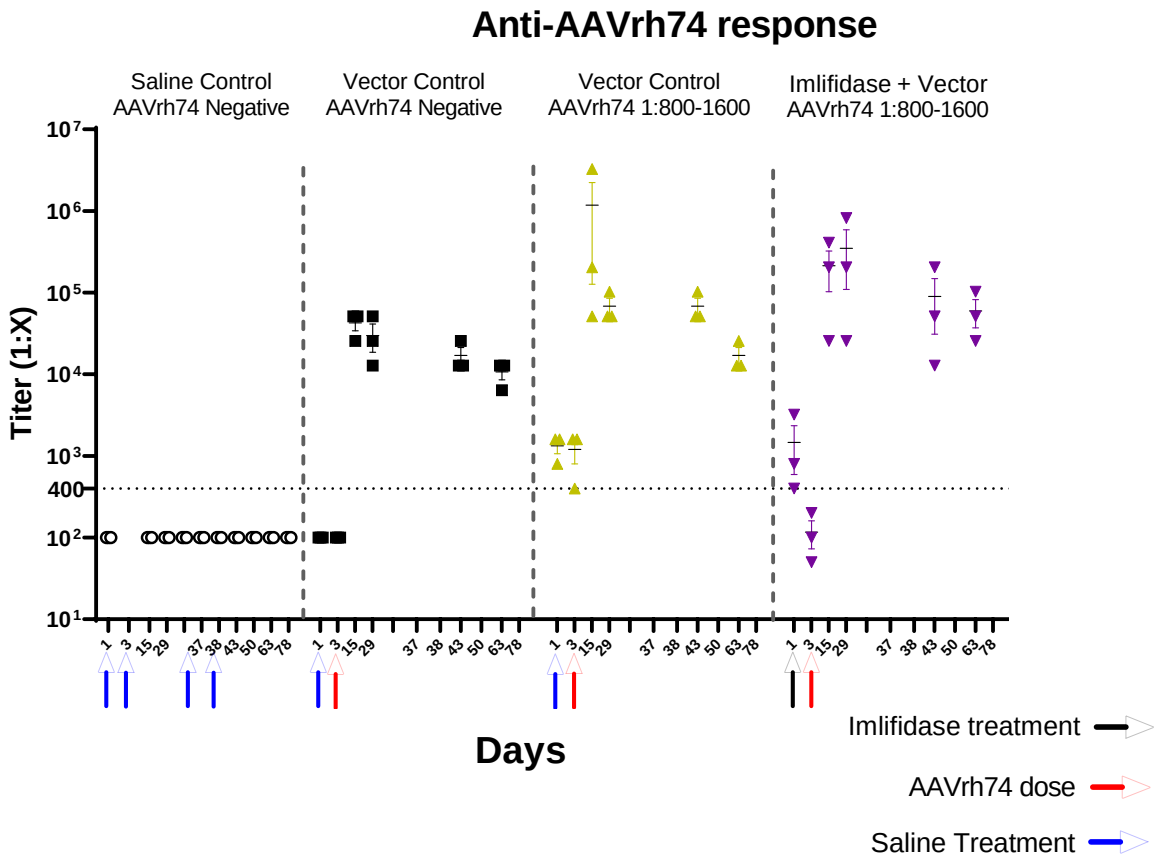
- Animals in Group 1 (AAVrh74 negative; n=3) were administered a single IV injection of imlifidase 1 mL/kg to examine initial tolerability of imlifidase in monkeys
- Animals in Groups 2–5 were administered prednisolone 1 mg/kg/day throughout the duration of the dosing phase through to the day prior to the terminal sacrifice. On days of control or test article administration, prednisolone was administered approximately 1 hour prior to dose administration
- Animals in Group 2 were observed for 59 days following control article administration on Day 38 and underwent terminal sacrif ice on Day 97. Animals in Groups 3–5 were observed for 60 days following control article or AAV administration on Day 3 and underwent terminal sacrifice on Day 63

