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

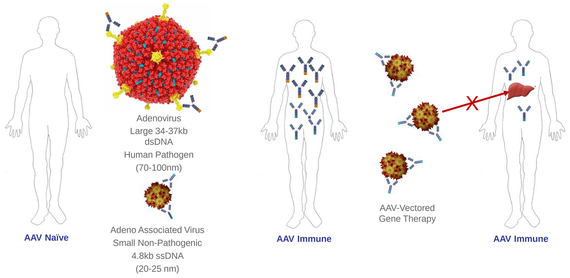
Brian R. Long[1], Benjamin M. Hock[1], Charles A. O'Neill[1], Jeremy Arens[1], Theresa Seitel[1], Lucy Crockett[1], Francis Relouzat[2], Claire-Maëlle Fovet[2], Nathalie Dereuddre-Bosquet[2], Pauline Maisonnasse[2], Helene Letscher[2], Roger Le Grand[2] Christian Vettermann[1] and Soumi Gupta[1]

1. BioMarin Pharmaceutical Inc. Novato CA, USA. 2. Commissariat à l'Energie Atomique et aux Energies Alternatives, IMVA-IDMIT Center DRF/Institut de Biologie François Jacob. 18, route du Panorama F92265, Fontenay-aux-Roses, France

Abstract

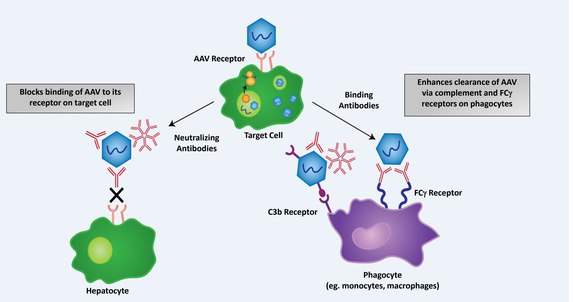
The investigation of adeno-associated virus (AAV) vectored gene therapies for the treatment of numerous monogenic disorders has increased exponentially over the past decade. However, the presence of pre-existing anti-AAV total binding antibodies (AAV TAb) and in vitro neutralizing antibodies (AAV NAb) may limit the efficacy of gene therapy. Moreover, a first administration of an AAV vector induces high titers of treatment emergent AAV NAb, which may compromise repeat dose administration with the same vector. Depletion of AAV NAb by immunoadsorption plasmapheresis (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 to remove AAV serotype 5 (AAV5) NAb from animals sensitized by an initial gene therapy dose. Five cynomolgus macaques (Macaca fascicularis) were included in this study; four were sensitized by administration of an AAV5 capsid encoding for the beta subunit of cynomolgus chorionic gonadotropin (AAV5-βCG) at a dose of 6E13vg/kg, and one control animal was naïve. All were subjected to IAP for a minimum of 1 day of 4 runs (plasma volume exchanges) to a maximum of 3 days of 3 runs. All 5 animals were challenged with the same AAV5 capsid encoding a different protein, human coagulation factor IX (AAV5-hFIX) at a dose of 6E13 vg/kg, administered within 10 minutes of the last run IAP. Efficacy of the IAP procedure was functionally evaluated by laboratory measures of plasma IgG and AAV5 total binding antibody (AAV5 TAb) titer, hFIX plasma protein concentration, and quantitation of vector genomes and transcripts in liver tissue. Maximal depletion of AAV5 TAb titer (>99%) was achieved in two animals resulting in a nadir titer of 61 and 59. These two animals achieved approximately 25% and 50% of reference hFIX plasma protein levels, respectively, compared to the naïve animal (0.8 IU/mL), and a proportional percentage of vector genome copies measured in liver tissue compared to the naïve animal (1.6E7 cp/µg DNA). Following a variable number of IAP sessions (plasma volume exchanges over consecutive days) and administration of the AAV5-hFIX challenge dose, there was significant perturbation of hematological and biochemical blood parameters; however, all parameters returned to baseline levels within hours or days of the procedure. These results demonstrate the viability of IAP as an immune modulation procedure to deplete AAV5 capsid-specific antibody titers sufficient to allow repeat dose administration. As such, IAP may enable AAV-based vector gene therapy in patients currently excluded from gene therapy clinical trials or commercial product use due to pre-existing antibodies. Furthermore, additional evaluation of the efficacy of this procedure may be worthwhile in subjects with pre-existing antibody titers resulting from natural exposure to AAV infections, which result in lower antibody titers than the treatment-emergent titers observed here.

