Immunoadsorption Plasmapheresis for the Removal of Plasma Immunoglobulins to Enable Repeat Dose Administration with an AAV5 Gene Therapy Vector

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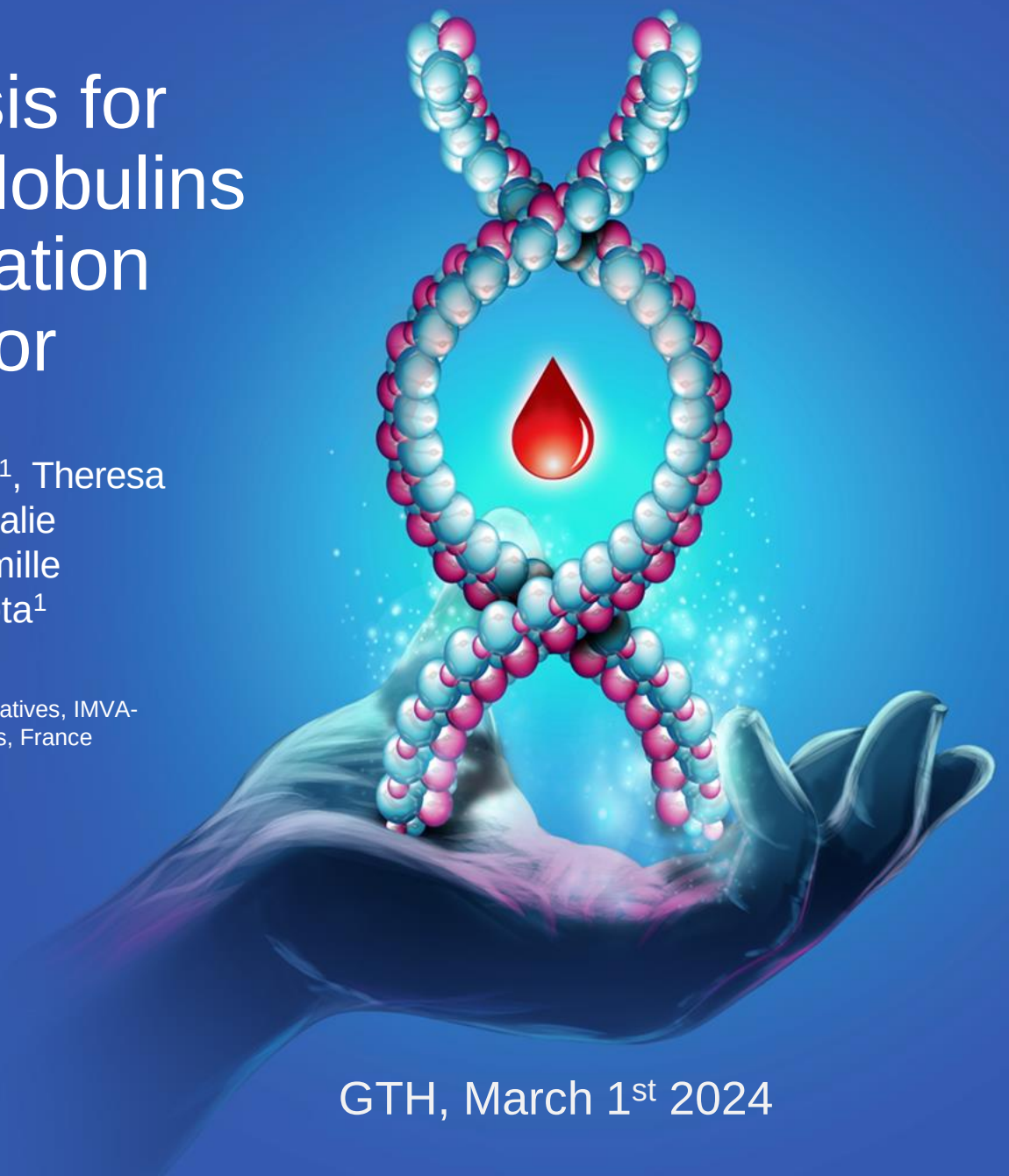

GTH, March 1st 2024

Disclosures

- Brian Long is an employee and stockholder of BioMarin Pharmaceutical Inc.

Executive summary

- A proportion of patients have pre-existing antibodies against AAV capsids through natural exposure to AAV
- The presence of pre-existing anti-AAV antibodies may limit the efficacy of AAV-delivered gene therapy
- To mitigate this potential lack of pharmacological activity, most clinical trials exclude patients with pre-existing immunity to AAV
- Moreover, a first administration of an AAV vector induces high titers of treatment-emergent AAV NAbs, compromising a second dose administration with the same vector
- Depletion of anti-AAV antibodies by IAP is a strategy that could allow successful vector administration in recipients with either pre-existing or treatment-emergent antibodies
- The objective of this study was to evaluate the effectiveness of IAP in removing AAV5 antibodies from animals sensitized by an initial AAV gene therapy dose to enable a successful second dose administration
- Results showed that multiple rounds of IAP over multiple days enabled up to 99% reduction of AAV-induced TAb titers in NHPs and enabled a successful second AAV dose administration as reflected in appreciable transgene protein production

AAV, adeno-associated virus; AAV NAbs, AAV neutralizing antibodies; AAV5, adeno-associated virus serotype 5; IAP, immunoadsorption plasmapheresis; NHPs, nonhuman primates; TAb, total binding antibodies.

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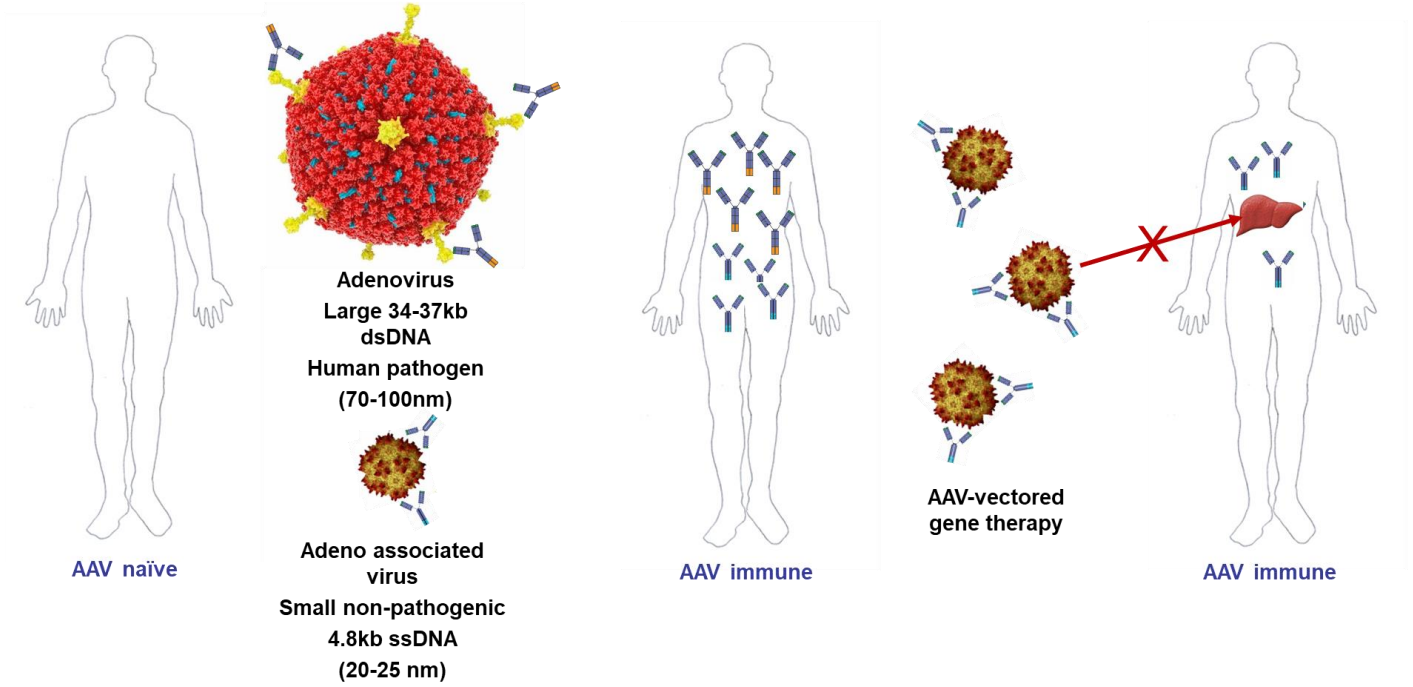
Pre-existing AAV antibodies postulated to arise from prior AAV and adenovirus exposure

- AAV belongs to the parvovirus family and is dependent on co-infection with other large DNA viruses, mainly adenoviruses, to replicate
- Antibodies are produced during recovery from virus-mediated infection
- Roughly 45% to 80% of adults carry AdHu5-neutralizing antibodies



4 AAV, adeno-associated virus; AdHu5, human adenovirus serotype 5; dsDNA, double-stranded DNA; ssDNA, single-stranded DNA.

