Low Seroprevalence of Neutralizing Antibodies to Adeno-Associated Virus Serotype 9 (AAV-9) in Preparation for MyPeak-1, the First-in-Human Study of TN-201, an Investigational AAV9-Mediated Gene Therapy for Individuals with *MYBPC3*-Associated Hypertrophic Cardiomyopathy (HCM)

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Patients with *MYBPC3*-associated HCM have low levels of pre-existing immunity to AAV9. The majority of this patient population may be eligible for TN-201 in clinical trials

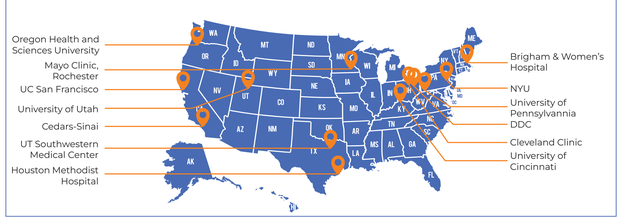
INTRODUCTION

- Adeno-associated viral vectors (AAV) are the leading approach for delivery of human genes to treat genetic disorders, with 6 AAV gene therapies approved and over 5,000 patients treated globally
- AAVs are comprised of different serotypes, each of which may exhibit distinct tropism for various tissue types. One specific serotype, AAV9, has shown superior selectivity for cardiac tissue relative to other serotypes, making it an ideal vector for genetic cardiomyopathies. Furthermore, AAV9 is the most studied and widely used gene therapy vector, with over 3,000 patients treated across nearly 50 countries[1]
- While AAV is not known to be pathogenic, prior exposure will lead to the development of circulating antibodies against that serotype and potentially others. High titers of circulating neutralizing antibodies (NAbs) to a specific serotype may inhibit the gene therapy from delivering the gene of interest to the target tissue and/or increase the risk of an inflammatory response[2]
- Low NAb titers to the selected serotype are often a pre-requisite to be eligible for AAV gene therapy. Sponsors have typically employed a range of titer thresholds, with nearly half requiring titers <1:50[3]
- Seroprevalence studies are often employed in advance of interventional trials to estimate the prevalence of pre-existing antibodies to one or more serotypes in a target population and to inform future clinical development
- Tenaya Therapeutics is developing TN-201, the first AAV9-mediated gene therapy for the treatment of *MYBPC3*-associated HCM. A Phase 1b study (MyPeak-1) is currently enrolling [see Robertson, et al., MyPeak-1: A Phase 1b Study to Evaluate Safety and Efficacy of TN-201, HCMS 2023 for more information]
- AAV9 seroprevalence has been quantified in other conditions, such as Duchenne muscular dystrophy (DMD) and spinal muscular atrophy (SMA).[4,5] Global estimates in the general population suggest 65-70% of adults have no or low pre-existing AAV9 NAbs, but rates can vary widely based on geography, demographics, and age[6]
- To date, there have been no studies to assess the seroprevalence of AAV9 immunity in *MYBPC3*-associated HCM patients and to estimate the proportion of the population who may be eligible for an AAV9-based gene therapy
- This poster describes the design and interim results of an AAV9 seroprevalence study among *MYBPC3*-associated HCM patients across HCM clinics across the US

METHODS

- Tenaya has initiated an exploratory seroprevalence study that is enrolling participants at 13 HCM clinics across the US to quantify the seroprevalence of AAV9 and pre-existing antibody titers in MYBPC3-associated HCM patients (**Figure 1**)

Figure 1. Site Map of AAV9 Seroprevalence Study



Sites collect demographic information, medical history, and serum from consented participants who meet the following criteria:

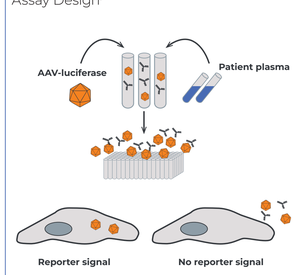
- Adults 18 to 65 years of age
- Diagnosis of HCM (maximal left ventricular wall thickness ≥13 mm)
- Confirmed pathogenic/likely pathogenic MYBPC3 truncating variant
- Symptomatic (NYHA Class II-V)
- Not currently receiving immunosuppressive therapy, chemotherapy, immunoglobulin or monoclonal antibody therapy
- Not previously dosed with a gene therapy

METHODS (continued)