Pre-Existing Immunity is Postulated to Arise from Prior Exposure to AAV and Adenovirus



- AAV belongs to the parvovirus family and is dependent on co-infection with other (large DNA) viruses, mainly adenoviruses, in order to replicate. They belong to the genus *Dependoparvovirus* within the family *Parvoviridae* and are amongst the smallest animal DNA viruses.
- Adenovirus infects mucoepithelial cells of respiratory and GI tract, conjunctiva and cornea. The virus persists in lymphoid tissues and antibody is essential for recovery from infection. Roughly 45 to 80% of adults carry AdHu5-neutralizing antibodies.

Neutralizing and/or Binding Abs Can Impact the Pharmacodynamic Efficacy of AAV-Vectored GT



Plasmapheresis: A Routine Medical Procedure with an Excellent Safety Profile

- Plasmapheresis is the removal, treatment, and return or exchange of blood plasma from and to the blood circulation
- Used clinically to treat multiple indications including Guillain–Barré syndrome, lupus, acquired Hemophilia A (HA) and removal of antibodies targeted at heparin (HIIT)

- Total Plasma Exchange (TPE)
  - Separates plasma from blood cells and replaces with donor plasma or other plasma surrogate (ex. 3% albumin solution)
  - 1-2 plasma volumes exchanged per treatment session
  - Does not selectively remove antibody and therefore comes with the risk of removing other beneficial plasma components
  - Removes up to ~80% of total immunoglobulin in plasma after multiple sessions

- Immunoadsorption Plasmapheresis (IAP)
  - Autologous plasma exchange; Separates plasma from blood cells, selectively removes antibodies from plasma and returns depleted plasma
  - Two to three plasma volumes exchanged per treatment session
  - Selectively removes antibodies (including protective antibodies) but reduces the risk posed by removing other beneficial plasma components
  - Removes ≥80% of total Ig in plasma after one session, increasing to ~98% after multiple sessions

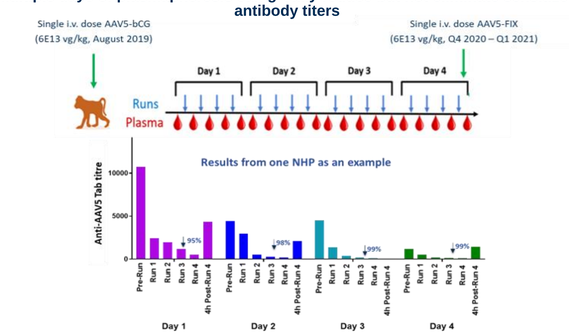
IA Plasmapheresis to Enable GT Dose Administration in Animals with Neutralizing Antibodies

- Multiple studies have supported the use of plasma filtering methods for the removal of anti-AAV antibodies *in vitro*[1] (in non-clinical species including mice[2], rats[3] and monkeys[4]) as well as clinically[5]
- UniQure presented a study in 2017 wherein immune adsorption in NHP resulted in the depletion of high-titer anti-AAV5 antibodies by >90%, allowing successfully redosing with a second AAV vector
- Bertin et al[4]. demonstrated that plasmapheresis allows for AAV vector readministration in non-human primates and efficient removal of capsid-specific antibody through use of Sepharose matrix crosslinked to AAV8 capsids.
- The non-clinical studies reported here were initiated in 2019 using cynomolgus macaques to confirm the antibody depletion results of previous studies and gain additional proof of concept data on the feasibility of re-dosing AAV vectors following multiple cycles of immunoadsorption plasmapheresis
- Non-clinical studies reported here utilized Miltenyi Biotec apheresis equipment paired with TheraSorb™ affinity columns (CE marked in the EU) to support use in clinical patients
- TheraSorb columns utilize a porous matrix coated with recombinant Camelid Ig to remove all subtypes of human antibodies (regardless of specificity)
- Proof of concept and optimization animal studies were conducted in a collaboration between BioMarin and Commissariat à l'Energie Atomique et aux Energies Alternatives, IMVA-IDMIT Center DRF/Institut de Biologie François Jacob, Paris, France
- Studies were initiated to establish conditions for use of the IA-plasmapheresis system in NHPs and evaluate procedure tolerance