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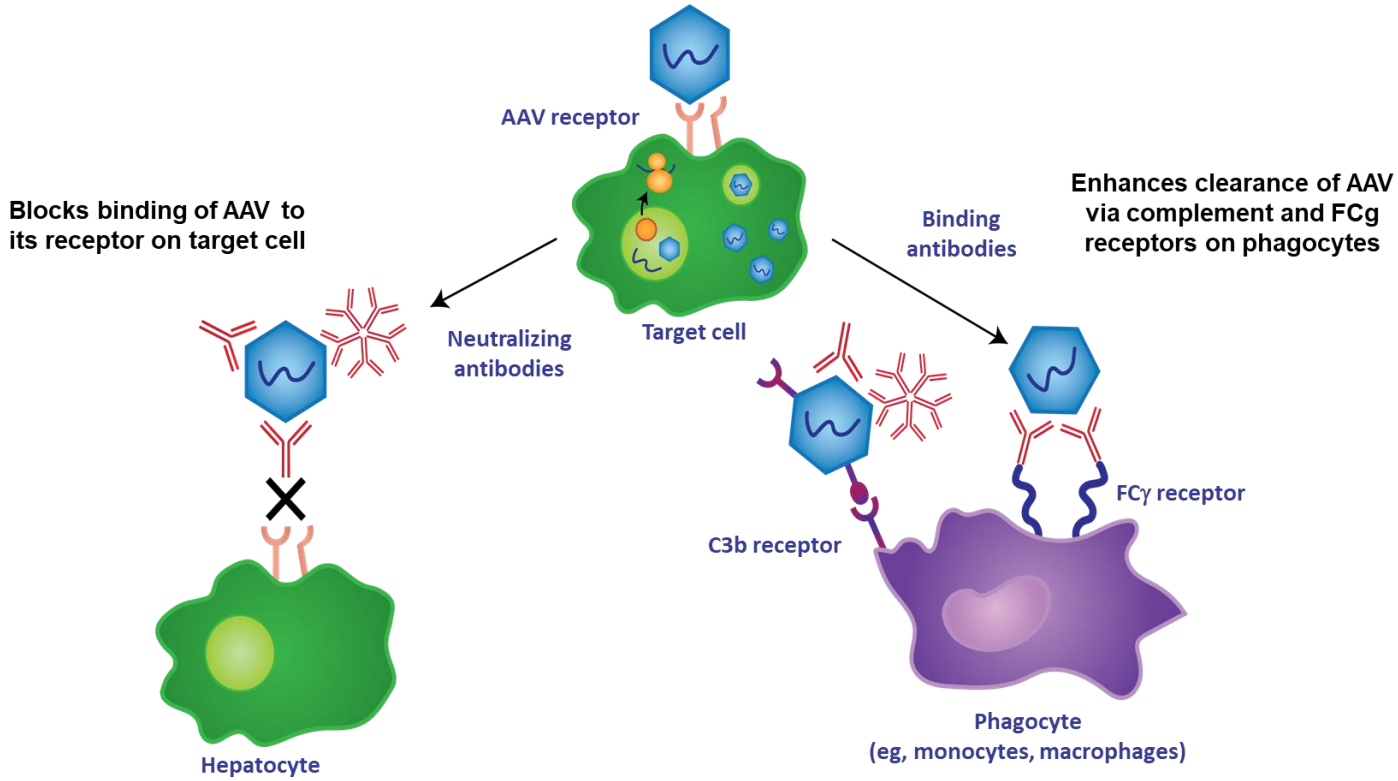
Neutralizing and/or binding antibodies impact the efficacy of AAV-vectored gene therapy

- Due to a reduction in the efficacy of AAV-gene therapy, patients who test positive for AAV5 TAb and AAV NAb antibodies are ineligible for treatment[1-4]



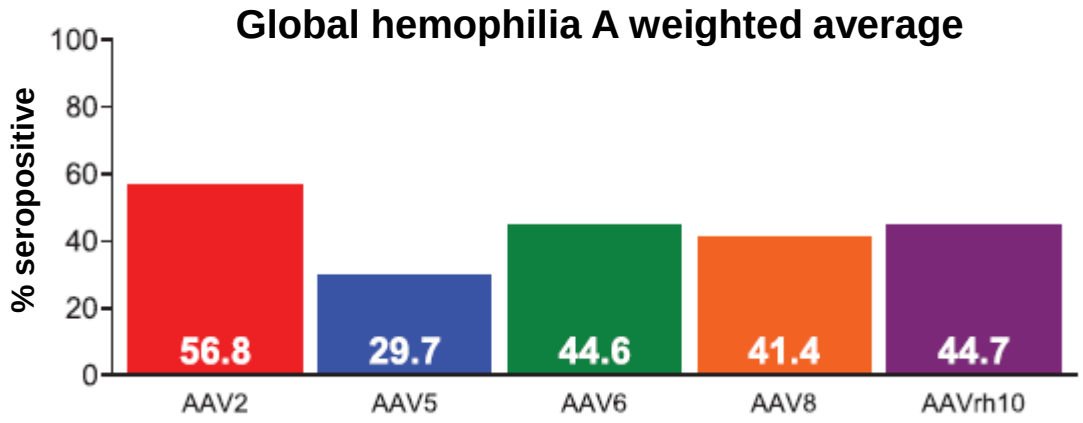
AAV, adeno-associated virus; AAV NAb, AAV neutralizing antibodies; AAV5 TAb, anti-AAV5 total binding antibodies; C3b, complement receptor type 3; FCγ, FC gamma receptor.

1. Rangarajan S, et al. *N Engl J Med*. 2017;377(26):2519–30; 2. Pasi KJ, et al. *N Engl J Med*. 2020;382(1):29–40; 3. Pasi KJ, et al. *Haemophilia*. 2021;27(6):947–56; 4. Ozelo M, et al. *N Engl J Med*. 2022;386(11):1013–25.

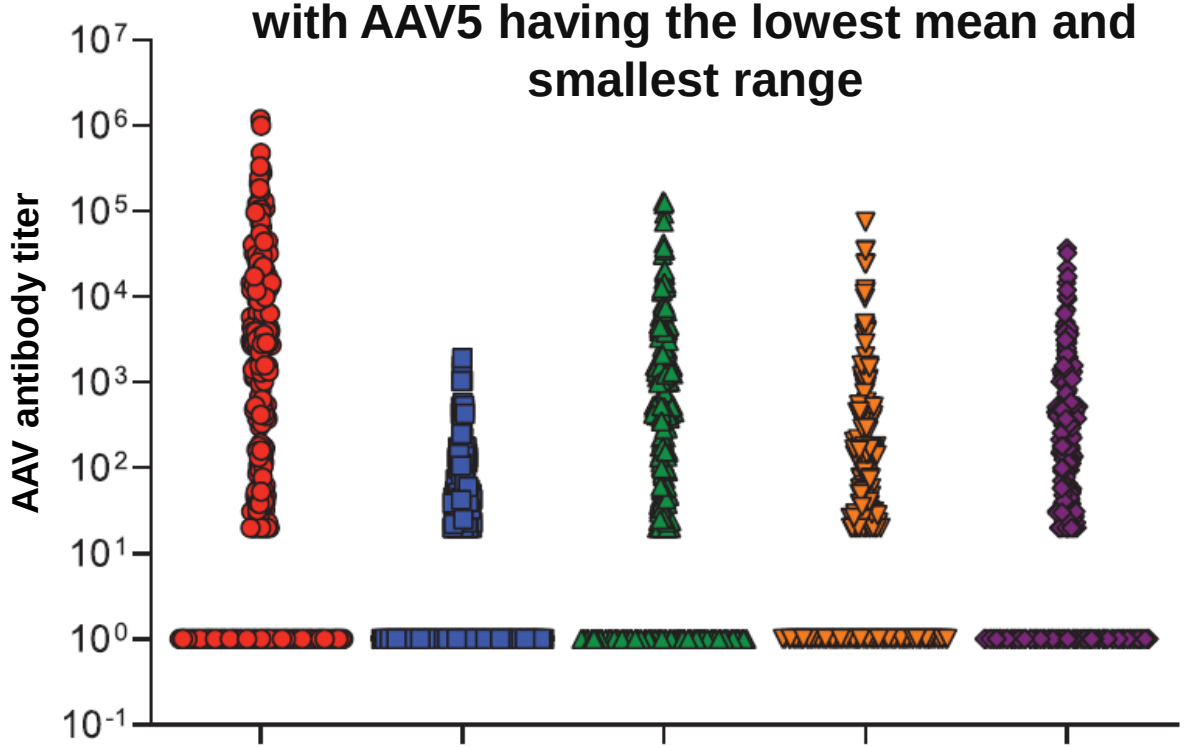
We have defined global seroprevalence of AAV by serotype

