1074

Characterization of pre-existing immunity to AAV5

Lau K[1], Vettermann C[1], de Hart G[1], Hock MB[1], Frazer-Abel S[2], Long B[1], Gupta S[1]

[1]BioMarin Pharmaceutical Inc., Novato, CA; [2]Exsera BioLabs, University of Colorado School of Medicine, Aurora, CO

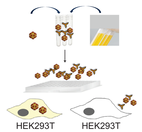
Objective

- The objective of this study was to further characterize pre-existing antibody responses to AAV serotype 5 (AAV5) in severe hemophilia A patients (n=540) who are naïve to gene therapy. Characteristics evaluated included:
  - AAV5 neutralizing factors (transduction inhibition, (TI) using a cell-based assay
  - AAV5 total binding antibodies (TAb) using a bridging immunoassay
  - AAV serotype specificity using a bridging immunoassay
  - AAV5 in vitro complement activation
  - AAV5 antibody isotypes

Methods

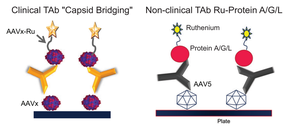
Assay methodologies for assessing AAV5 TI and TAb pre-existing immunity

Cell-based Transduction Inhibition (TI) Assay



- Measures ability of plasma to block transduction of a cell line by AAV
- Differing amounts of capsid needed for different serotypes can make TI titer comparisons across capsids more difficult

Anti-AAV Total Antibody (TAb) Assay

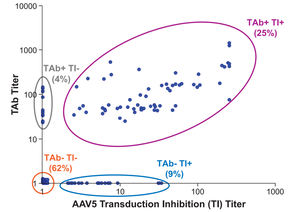


- Electrochemiluminescent assay (ECLA) on the MSD platform
- Ease of qualifying divergent capsid serotype assays in identical format

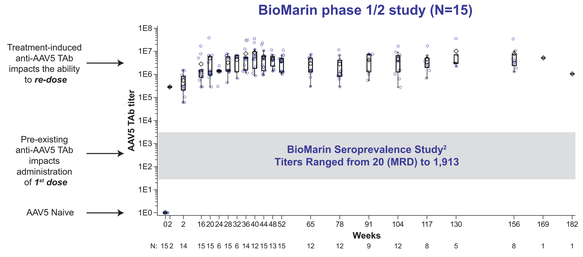
Results

The majority of subjects with pre-existing immunity to AAV5 were positive for both TAb and TI[1]

- Majority of Hemophilia A subjects are negative or positive in both TAb and TI assays
  - 62% TAb and TI negative
  - 25% TAb and TI positive
- A small subset of subjects have discordant results
  - 4% TAb+ and TI-
  - 9% TAb- and TI+

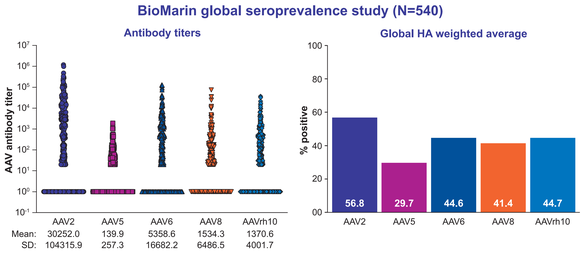


Pre-existing AAV5 TAb titers were much lower than AAV5 gene therapy post-treatment titers



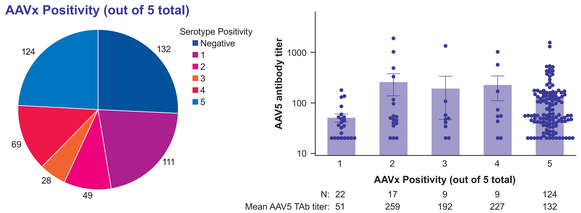
Global seroprevalence of AAV varies by serotype

- We previously assessed the seroprevalence of pre-existing immunity to AAV5 and four additional AAV serotypes[3]: AAV2, AAV6, AAV8, and AAVrh10



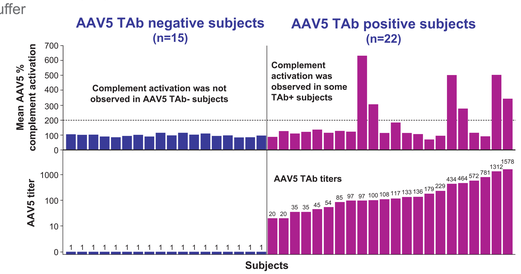
Higher AAV5 TAb titers were not correlated with more AAV serotype positivity

- We assigned subjects from the global seroprevalence study to AAVx positivity groups (Negative, 1, 2, 3, 4, 5) based on the number of serotypes for which they were positive
- There was no significant association between higher AAV5 TAb titers and the presence of antibodies reactive with other serotypes, suggesting that AAV5-specific TAb titers were not a result of broad previous AAV exposures (and subsequent cross-reactivity)



