Development of Rational Formulation for the Delivery of AAV Viral Vector for Treatment of Heart Disease

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Abstract

Adeno-associated virus (AAV) vectors have emerged as the leading platform for delivering gene therapy to the heart to treat genetic diseases. However, due to challenges associated with manufacturing scale up and long-term storage, AAV can be prone to degradative, aggregative and adsorptive loss which can negatively impact the purity, potency and quality of the final drug product. Additionally, conventional drug product formulation excipients, such as phosphate and acetate, can have negative effects on heart function, which limit the options for excipient selection.

Using rational design, Tenaya Therapeutics, Inc. has developed a robust formulation for AAV that is stable under different storage temperatures and stress conditions. The formulation is photostable and supports in-use conditions including multiple freeze-thaw cycles and is compatible with devices for both intravenous and local delivery via cardiac catheters. It is expected that this novel formulation will greatly enhance clinicians' ability to deliver AAV viral vector drug products to patients suffering from genetic heart diseases.

Genetic Medicines Manufacturing Center

48K
square foot facility with ~50K square feet for expansion

~45
FTE in-house team conducting Process Development, Analytical Development, Quality Control

Non-GMP thru cGMP Productivity

- IP and know-how to enable scale from starting materials to large (> 5000L) bioreactors
- Maintenance of high potency from small to large volumes
- Consistently high purity vector production

Analytical and Assay Development

- Robust internal development of assay to support DS, DP release
- FDA supports Tenaya CMC strategies (Type B meeting 2021)

Ongoing Optimization Efforts

- Development and validation of proprietary technologies to increase yield in Sf9 and HEK293 systems

Background

Developing a suitable formulation for the delivery of AAV gene therapy to cardiac tissue presents unique challenges as most excipients commonly used in systemic delivery, when delivered locally, can cause electric imbalance to cardiomyocytes and potentially result in serious adverse events (SAEs). To select an appropriate buffer for the unique demands of delivery to the heart, it must support the manufacturing process, remain stable for short- and long-term storage, and in-use in a clinical setting. Aggregation, Vector Genome (Vg) /Capsid titer (Cp) loss, photostability, thermal stability and infectivity and/or *in vitro* expression are key data points to consider when testing different stress conditions on the drug product.

Excipient	Systematic Delivery	Local Delivery	Excipient	Systematic Delivery	Local Delivery
Magnesium stearate	✓✓		Sodium lactate	✓✓	✓
Potassium Chloride	✓✓	x	Potassium Phosphate	✓✓✓	✓
Calcium Chloride	✓✓	x	Sodium Acetate	✓✓✓	✓
Sodium Gluconate	✓✓	x	Sodium Chloride	✓✓	✓
Sodium Bicarbonate	✓✓		Magnesium Sulfate	✓✓	✓

Thermal Stability

The thermal stability of capsid two was tested in three formulation buffers with varying amounts surfactant and anticoagulant. The Melting Temperature (Tm) of capsid two remained consistent for all the conditions tested.

1:

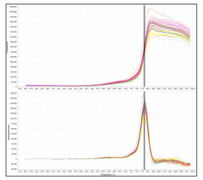


Image 1: All the conditions assessed were comparable with the melting point at ~78 +/- 1.0ºC for both Boltzmann and first derivative fit of the data

2:

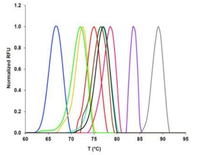
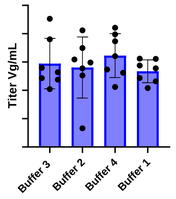


Image 2: Sample Evaluation and Stability of Full rAAV1–rAAV9-gfp and rAAVrh.10-gfp, Packaging the green fluorescent protein (GFP), Vectors in PBS from (Bennett, et, al, Molecular Therapy: Methods & Clinical Development, 2017).

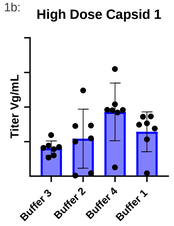
Local Delivery

To ensure that the drug product is stable in the selected formulation buffer through dosing, the proposed injection equipment and procedure was tested for both a systemic and local delivery method. Testing of Vg titer for absorptive loss, size exclusion chromatography (SEC) for aggregation, and *in vitro* potency for the affect of the dosing system on material was performed. These data points were compared to the total Vg delivered through the system over the course of the theoretical injection.