BMN 270-901 Global Seroprevalence Study (N = 540)

AAV5 exhibits the lowest seroprevalence globally (~30%)



Pre-existing titers range widely over 5 logs, with AAV5 having the lowest mean and smallest range



	AAV2	AAV5	AAV6	AAV8	AAVrh10
Mean	30,252.0	139.9	5358.6	1534.3	1370.6
SD	104,315.9	257.3	16,682.2	6486.5	4001.7
n	294	188	247	227	233

AAV, adeno-associated virus; AAVx, adeno-associated virus serotype 2, 5, 6, 8, rh10; MRD, minimum required dilution; n, number; SD, standard deviation.

1. Klamroth R, et al. *Hum Gene Ther*. 2022;33(7-8):432-441; 2. Boutin S, et al. *Hum Gene Ther*. 2010;21(6):704-712.

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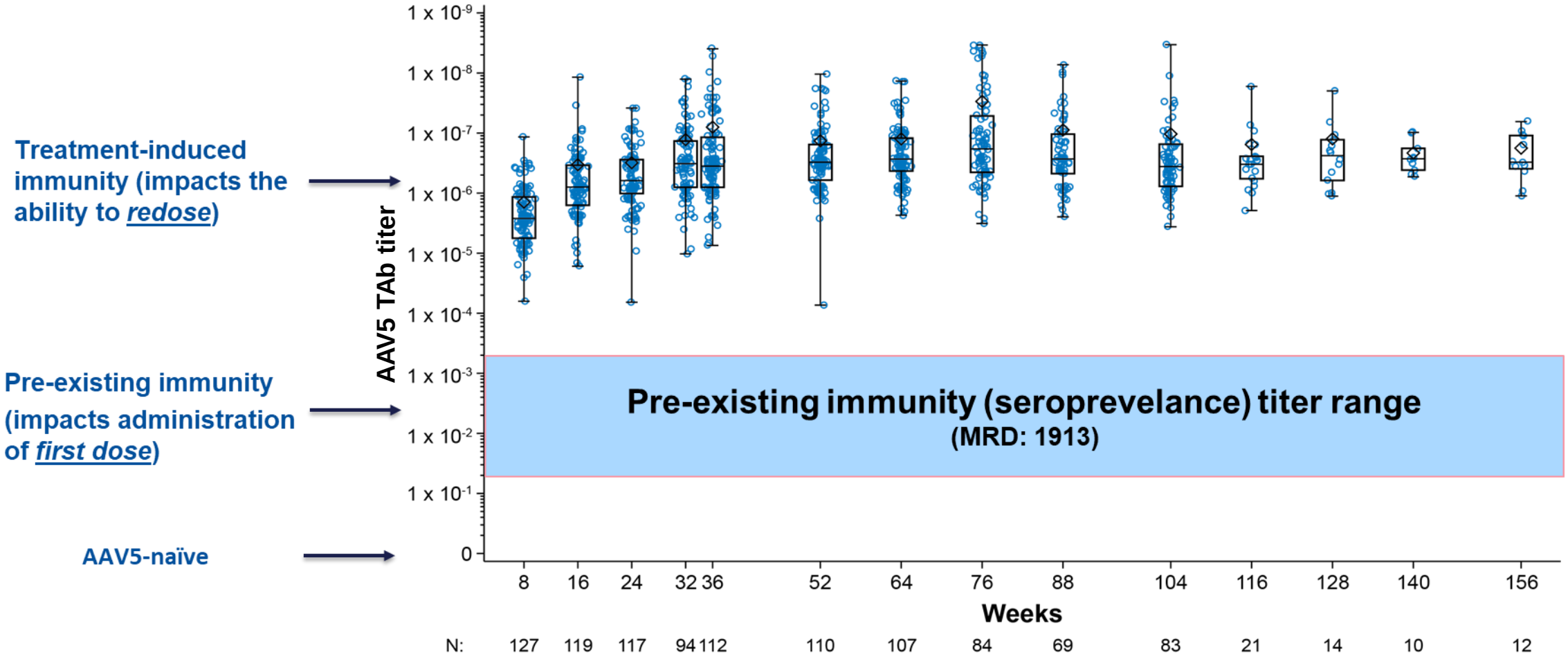
Pre-existing antibody titers are lower and more manageable than treatment-emergent titers in patients with hemophilia A



AAV5, adeno-associated virus serotype 5; AAV5 TAb, anti-AAV5 total binding antibodies; ITT, intention to treat; MRD, minimum required dilution.

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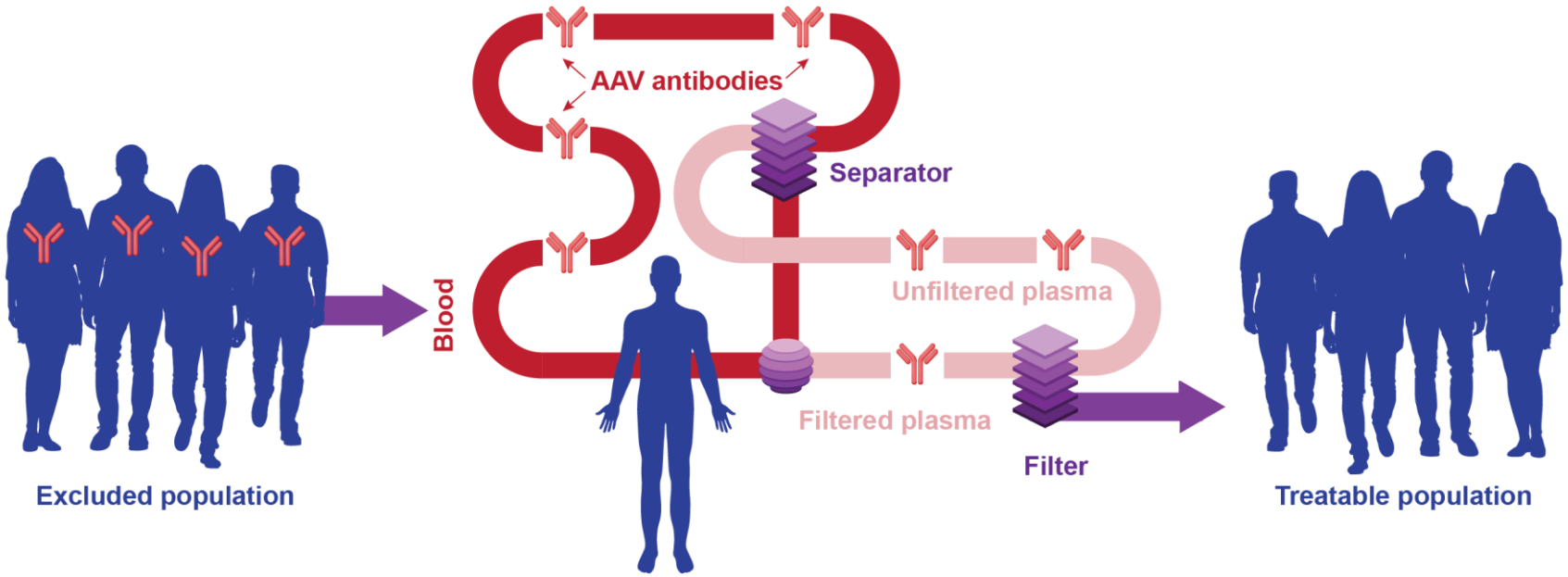
Rationale

- Develop strategies that enable patients with pre-existing antibodies to be considered for treatment and those with treatment-induced antibodies to be considered for additional AAV treatment

Aims:
Investigate the applicability of immunoadsorption plasmapheresis to deplete both pre-existing AAV5 capsid-specific antibody titers and treatment-emergent titers



AAV, adeno-associated virus; AAV5, AAV serotype 5.

