



Development of a Flexible High Yielding, High Performing Process for Manufacturing of AFTX-201, a Novel Investigational AAV Gene Therapy for Treatment of BAG3 Dilated Cardiomyopathy

2025 ASGCT

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BAG3 DCM is a devastating disease



Disease biology and unmet medical need

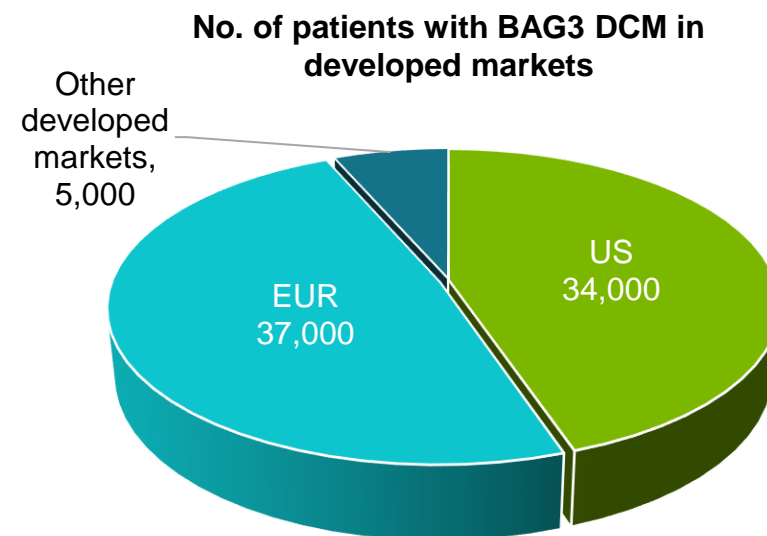
- Monogenic disease with haploinsufficiency
- Diagnosed at a mean age of 37 years
- 64% are in NYHA Class II – IV at presentation
- Current treatments for symptomatic improvement
- 22% with DCM require a heart transplant

A significant market for gene therapy

76K patients in developed markets

Increasing genotyping and diagnosis

Forecasted peak annual WW sales >\$2B²

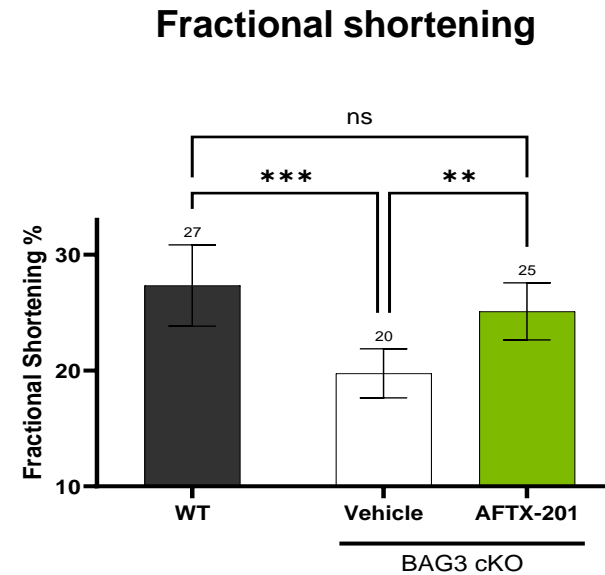
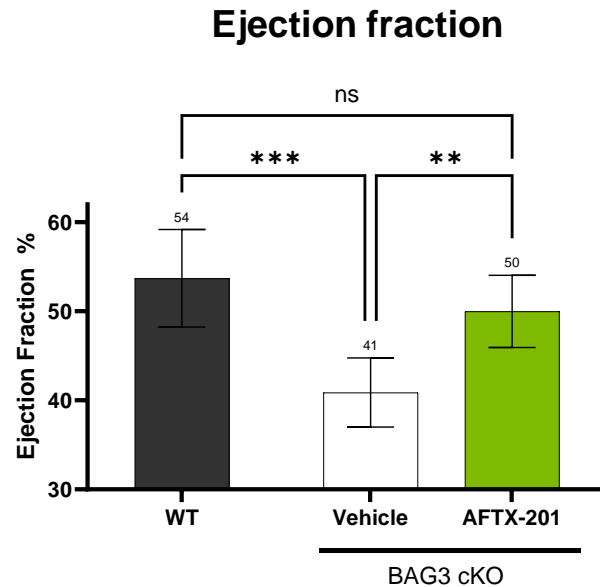


1) DeGroot, S. (2024, August 16). Facebook; accessed October 2, 2024.