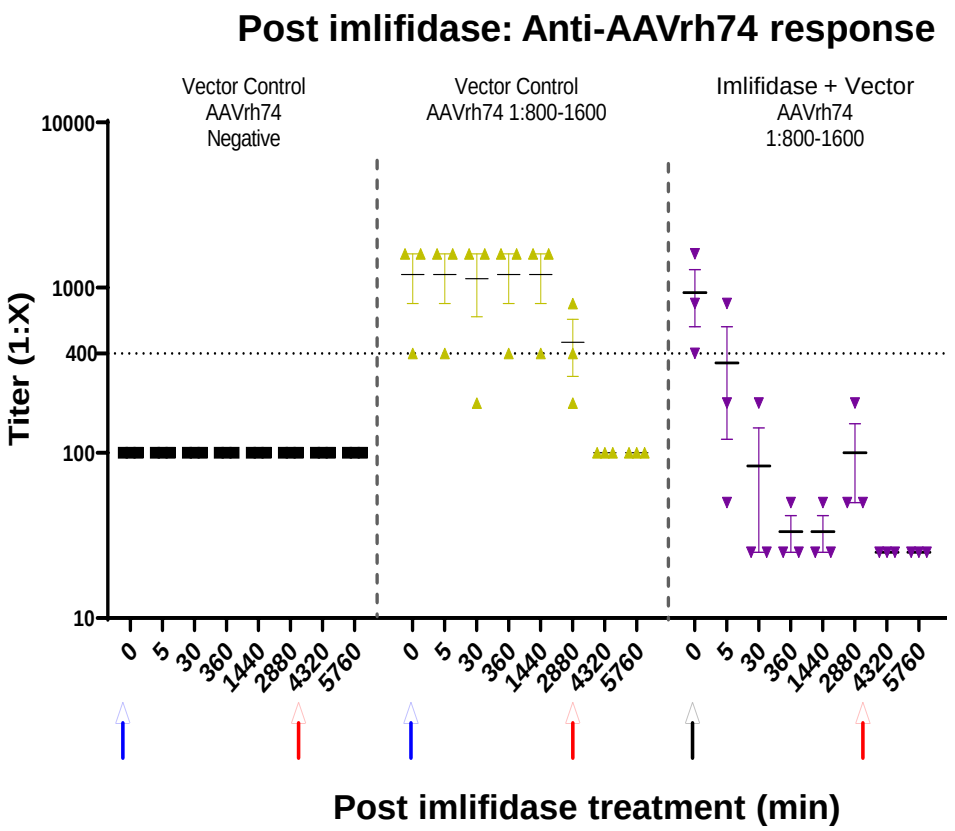
* Anti-AAVrh74 antibody status was based on the titer values obtained during the predose phase. Anti-AAVrh74 negative animals in Groups 2 and 3 were defined as those with anti-AAVrh74 antibody titers ≤ 1:400. Anti-AAVrh74 positive animals in Groups 4 and 5 were defined as those with anti-AAVrh74 antibody titers of 1:800–1:1600. Anti-AAVrh74 positive animals in Group 6 were defined as those with anti-AAVrh74 antibody titers of 1:3200–1:25600.

AAV, adeno-associated virus; AAVrh74, AAV rhesus isolate serotype 74; CMV, cytomegalovirus; eGFP, enhanced green fluorescent protein; IV, intravenous; vg, viral genome.

Immunologic response demonstrates reduction in pre-existing anti-AAVrh74 with imlifidase pre-treatment

- Treatment with imlifidase prior to AAVrh74-eGFP in animals with pre-existing anti-AAVrh74 antibodies (titer range: 1:800–1:1600) led to decreased anti-AAVrh74 antibody titers