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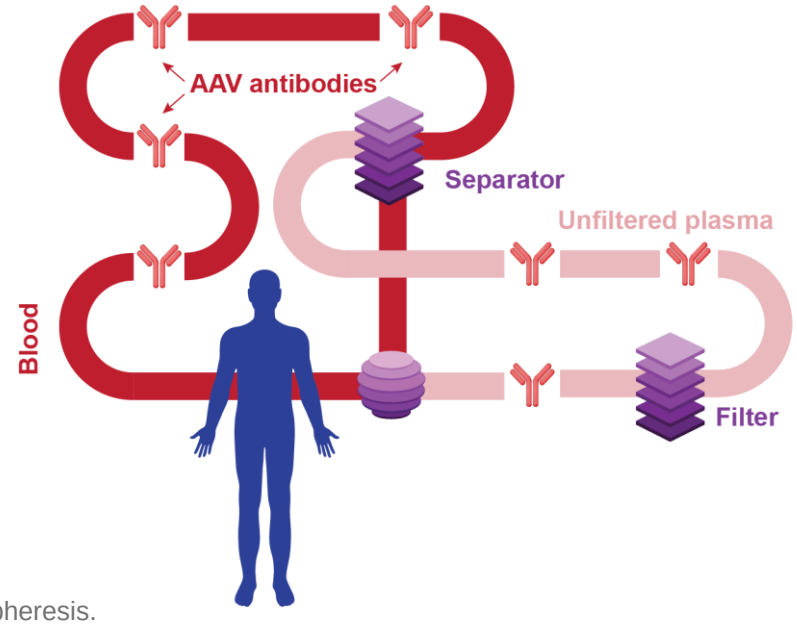
Plasmapheresis: a routine medical procedure with an excellent safety profile

- Plasmapheresis: removes and returns or exchanges blood plasma from and to the blood circulation
- IAP separates plasma from blood cells, selectively removes antibodies by ex vivo immunoadsorption, and returns antibody-depleted plasma to circulation
  - Removes ≥80% of total immunoglobulin in plasma after one session, increasing to ~98% after multiple sessions
  - Preclinical[1,2] and clinical[3] studies have demonstrated successful AAV antibody reduction



AAV, adeno-associated virus; IAP, immunoadsorption plasmapheresis.

1. Orlowski A, et al. *Mol Ther: Methods Clin Dev.* 2020;16:192-203; 2. Bertin B, et al. *Sci Rep*. 2020;10:864; 3. Monteilhet V, et al. *Mol Ther.* 2011;19(11):2084-2091.

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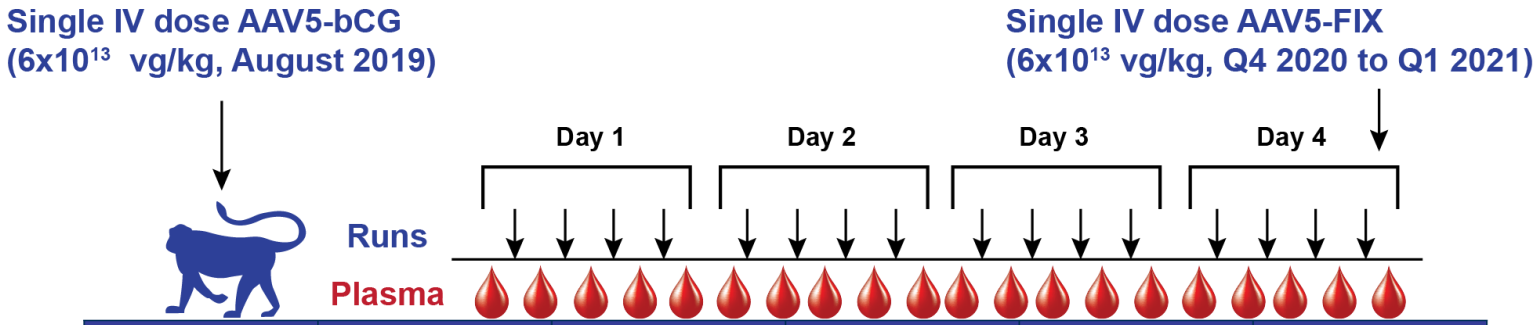
Experimental outline

- Animals were sensitized with a single IV dose of AAV5-bCG at $6x10^{13}$ vg/kg
- Multiple rounds of IAP were performed over multiple days as tolerated by each animal; at minimum, 1 day with 4 rounds of IAP
- Following IAP procedure, animals were administered repeat (challenge) dose of AAV5-hFIX gene therapy
  - All animals had significant perturbation of hematological and biochemical blood parameters and cytokines; however, all parameters returned to baseline levels before end of study



Group	Sex	ID	Approximate age	Weight	AAV5 antibody
Group 1	Female	NHP0 (neg control)	7 years	3.99 kg	Negative
Group 2	Male	NHP1	11 years	7.2 kg	Positive (previously sensitized)
		NHP2	7 years	8.07 kg	Positive (previously sensitized)
		NHP3	11 years	8.7 kg	Positive (previously sensitized)
		NHP4	10 years	10.6 kg	Positive (previously sensitized)

AAV, adeno-associated virus; AAV5, AAV serotype 5; AAV5 TAb, anti-AAV5 total binding antibodies; bCG, beta subunit of cynomolgus chorionic gonadotropin; hFIX, human anticoagulation factor IX; IAP, immunoadsorption plasmapheresis; IV, intravenous; neg, negative; NHP, nonhuman primate; Qx, quarter number.

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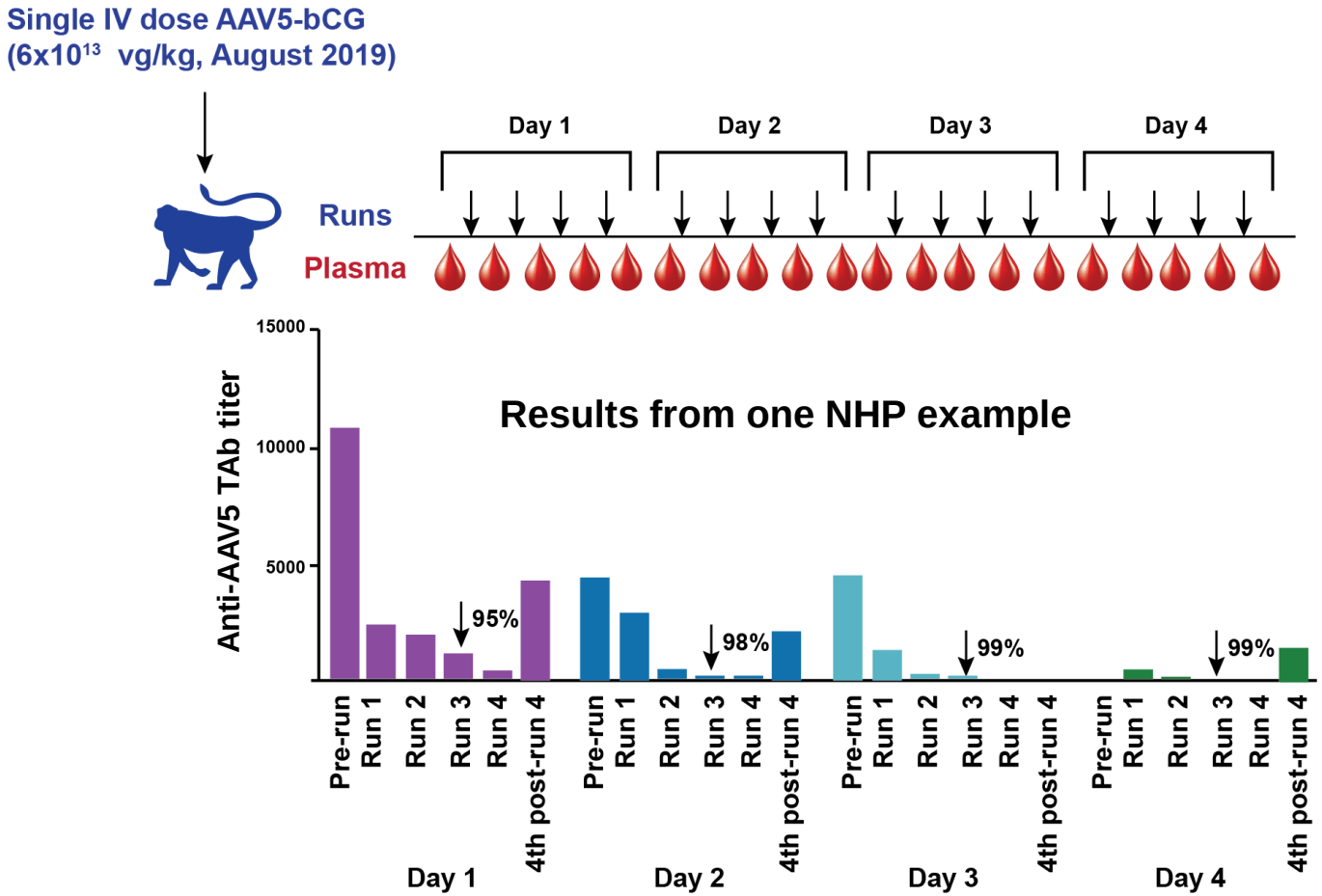
Multiple rounds of IAP over multiple days enabled up to 99% reduction of anti-AAV5 TAb titers



AAV5, adeno-associated virus serotype 5; anti-AAV5 TAb, anti-AAV5 total binding antibodies; bCG, beta subunit of cynomolgus chorionic gonadotropin; IAP, immunoadsorption plasmapheresis; IV, intravenous; NHP, nonhuman primate.