2) Sentero Pharma market research & forecast

AFTX-201 reversed disease pathology in the BAG3 cKO mouse model: increased EF and improved dilated cardiomyopathy

BAG3 cKO mouse model closely mimics the structural, functional, and molecular defects observed in patients affected by BAG3 DCM



**Affinia solution:
AFTX-201**

- Restore BAG3 protein to normal level with a full-length, fully-human BAG3 transgene using Affinia's novel cardiotropic capsid
- Reverse disease pathology: increase contractility, reduce dilation, increase ejection fraction and exercise capacity



**BAG3
protein**



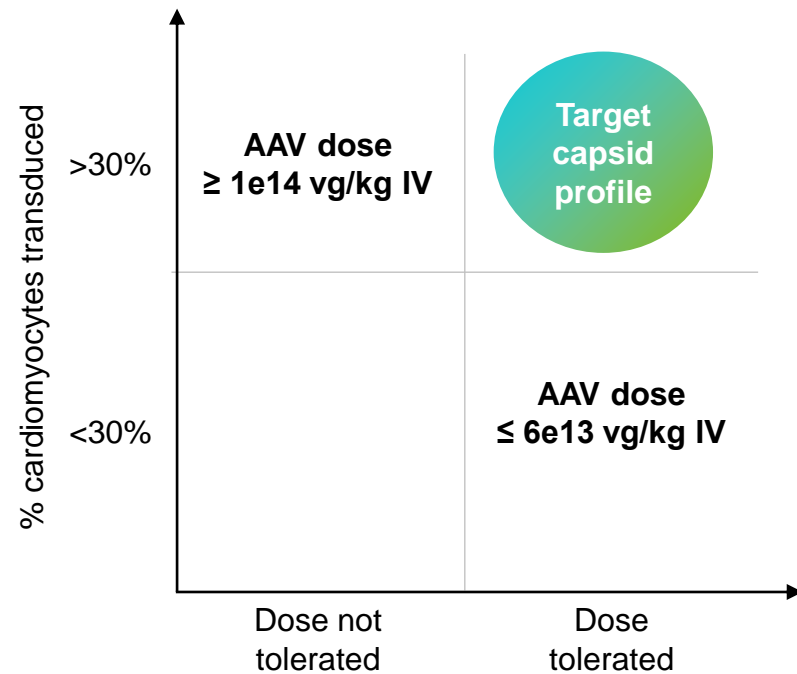
**Cardiac
contractility**



Dilated heart
Patient exercise capacity

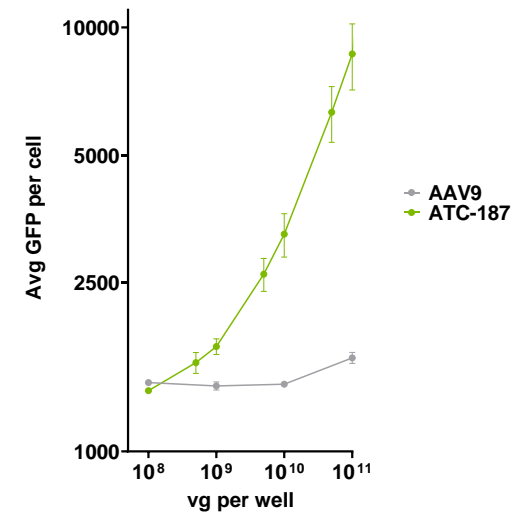
Affinia's novel cardiotropic capsids are engineered to address the limitations of conventional capsids in heart disease