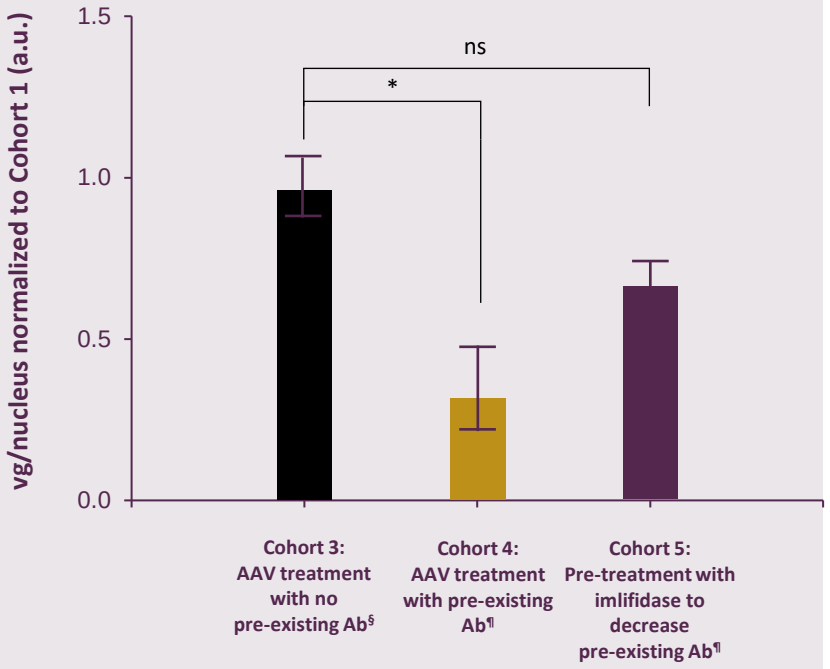


* Titer cut-off is defined as <1:400.

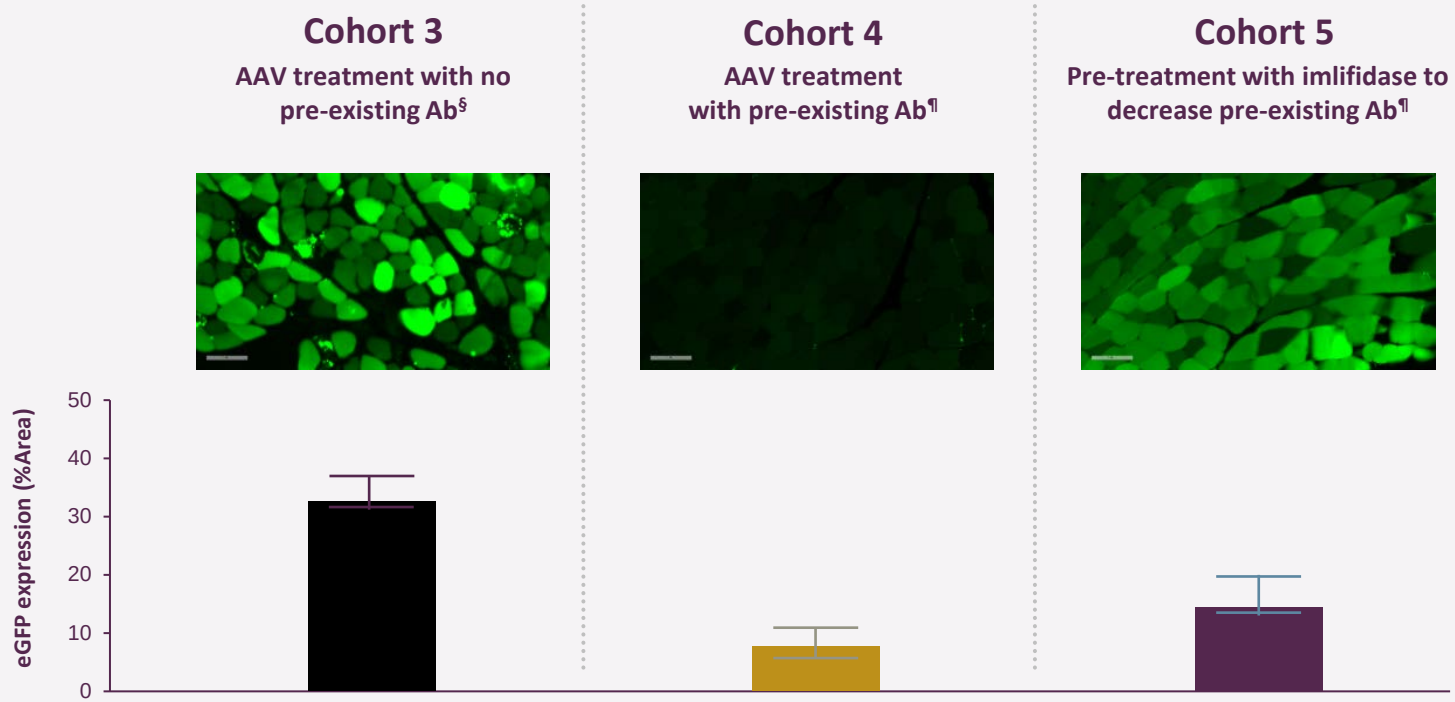
AAVrh74, adeno-associated virus rhesus isolate serotype 74; eGFP, enhanced green fluorescent protein.