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Percent reduction in AAV5 TAb titer is dependent on number of days and number of runs per day of successful IAP

- Number of days and runs achieved for each subject varied based on the health of each animal following each run

Animal	NHP0 (neg control)	NHP1	NHP2	NHP3	NHP4
Number of plasma volumes/day of IAP	4	3-4	4	4	4
Number of days of IAP	2	3	2	1	1
Starting TAb titer	Neg	3714	43,406	52,945	42,181
TAb titer on day of rechallenge	Neg	59	158	599	2416
Percent TAb titer reduction	NA	98.4%	99.6%	98.9%	94.3%

AAV5, adeno-associated virus serotype 5; AAV5 TAb, anti-AAV5 total binding antibodies; IAP, immunoadsorption plasmapheresis; NA, not applicable; NHP, nonhuman primate.

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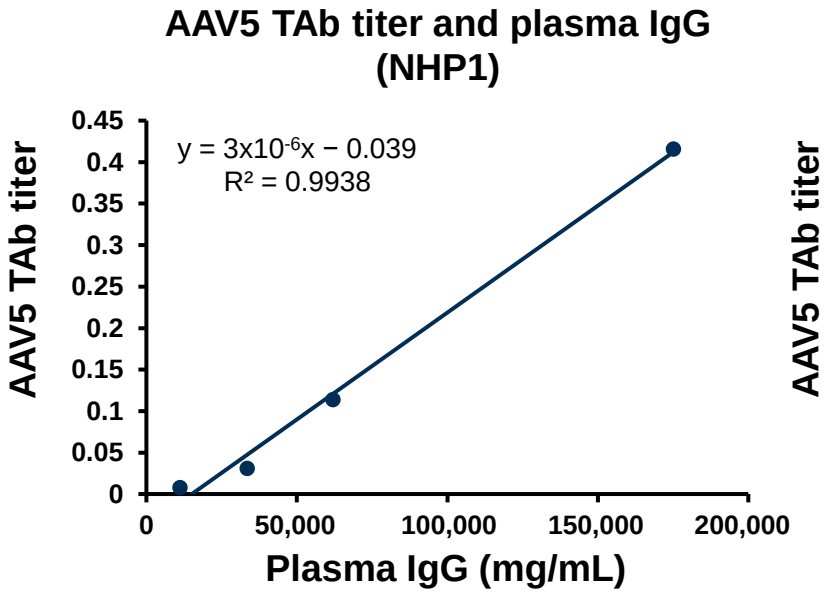
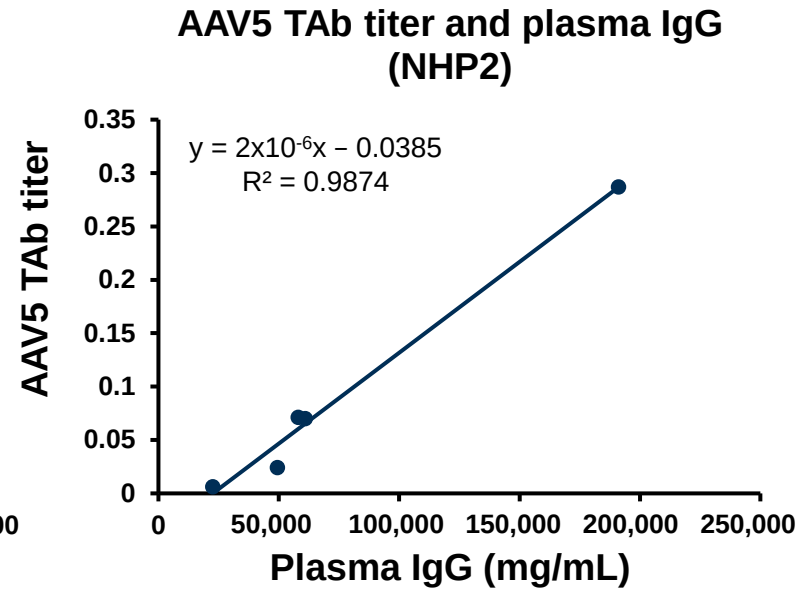
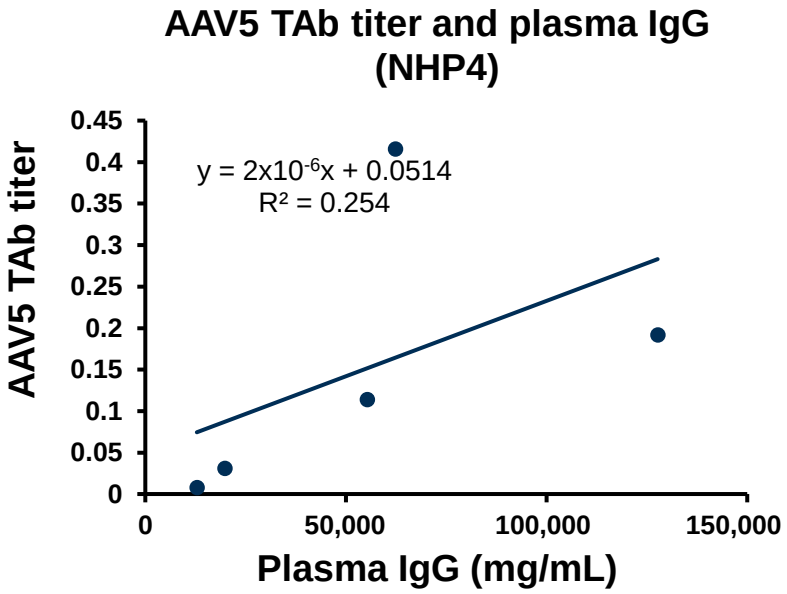
Depletion of IgG correlates with declining TAb titer in NHPs

- Potential “real-time” point-of-care dosing surrogate to extrapolate the reduction in AAV5 TAb







AAV5, adeno-associated virus serotype 5; AAV5 TAb, anti-AAV5 total binding antibodies; IgG, immunoglobin; NHPs, nonhuman primates.

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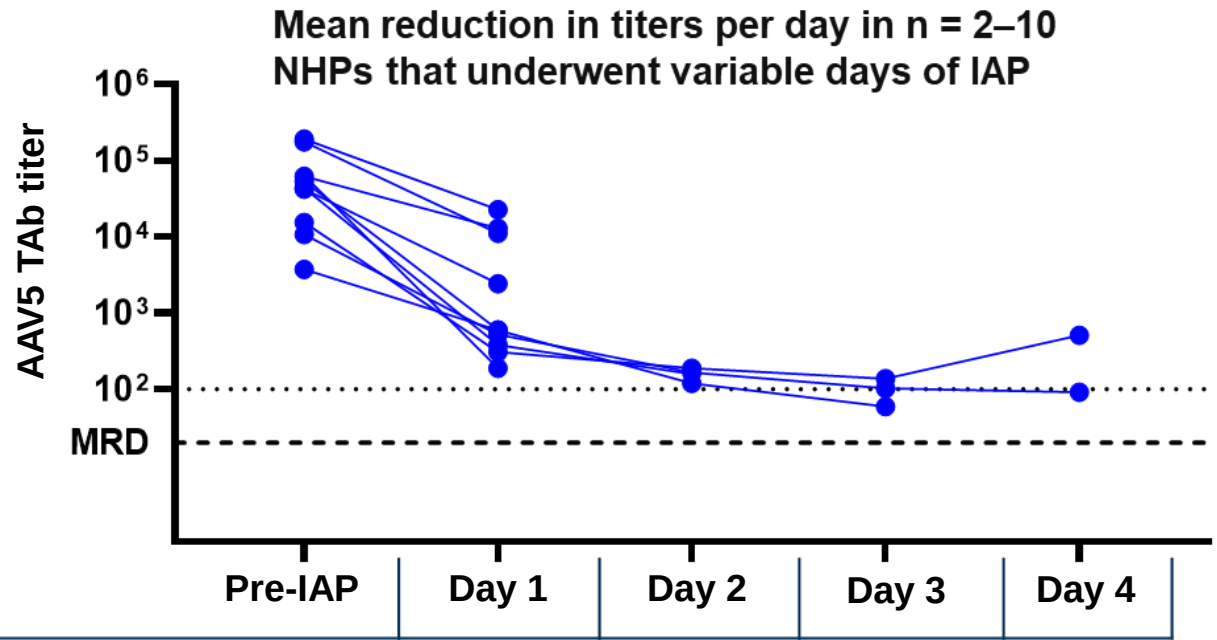
Data collected over several experiments showed that 2 plasma volumes of IAP per day resulted in an 83% decrease in AAV5 TAb titer after 1 day and a 95% reduction in AAV5 TAb titer after 2 days