AAV9 Neutralizing Antibody (NAb) and Total Antibody (TAb) Assays

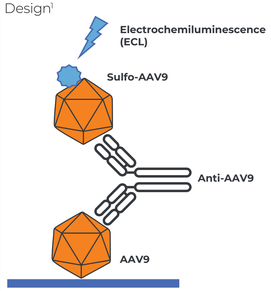
- Blood samples were collected locally at centres and then analyzed centrally at a research organization with expertise in quantifying pre-existing immunity to AAV serotypes
- The AAV9 NAb assay is an *in vitro* assay designed to determine eligibility for MyPeak-1, wherein cells exposed to a custom AAV9 vector containing a gene that encodes a protein that emits a luminescent signal upon entering a cell (BioAgilytix, Durham, NC, USA)
- The custom vector is incubated with participant serum and the assay cells. Pre-existing AAV9 NAb in participant serum will inhibit the custom vector from cellular transduction, thereby reducing the luminescent signal from the assay (**Figure 2**)
- AAV9 NAb titer is determined through serial dilution of participant serum. Participants with titers ≤1:10 were classified as having low pre-existing AAV9 NAb titers and seronegative; those with higher titers were classified as seropositive. This classification is informed by TN-201 preclinical studies and more conservative than most other AAV gene therapy clinical trials
- The AAV9 total antibody (TAb) assay is an in vitro assay wherein AAV9 capsids are coated onto a plate and exposed to participant serum. Anti-AAV9-specific antibodies are then detected by a chemically tagged AAV9 capsid that emits a luminescent signal upon binding to the anti-AAV9 antibody (**Figure 3**)
- AAV9 TAb titer was assessed to compare with AAV9 NAb titers to evaluate the concordance between the two assays

Figure 2. AAV9 Neutralizing Antibody Assay Design[1]



The firefly luciferase (FLuc) gene is placed in the AAV9 vector and exposed to cells along with patient's serum. If low to no NAbs are present in the serum, AAV9 can enter the cell and result in expression of the FLuc gene, emitting a luminescent signal. High NAbs in serum will bind to the AAV9 and inhibit the entry of AAV9 into the cell, thereby resulting in no expression of FLuc and no luminescent signal.

Figure 3. AAV9 Total Antibody Assay Design[1]



To assay total antibody to AAV9 vector, the surface of a plate is coated with AAV9 vector and exposed to patient's serum. Anti-AAV9 antibody will bind to the vector bound on the surface of the plate. Next, the plate is exposed with a chemically tagged AAV9 vector that emits a luminescent signal once bound to the anti-AAV9 antibody.

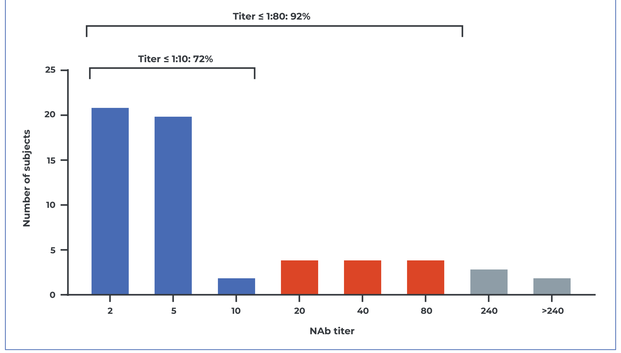
Evaluation of Participants Demographic and Medical History Characteristics

- Participants' age, sex, and race are collected as part of demographic data. Geographic location is assigned according to the location of the clinical trial site
- Medical history is collected as an open, free text field. Participants with medical history of surgery, autoimmune disease (e.g., lupus), or endocrine disorders (e.g., diabetes) are identified to explore whether pre-existing conditions might influence seronegativity
- Though exploratory, the study sample size is large enough to detect clinical or demographic differences between seropositive and seronegative participants
- Comparisons of demographic and medical history characteristics between seronegative and seropositive participant populations were done using unpaired t-tests for continuous data and Fisher's exact test for categorical data using R statistical software (R 4.1.0)

RESULTS

- As of August 2023, 76 participants enrolled in this seroprevalence study across 10 clinics and 60 participants had their sera tested for AAV9 NAb and TAb titers
- Interim analyses indicate that 43 of 60 (72%) *MYBPC3*-associated HCM participants had AAV9 NAb titers ≤1:10, the current threshold required, among other medical eligibility criteria, to be eligible to enroll into MyPeak-1, the TN-201 Phase 1b. This estimate is consistent with other studies of AAV9 seroprevalence[4–6] (**Figure 4**)
- Moreover, 55 of 60 (92%) had titers ≤1:80, the maximum allowable AAV9 NAb titer threshold that could be adopted in MyPeak-1 and roughly the median titer threshold used in other gene therapy trials.[3]. The vast majority of patients may be eligible for TN-201 trials, including MyPeak-1, if and when AAV9 NAb titer threshold is relaxed from ≤1:10 to ≤1:80