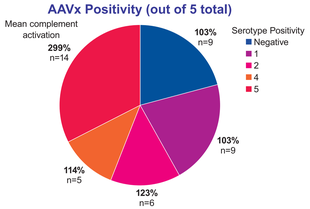
Some AAV5 TAb positive subjects demonstrated complement activation in response to AAV5

- AAV5-induced complement activation was assessed in vitro by measuring complement split products. Sera was incubated with either AAV5-bCG or control buffer



- In some AAV5 TAb+ subjects, a >200% increase in activated complement was observed; however, this was not strictly correlated with the magnitude of AAV5 TAb titers as complement activation was not observed in all subjects with higher titers

Complement activation to AAV5 >200% in vitro was observed only in subjects that were positive for all five serotypes (AAV5/2/6/8/rh10)

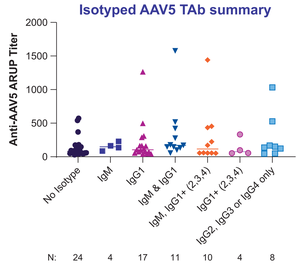


		Complement Activation (Mean % Increase)				
AAVx Positivity	N	Bb	C5a	C3a	C4a	Average
Negative	9	102%	98%	96%	115%	103%
1	9	101%	104%	99%	109%	103%
2	6	110%	105%	121%	155%	123%
3	0	–	–	–	–	–
4	5	105%	114%	106%	131%	114%
5	**14**	**260%**	**257%**	**271%**	**407%**	**299%**

C3a: central point of activation
Bb: alternative pathway activation
C4a: classicalfectin pathway activation
C5a: terminal pathway activation

AAV5 TAb responses were predominantly the IgG Isotype

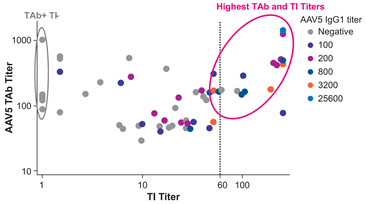
- In a subset of subjects (n=78) with pre-existing AAV5 TAb positivity, we characterized the isotype and subtype of the anti-AAV5 response (IgM, IgG1, IgG2, IgG3, IgG4, IgA)
  - Different isotypes reflect maturation of antibody response, and can have variable effector functions (neutralization and Fc receptor affinities)
  - IgG represents a more affinity mature and likely neutralizing response
  - IgM is the first to arise and reflects an immature response



N = 7 with IgA titers of 100-200, randomly distributed with respect to other isotypes

Subjects that were AAV5 TAb+ and TI negative or had low TI titers were predominantly AAV5 IgG1 negative

- Higher AAV5 TAb titers (~ >100) and TI titers (~ >60) were typically associated with detection of IgG1, but higher AAV5 TAb titers that were TI negative or low TI titer (~ <60) did not reveal a similar trend
- Consequently, highly neutralizing antibody responses were associated with detection of AAV5-specific IgG1



Summary

- The presence of pre-existing AAV antibodies may impact the successful administration and efficacy of gene therapy
- The majority of subjects with pre-existing immunity to AAV5 were positive for both TAb and TI, with a small subset showing discordant results. Pre-existing AAV5 TAb titers were much lower than AAV5 gene therapy post-treatment titers
- There was no association between higher AAV5 TAb titers and the presence of antibodies reactive with other serotypes, suggesting that AAV5-specific TAb titers were not a result of broad previous AAV exposures (and subsequent cross-reactivity)
- In some AAV5 TAb+ subjects, a >200% increase in activated complement was observed; however, this was not strictly correlated with the magnitude of AAV5 TAb titers. Complement activation was not observed in TAb-negative subjects, and not all TAb-positive subjects exhibited complement activation
- Complement activation to AAV5 >200% in vitro was observed only in subjects that were positive for all five serotypes (AAV5/2/6/8/rh10)
- The isotype and subtype of the anti-AAV5 response (IgM, IgG1, IgG2, IgG3, IgG4, IgA) was predominantly the IgG isotype. Higher AAV5 TAb titers and TI titers were typically associated with detection of IgG1
- Understanding the most important immunological determinants of pre-existing immunity that impact efficacy could enable more patients to gain access to gene therapy

References

1. Hayes et al. ISTH Presentation. 2019. **2.** Greg Hayes, Global Seroprevalence of pre-existing immunity against various AAV serotypes in the hemophilia A population, ISTH 2019. **3.** Klamroth et al. *Human Gene Therapy.* 2021.

Disclosures

Kelly Lau, Christian Vettermann, Greg de Hart, Benjamin Hock, Brian Long, and Soumi Gupta are employees of BioMarin Pharmaceutical Inc.

Presented at the ASGCT 26th Annual Meeting: May 16–20, 2023, Los Angeles, CA