Number of animals	10	4	3	2
Mean % reduction in TAb	93.1%	98.5%	98.8%	98.1%
Mean % reduction in TAb after 2 cycles	83%	95%	95%	98%

AAV5, adeno-associated virus serotype 5; AAV5 TAb, anti-AAV5 total binding antibodies; IAP, immunoadsorption plasmapheresis; MRD, minimum required dilution; NHPs, nonhuman primates.

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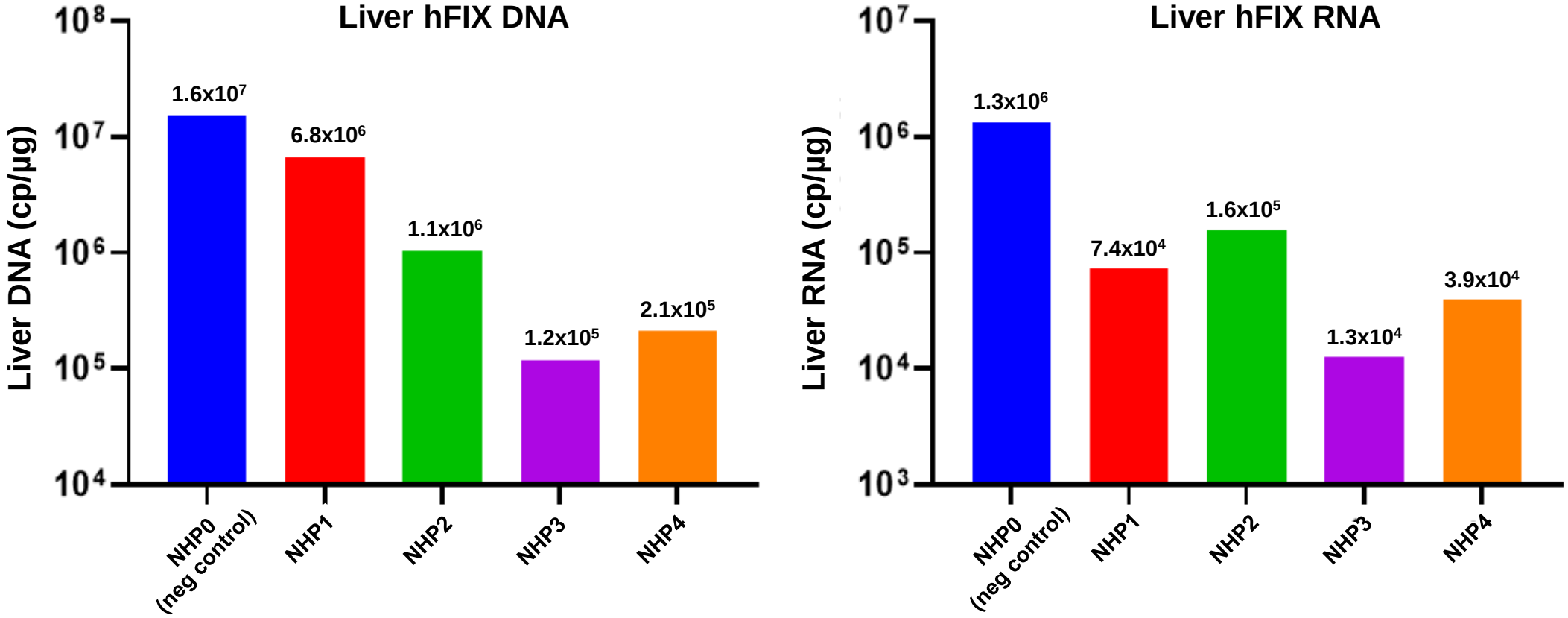
Mean reduction of 98.5% in AAV-induced antibody titers and successful second transduction achieved after 2 days of IAP in NHPs

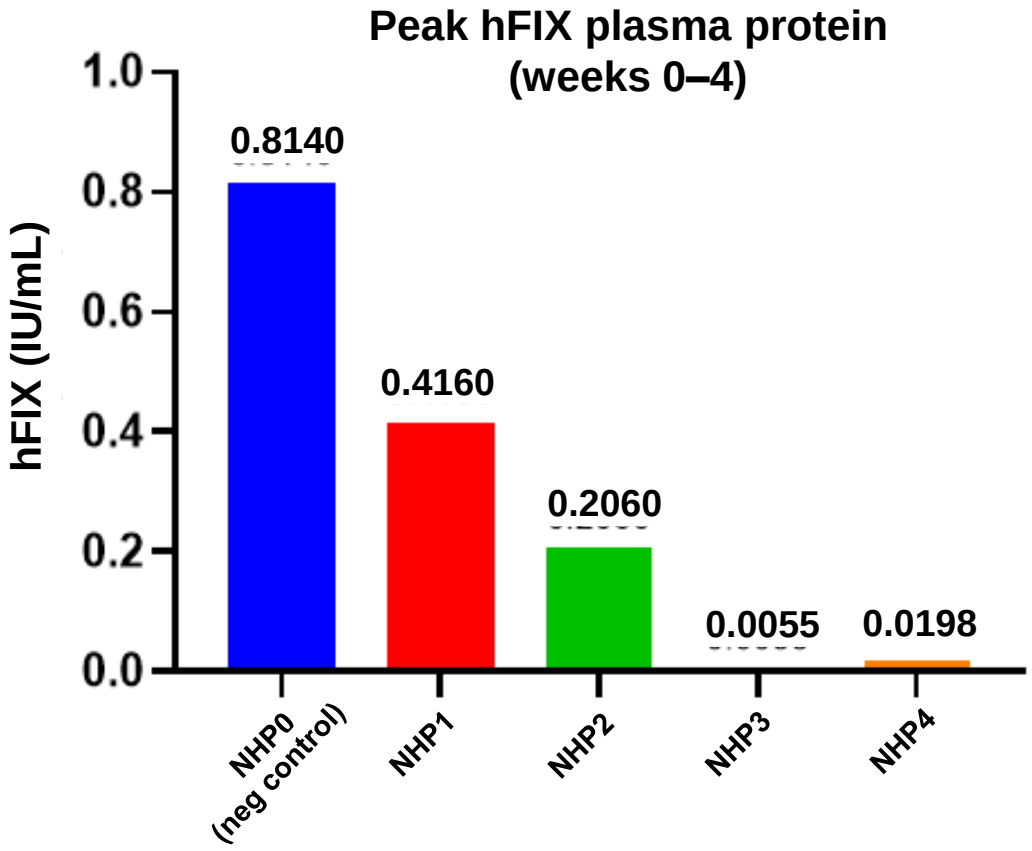
- Liver hFIX vector DNA and RNA:
  - Liver samples collected ~8 weeks following challenge dose and assessed via ddPCR