Imlifidase pre-treatment decreases pre-existing antibodies and enhances transduction and transgene expression in NHPs

Transduction†



Expression in Skeletal Muscle‡



Imlifidase treatment enhances transduction and expression in NHPs in the presence of pre-existing anti-AAVrh74 antibodies

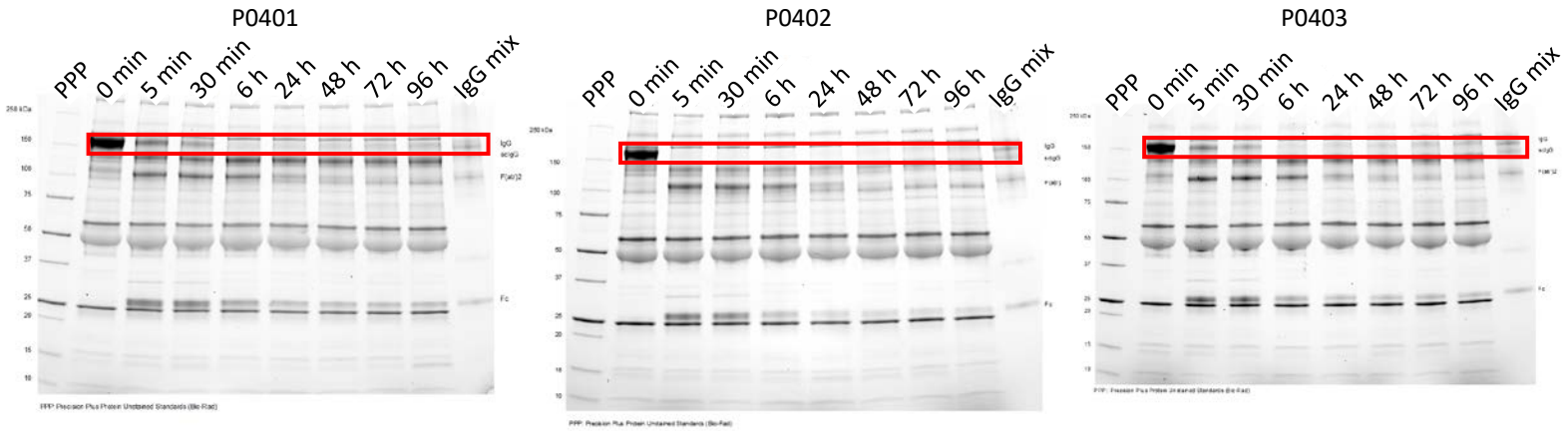
*P<0.05. †Data are represented as mean ± SEM and analyzed by one-way ANOVA followed by post-hoc analysis with Dunnett's multiple comparison test . ‡Data are represented as the mean ± SEM for the percent area for all of the muscle tissues analyzed at terminal necropsy. §AAVrh74 titer ≤1:400. ¶AAVrh74 titer 1:800–1:1600.