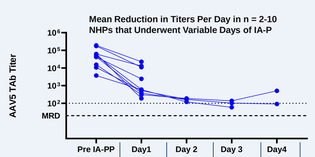
1 Kasprzyk, et al, *Mol. Ther.: Methods & Clin. Dev.*, 2022
2 Majowicz, et al, *Molecular Therapy*, 2017
3 Orlowski, et al, *Mol. Ther.: Methods & Clin. Dev.*, 2020
4 Bertin, et al, *Nature: Scientific Reports*, 2020
5 Monteilhet, et al, *Molecular Therapy*, 2011

Multiple Rounds of IAP Over Multiple Days Enabled up to 99% Reduction of Anti-AAV5 TAb titers

Multiple days of plasmapheresis can greatly reduce but not eliminate sensitized antibody titers



Two plasma volumes of Immuno-adsorption Plasmapheresis per day resulted in an 83% decrease in AAV5 TAb titer after 1 day, and a 95% reduction in AAV5 TAb titer after two days

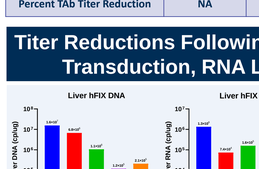


	Pre IA-PP	Day1	Day 2	Day 3	Day4
Number of Animals by Day		10	4	3	2
Mean % Reduction in TAb Per Day		93.1%	98.5%	98.8%	98.1%
Mean % Reduction in TAb after 2 Cycles Per Day		83%	95%	95%	98%

Optimized IAP Procedure Allows for AAV Re-Dosing

- Additional studies evaluated the effectiveness of IAP for removal of AAV5 capsid specific antibodies from AAV5-sensitized animals following an initial gene therapy administration, culminating in further optimization of the safety and efficacy of the IAP procedure in NHP and administration of a repeat (challenge) dose of gene therapy in animals sensitized to AAV5
- Results of the final study in which AAV5 sensitized NHPs were subjected to up to four consecutive daily immunoadsorption plasmapheresis procedures circulating up to four plasma volumes per day (as safely tolerated by 5 individual animals including one unsensitized control) prior to administration of repeat dose of AAV5 vector gene therapy are reported here.

- Aim was to use the optimized 4-day IA-P procedure, followed by a single dose of AAV5-FIX within 30 minutes of the final run of IA-P
- Number of successful IAP runs could vary between animals and would be dependent on animal health
- Decision tree would be implemented by the veterinary staff as shown below for AAV5-FIX dosing
- Efficacy was assessed by measuring Vector DNA and RNA levels in liver as well as anti-AAV5 TAb titers and hFIX protein production in serum



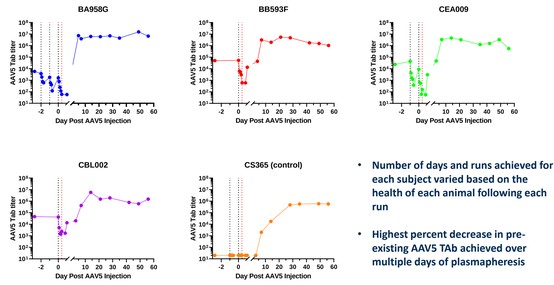
Decision tree for AAV Vectored Gene Therapy Challenge Dose Administration

Administration of test item will be performed after the 4th run on Day 4. Test item may be injected as soon as the end of 2 runs on the first day if an IA-PP issue is anticipated for the next days, according to scheme at left.

Five cynomolgus macaques (*Macaca fascicularis*) imported from Mauritius, four males weighing 7-11 kg and one female weighing 3.5-4 kg were included

Group	Gender	ID	Approximate Age	Weight	AAV5 Antibody
Group 1	Female	Neg Control CS365	7 years	3.99 kg	Negative
Group 2	Male	NHP1 (BA958G)	11 years	7.2 kg	Positive (Previously Sensitized)
		NHP 2 (CEA009)	7 years	8.07 kg	Positive (Previously Sensitized)
		NHP 3 (BB593F)	11 years	8.7 kg	Positive (Previously Sensitized)
		NHP 4 (CBL002)	10 years	10.6 kg	Positive (Previously Sensitized)

AAV5 Total Antibody (TAb) Titer Reduction Following IA-P



- Number of days and runs achieved for each subject varied based on the health of each animal following each run
- Highest percent decrease in pre-existing AAV5 TAb achieved over multiple days of plasmapheresis

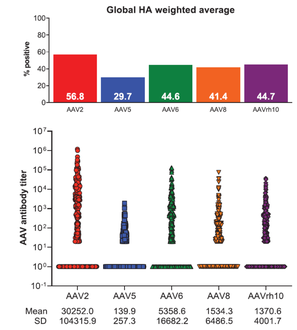
AAV5 TAb titer. Black dotted lines mark the beginning of each day of IAP (before the first run), while the red dotted line marks the administration of AAV5-hFIX.