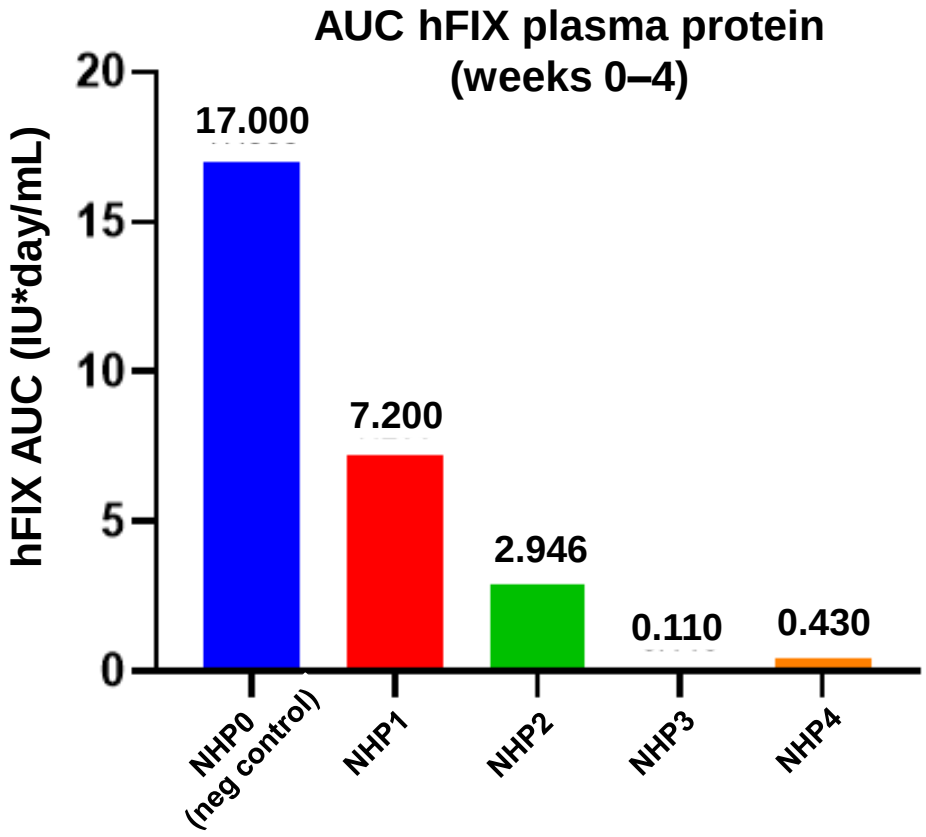


ddPCR, digital droplet polymerase chain reaction; IAP, immunoadsorption plasmapheresis; hFIX, human anticoagulation factor IX; NHPs, nonhuman primates.

Mean reduction of 98.5% in AAV-induced antibody titers and successful second transduction achieved after 2 days of IAP in NHPs

- Plasma hFIX protein concentration:
  - Peak hFIX expression and AUC are shown from day 0 through 4 weeks post challenge dose





AUC, area under the curve; IAP, immunoadsorption plasmapheresis; hFIX, human anticoagulation factor IX; NHPs, nonhuman primates.

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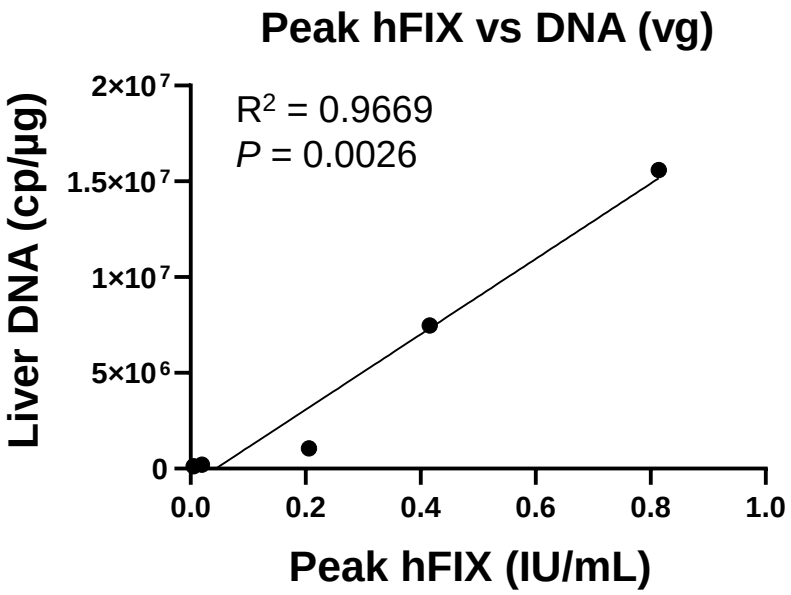
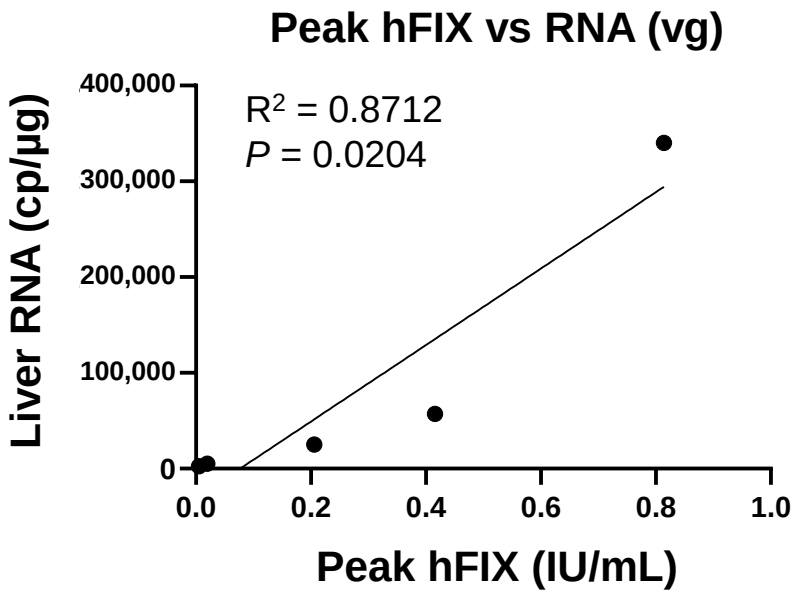
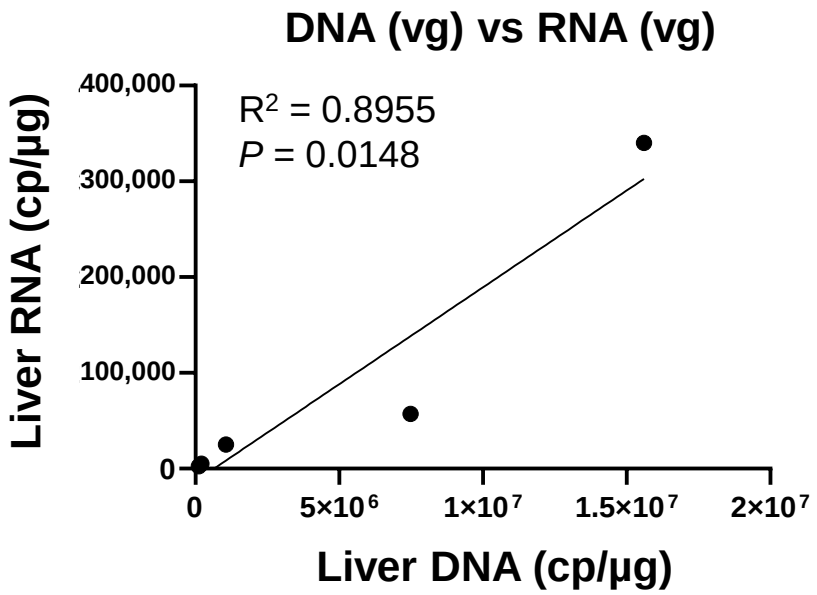
Vector genome DNA and RNA well correlated with peak hFIX in NHPs







hFIX, human anticoagulation factor IX; NHPs, nonhuman primates; vg, vector genome.