Figure 4. Histogram of Participant Count by Pre-Existing AAV9 NAb Titer



- Comparisons between AAV9 seronegative and seropositive participants suggest age, sex, race, geography, or medical history are not strongly associated with serostatus (Table 1)

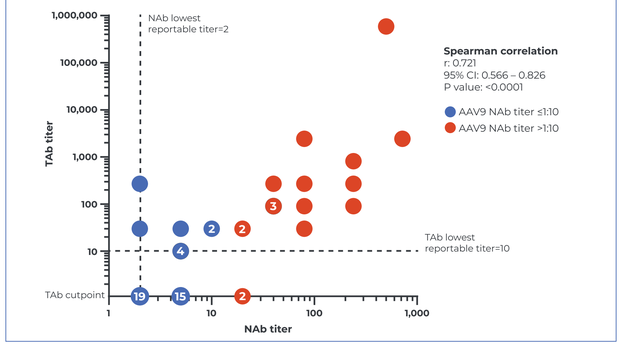
Table 1. Comparison of Demographic and Medical History Characteristics Between AAV9 NAb Seronegative and Seropositive *MYBPC3*-Associated HCM Participants

Characteristics	AAV9 NAb Seronegative (n=43)	AAV9 NAb Seropositive (n=17)	p-value
Age, mean years (SD)	42.8 (14.0)	48.8 (12.6)	0.13
Sex, Female, n (%) (n=20)	17 (85.0)	3 (15.0)	0.14
Race, n (%)			0.99
White (n=55)	39 (70.9)	16 (29.1)	
Black/African American (n=3)	2 (66.7)	1 (33.3)	
Asian (n=1)	1 (100.0)	0 (0.0)	
Not Reported (n=1)	1 (100.0)	0 (0.0)	
Geography, n (%)			0.42
Northeast (n=15)	13 (86.7)	2 (13.3)	
South (n=3)	2 (66.7)	1 (33.3)	
Midwest (n=34)	22 (64.7)	12 (35.3)	
West (n=8)	6 (75.0)	2 (25.0)	
Medical History, n (%)			
Surgery (n=28)	22 (78.6)	6 (21.4)	0.40
Autoimmune disorder (n=6)	6 (100.0)	0 (0.0)	0.17
Endocrine disorder (n=7)	4 (57.1)	3 (42.9)	0.39

RESULTS (continued)

- Pre-existing AAV9 NAb titer was strongly correlated with pre-existing AAV9 TAb titer (Spearman correlation coefficient = 0.721) and confirms that the AAV9 NAb titer is an appropriate surrogate for detection of high total antibody titer (**Figure 5**)

Figure 5. Key Patient Eligibility Criteria



CONCLUSIONS

- Results suggest that *MYBPC3*-associated HCM patients have low levels of pre-existing immunity to AAV9 and that the majority of patients could be eligible for TN-201 in clinical trials
- Though the study is exploratory, comparisons suggest that key patient characteristics, such as age and medical histories, do not appear to be significantly associated with serostatus
- AAV9 TAb titer was strongly correlated with AAV9 NAb titer, suggesting that the faster, less resource-intensive AAV9 TAb assay could be used for assessment of eligibility for future TN-201 studies. The association of pre-existing NAb and TAb titer requires further investigation and will be studied in TN-201 clinical trials
- Tenaya intends to expand this study to evaluate AAV9 seroprevalence in different patient populations, including in pediatric and international patients, through clinical development of TN-201
- Potential eligibility for TN-201 can be assessed by performing AAV9 NAb testing via participation in this seroprevalence study. Additionally, MyPeak-1, a Phase 1b interventional trial of TN-201 (clinicaltrials.gov: NCT05836259), is open and enrolling participants. For more information, including indicating interest in participating in these studies, email clinicaltrials@tenayathera.com

REFERENCES

1. Novartis, Q4 2022 Results
2. Mendell J., et al., *Mol Ther Methods Clin Dev*, 2022
3. Shen, W., et al., *Front Immunol*, 2022
4. Day, J.W., et al., *Mol Ther Methods Clin Dev*, 2023
5. Verma, S., et al., *Hum Gene Ther*, 2023
6. Rasko, J., et al., *Blood*, 2022

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