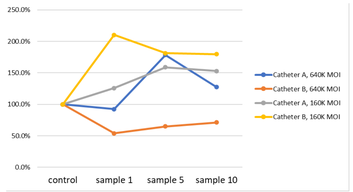
1a: **Low Dose Capsid 1**



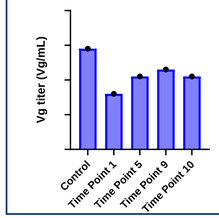
1b: **High Dose Capsid 1**



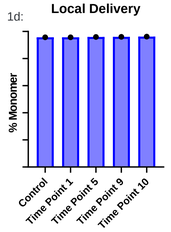
1e: **Local Delivery mRNA expression percent of control**



1c: **Local Delivery**



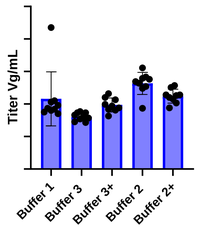
1d: **Local Delivery**



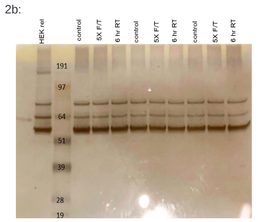
Graph 1a and 1b display the Vg titer changes under different storage and freeze thaws for at two doses. The change in Vg titer across a mock local delivery is shown in graph 1c. The SEC percent monomer is tested for the local delivery in graph 1d. mRNA expression was tested and normalized to the control in graph 1e.

Systemic Delivery

2a: **Vg Stability Capsid 2**

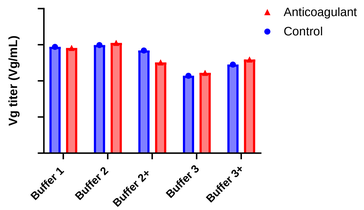


2b:

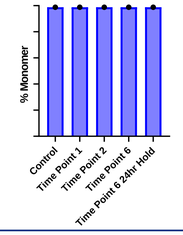


The image above is the SDS-PAGE of three buffers after the longest stress conditions.

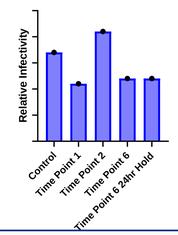
2c: **Addition of anticoagulant**



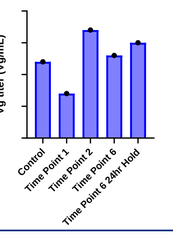
2d: **Systemic Delivery**



2e: **Systemic Delivery**



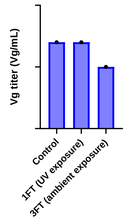
2f: **Systemic Delivery**



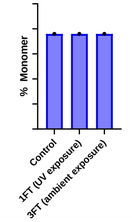
Capsid 2 Vg stability was tested for three buffers and multiple hold conditions graph 2a. Vg titer 2b. Capsid degradation by SDS-PAGE, 2c. Effects of Anticoagulant; Additional data of capsid 2 with the addition of anticoagulant was tested for Vg titer 2d-2f. The systematic delivery device was tested at varying points along the injection.

Photostability

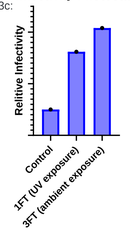
3a: **Vg Photostability**



3b: **SEC Photostability**



3c: **Infectivity Photostability**



It is important that material is tested after it is exposed to multiple freeze thaws or to excess light. All the conditions tested were evaluated for decrease in Vg titer (3a), increased aggregation(3b), and *in vitro* potency(3c).

Results

Four buffers were evaluated at different concentrations for two capsids. Vg and capsid degradation, thermal stability, and aggregation were used to select a formulation buffer suitable for additional testing across the platform. Capsid 1 Vg titer by qPCR showed a significant decrease during the freeze thaws in buffer 2 at both concentrations. Buffer 1 and buffer 4 for capsid 1 showed a decrease in titer by qPCR for single freeze thaws in the high concentration group. Aggregation was not observed in any of the conditions for either capsid 1 or 2. Thermal stability for the capsid was not changed based on the formulation buffer and remained within 1C of the literature value. Anticoagulant or surfactant concentration added to the formulation buffer did not affect the stability of both capsids.

Conclusion

Buffer 1 and buffer 2 both contained components considered non ideal (phosphate and acetate respectively) for heart function. Buffer 4 did not have comparable stability to buffer 3 after initial screening. Buffer 3 was identified as the ideal formulation for use in the manufacture of Tenaya's gene therapies based on lack of aggregation, Vg and capsid stability. This buffer also contained no excipients known to impact heart function. After determining that buffer 3 was the ideal formulation, additional compatibility tests were performed that were specific to stress, storage and dosing conditions. Photostability and device compatibility did not show any negative impact to the product. Data supports buffer 3 as the best formulation for the Tenaya Therapeutics platform and for future products.