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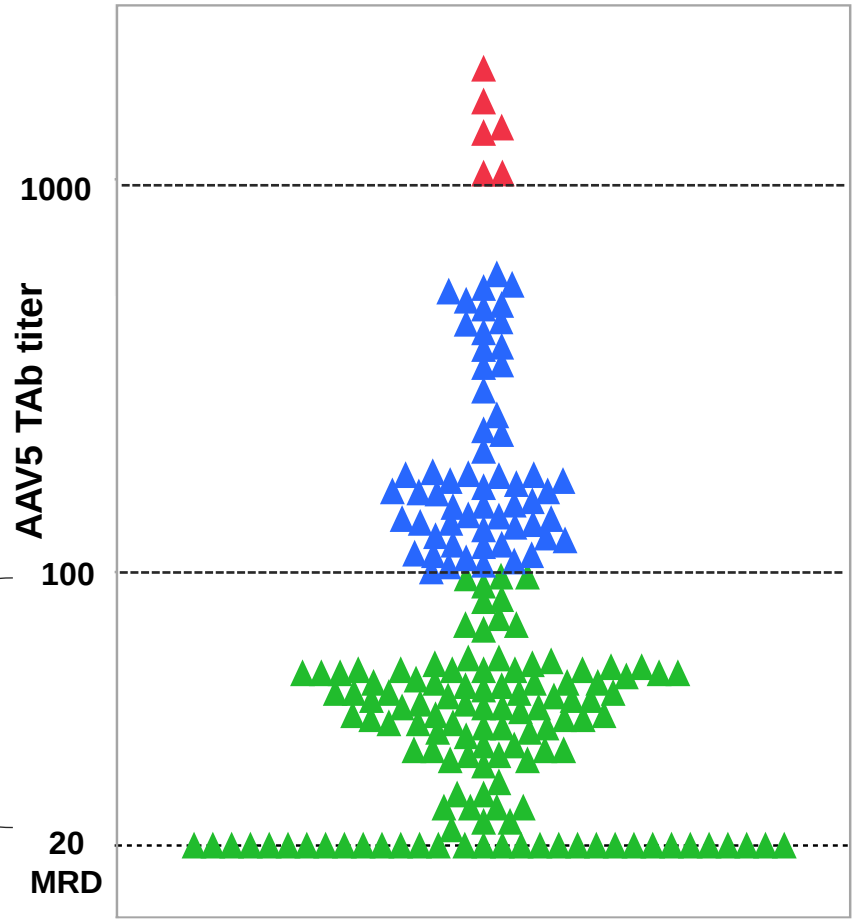
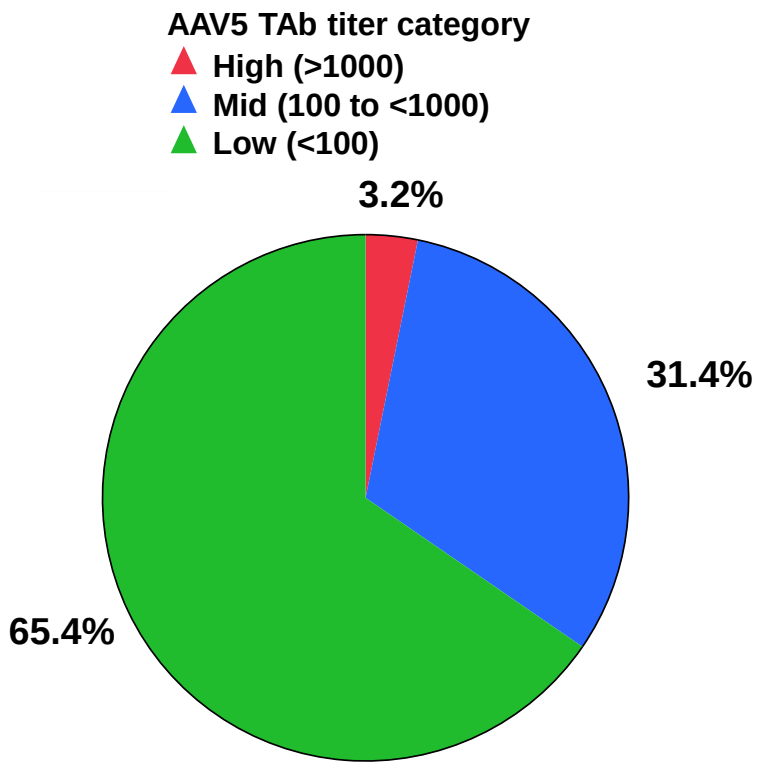
In patients, pre-existing AAV5 antibody titers span 3 logs, suggesting patients may require different numbers of IAP cycles to achieve transduction

97% addressable after 2 days (4 cycles) of IAP

65% addressable after 1 day (2 cycles) of IAP



Seroprevalence AAV5 TAb data
(N = 188 TAb positive)



AAV5, adeno-associated virus serotype 5; AAV5 TAb, anti-AAV5 total binding antibodies; IAP, immunoadsorption plasmapheresis; MRD, minimum required dilution.

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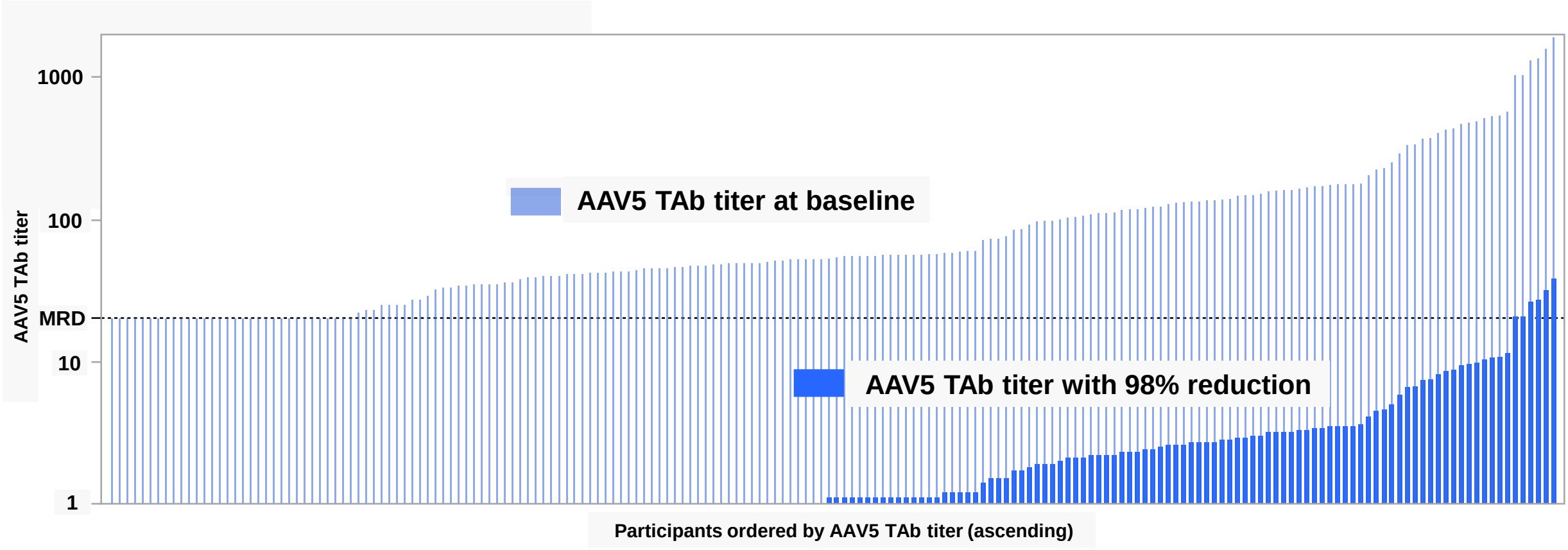
A 98% decrease would reduce TAb titers in all patients from BMN270-901 to below cut-point, assuming IAP was equally effective in patients



AAV5, AAV serotype 5; AAV5 TAb, anti-AAV5 total binding antibodies; IAP, immunoadsorption plasmapheresis; MRD, minimum required dilution.

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Summary: plasmapheresis is likely to reduce TAb titers and make liver transduction achievable

- Pre-existing immunity to AAV is postulated to arise from prior exposure to wild-type infection and could prevent patients who test positive from being considered for gene therapy treatment
- Developing an approach to reconcile this is important
- IAP has been demonstrated as a safe and routine medical procedure
  - Tolerance can be easily be monitored by regularly checking blood pressure, temperature, and pulse during treatment[1]
- Multiple sessions (days) and plasma volume (runs) of IAP brought antibody titers below 100 in a subset of previously sensitized NHPs
- Increasing the number of sessions and plasma volumes reduced antibody titers to a lower nadir
- Achieving an AAV5 TAb titer below 100 was associated with an improved level of pharmacological activity as reflected by peak transgene DNA and RNA as well as transgene protein production after repeat dose administration of AAV5-hFIX in NHPs
- One day of plasmapheresis (2 plasma volumes) may address ~65% of persons with hemophilia A with pre-existing immunity, and 4 plasma volumes over 2 days would likely capture patients with preexisting and treatment-induced immunity

AAV, adeno-associated virus; AAV5, AAV serotype 5; AAV5 TAb, anti-AAV5 total binding antibodies; hFIX, human anticoagulation factor IX; IAP, immunoadsorption plasmapheresis; NHPs, nonhuman primates.

1. Altobelli C, et al. *Kidney Blood Press Res*. 2022 48(1):66–78.

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Acknowledgments

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- Medical writing and editorial support were provided by Taryn Bosquez-Berger, PhD, of AlphaBioCom, a Red Nucleus company, and funded by BioMarin Pharmaceutical Inc.

Questions?