Development, Optimization, and Qualification of a Comprehensive Analytical Panel for AFTX-201, an Investigational AAV Gene Therapy for BAG3-Associated Dilated Cardiomyopathy

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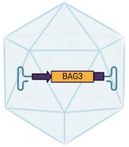
Introduction

AFTX-201 is an investigational rAAV gene therapy designed to restore BAG3 function in patients with BAG3-associated dilated cardiomyopathy. The therapy utilizes an engineered cardiotropic capsid (ATC-0187) to enable efficient myocardial transduction at reduced doses and deliver a fully human, full-length BAG3 transgene. To support GMP manufacturing and clinical evaluation in the UPBEAT trial, a robust, product-specific analytical strategy was developed to ensure consistent product quality and phase-appropriate regulatory readiness.

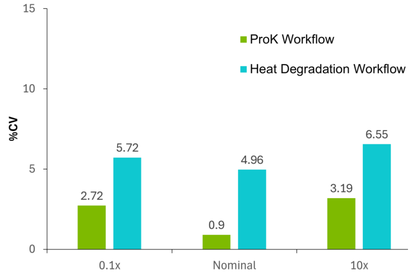


ATC-0187 Capsid
(Engineered AAV9)
+
Muscle-Specific Promoter
+
BAG3 cDNA

Droplet Digital PCR (ddPCR)

Fig 3 – ddPCR determination for ATC-0187

For ddPCR-based vector genome titer determination of the engineered cardiotropic AAV capsid ATC-0187, a proteinase K (ProK) treatment was evaluated and compared to heat-based capsid disruption. ProK treatment resulted in improved assay precision, dilution linearity, and run-to-run reproducibility, with increased genome recovery observed across inputs. These improvements are consistent with enhanced capsid disruption and the high full-capsid content enabled by the optimized plasmid design. Collectively, these optimizations supported successful qualification of the ddPCR assay with high precision and accuracy for GMP lot testing.



AFTX-201 Qualification Preliminary Runs		
vg/mL	Average	%CV
1.03E+14	1.04E+14	6.7%
1.11E+14		
9.72E+13		

Accuracy	vg/mL	%RE	Intra-assay precision (%CV)
0.1-fold nominal	1.07E+13	2.7	5.3
0.2-fold nominal	2.10E+13	1.5	3.6
Nominal	1.09E+14	4.8	3.5
Nominal	1.20E+14	15.9	2
5-fold nominal	5.08E+14	2	3.1
10-fold nominal	1.09E+15	5.3	3.6
Inter-assay precision	1.09E+14		5.80%
R2 (Linearity)		1.00	

Relative Potency & Infectious Titer ($TCID_{50}$)

Fig 1 – Relative Potency Assay

A cell-based relative potency assay was developed early in the product life cycle to quantify BAG3 protein expression in support of process development.

For the relative potency assay, transducibility across multiple cell lines, transduction duration, seeding density, and the use of different cell bank lots were evaluated to identify conditions that produced a consistent dose–response relationship.

These optimizations resulted in improved assay sensitivity and inter-assay consistency.

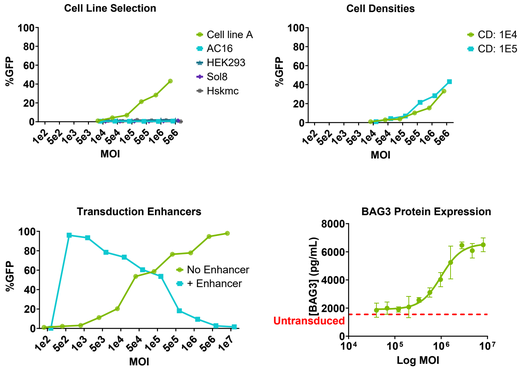
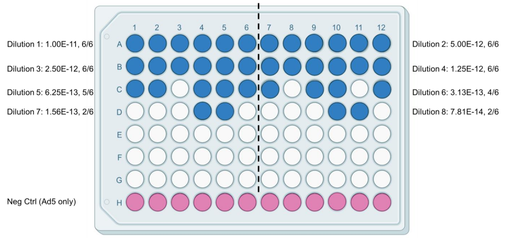


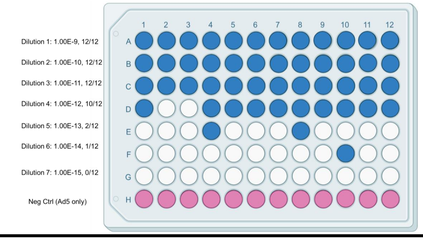
Fig 2 – $TCID_{50}$ Assay Optimization

Infectious titer is typically determined by a $TCID_{50}$ assay using qPCR with 10-fold dilutions. Alignment of ddPCR-based genome titration with a two-fold dilution scheme for the $TCID_{50}$ assay generated reliable measurements while yielding comparable infectivity estimates to the log dilution (4.00E+13 $TCID_{50}$/mL for 2-fold dilution vs 3.83E+13 $TCID_{50}$/mL for log dilution with 3%CV).

1:2 dilution scheme



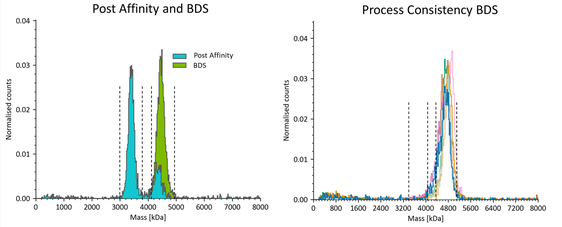
1:10 dilution scheme



Capsid Packaging Quality

Fig 4 – Mass Photometry Analysis

Capsid packaging quality during development was assessed using mass photometry, allowing direct differentiation and quantification of empty, full, and partially packaged AAV capsids generating comparable results to AUC.



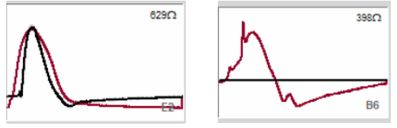
BDS			
Method	Empty	Partial	Full
Mass Photometry	1.9%	2.0%	96.1%
AUC	1.0%	4.0%	95.0%

Mass photometry demonstrated consistent profiles across multiple lots.

Functional Potency Assays

Fig 5 – Functional Potency

A functional potency assay is under development. Cardiomyocyte contractility was evaluated as an exploratory functional readout. Impedance measurements demonstrate stable rhythmic activity in control iPSC-derived cardiomyocytes and reduced contractile function following BAG3 knockdown (day 5 post-transfection). These data highlight a biologically relevant in vitro phenotype associated with BAG3 loss-of-function.



Control | **BAG3 siRNA at day 5 post-transfection**

Baseline traces (**red**) overlayed with siRNA traces (**Black**).

Conclusions

- This analytical panel provides a high-resolution assessment of AAV product quality, strengthening lot-to-lot comparability and enabling informed decision-making across process development and clinical product manufacturing.
- This approach supports the advancement of AFTX-201 while demonstrating a scalable framework for analytical characterization of next-generation AAV gene therapies.
- The framework and assays can be readily leveraged for developing additional product candidates in cardiac or skeletal muscle indications using the ATC-0187 capsid.

References:

Portions of the data presented here were generated in collaboration with Forge Biologics and Ncardia.
Duong, Tam, et al., 2023, https://doi.org/10.1089/hum.2023.014

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