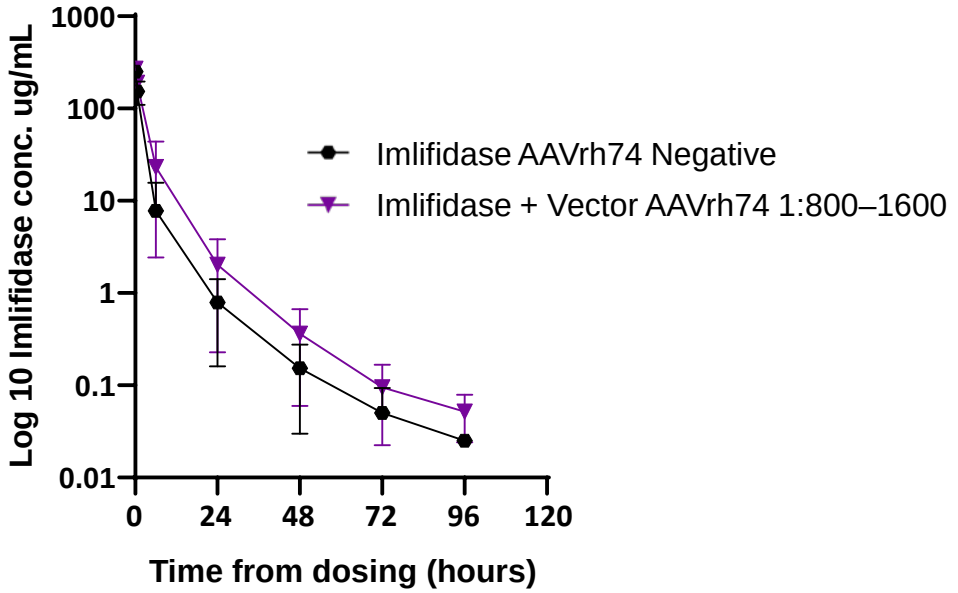
AAV, adeno-associated virus; AAVrh74, adeno-associated virus rhesus isolate serotype 74; Ab, antibody; a.u., arbitrary units; eGFP, enhanced green fluorescent protein; NHP, non-human primate; ns, not significant; vg, viral genome.

PD/TK analysis and histopathology

- Animals treated with imlifidase (10 mg/kg) showed significant IgG cleavage around 24 hours
- The kinetic analysis of imlifidase over time suggested that the groups received comparable amounts of imlifidase and exhibited similar rates of clearance

Imlifidase + vector AAVrh74 1:800–1600





No adverse clinical events or deaths occurred subsequent to dosing with gene therapy, and no adverse immuno-toxicologic or histopathologic findings related to imlifidase pre-treatment were found, including in the reproductive organs

AAVrh74, adeno-associated virus rhesus isolate serotype 74; IgG, immunoglobulin G; PD, pharmacodynamic; TK, toxicokinetics.

Conclusions

- Treatment with imlifidase prior to AAVrh74-eGFP in animals with pre-existing anti-AAVrh74 antibodies (titer range: 1:800–1:1600) led to decreased anti-AAVrh74 antibody titers
- The decrease in anti-AAVrh74 antibodies observed in animals that received imlifidase prior to gene therapy resulted in efficient transduction and expression of AAVrh74.CMV.eGFP relative to animals with the same antibody titers that did not receive imlifidase
- No adverse clinical events or mortality occurred subsequent to dosing with gene therapy, and no adverse immuno-toxicologic or histopathologic findings related to imlifidase pre-treatment were found, including in the reproductive organs
- These results suggest that imlifidase pre-treatment can permit AAV transduction in seropositive animals. These findings may help enable treatment in patients currently excluded from AAV-based gene therapy due to pre-existing antibodies

AAV, adeno-associated virus; AAVrh74, AAV rhesus isolate serotype 74; CMV, cytomegalovirus; eGFP, enhanced green fluorescent protein.