Animal	CS365 - Control	BA958G	CEA009	BB593F	CBL002
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%

Titer Reductions Following IAP is Associated with Improved Transduction, RNA Levels and Protein Expression

Human FIX transgene DNA and RNA was evaluated by ddPCR analysis from cynomolgus monkey liver samples collected at termination ~8 weeks following challenge dose administration. Plasma was collected at multiple time points for assessment of hFIX plasma protein concentration. Peak hFIX expression and area under the concentration time curve (AUC) are shown from the time of challenge dose administration through 4 weeks post challenge.

Seroprevalence Studies Indicate ~30% of Persons with Hemophilia A are Positive for AAV5 Antibody

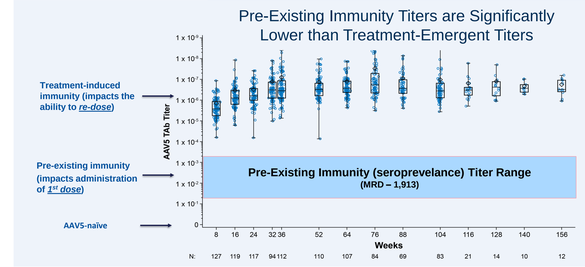


AAV seropositivity using the global HA weighted average calculated by multiplying the percentage of participants who tested positive in each country by the number of people with HA in that country, divided by the total number of people with HA in all countries in the study

Individual participant titers for individual participants in the global population. Participants with negative titers plotted as a value of 1. Participants with positive titer results and a titer <20, the MRD, are shown as 20. Width is representative of the number of points at a particular value. Mean (SD) values are for participants with quantifiable titers only: AAV2, n = 294; AAV5, n = 188; AAV6, n = 247; AAV8, n = 227; AAVrh10, n = 233.

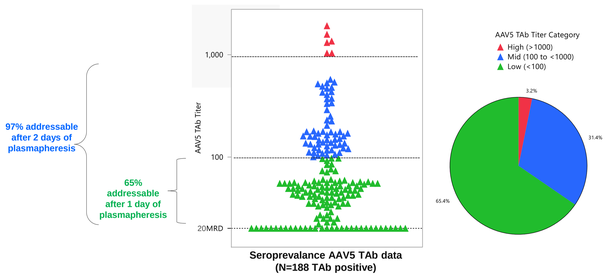
Klamroth R, et al. Global Seroprevalence of Pre-existing Immunity Against AAV5 and Other AAV Serotypes in People with Hemophilia A. Hum Gene Ther. 2022 Apr;33(7-8):432-441

Pre-Existing Immunity Titers are Significantly Lower than Treatment-Emergent Titers



Application to HA Patients with Pre-Existing Antibody

~65% of participants in the seroprevalence study with pre-existing immunity would be potentially addressable in 1 Day (2 Cycles), 97% may be transduced after 2 days (4 cycles)



Conclusions

- Pre-existing immunity to AAV is postulated to arise from prior exposure to wild type infection
- Patients that test positive for AAV5 antibody may be ineligible for gene therapy (~30% of the global population)
- Finding an approach to reduce antibody titers in seropositive people may enable access to gene therapy
- Plasmapheresis is accepted as a safe and routine medical procedure
- Multiple sessions (days) and plasma volume (runs) of IA-plasmapheresis brought antibody titers down below 100 in a subset of previously sensitized NHP
- Increasing the number of sessions and plasma volumes reduced antibody titers more effectively
- Achieving an anti-AAV5 TAb titer below 100 was associated with an improved level of efficacy following repeat dose administration
- One day of plasmapheresis (2 plasma volumes) may address ~65% of persons with HA with pre-existing immunity, and 4 plasma volumes over two days would likely capture almost everyone

Acknowledgements

Thank you to the following people for their considerable input and discussion of these studies: Stephanie MacLeod, Russell Soon, Ryan Boyer, Anu Cherukuri, Justin Ishida, Suresh Agarwal and Randall Lee. Thank you to the Roger LeGrand lab and staff at the Paris-SUD university for carrying out the NHP studies.