In clinical trials, conventional capsids have been unable to transduce >30% cardiomyocyte at tolerated doses¹

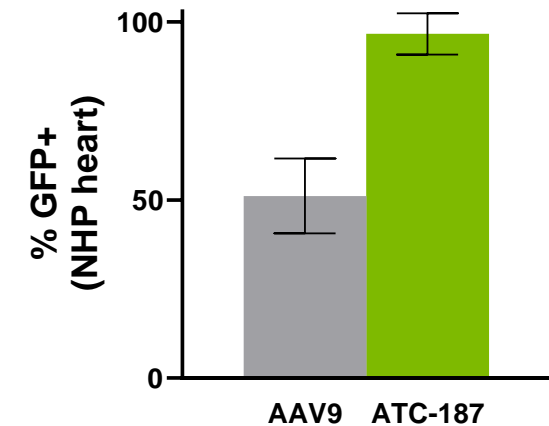


Affinia's novel cardiotropic capsids have demonstrated superiority vs. conventional capsids in NHP and in human cardiomyocytes

Head-to-head capsid performance in human cardiomyocytes



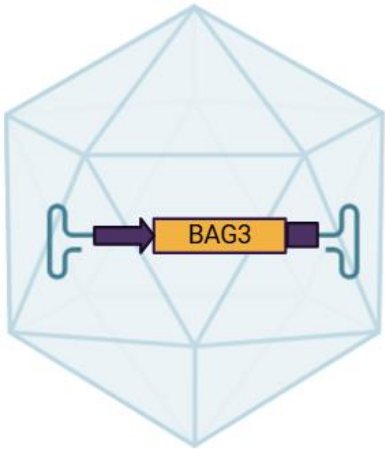
Head-to-head capsid performance in NHP heart at $3e13$ vg/kg IV



¹) Internal analysis based on public information from AAV9-based clinical programs
Left: PR-0025 cynos (n=3-4), CAG.GFP $3e13$ vg/kg IV, day 28; heart LV % cardiomyocytes GFP+
Right: iPSC derived cardiomyocytes, 96-well assay, 50K cells per well, 72h incubation, in duplicate

Process development for AFTX-201

AFTX-201

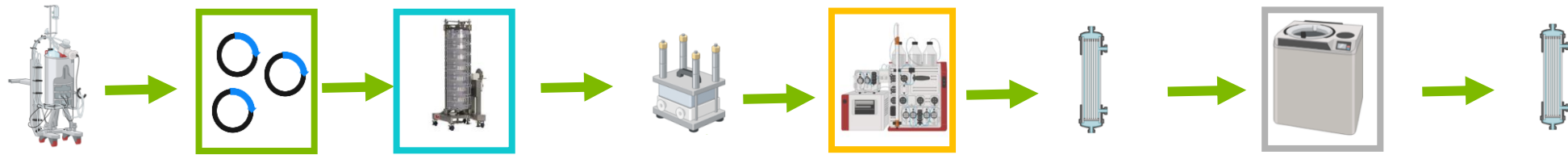


ATC-0187
AAV9 with peptide insertion

Goal was to develop a robust process for a **novel capsid** to support a Phase 1/2 clinical trial for BAG3 DCM

- High-Yielding
 - Conduct manufacturability assessment during capsid selection to ensure good productivity of novel capsid
 - Enable lower manufacturing costs
- High performing
 - Achieve low residuals and high % full capsids
- Stability
 - Conduct manufacturability assessment to ensure stability of novel capsids
- Flexibility
 - Demonstrate strong performance across multiple payloads and capsids

Process flow diagram for AFTX-201

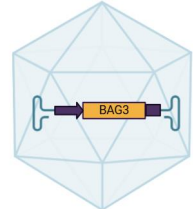


Utilize Affinia's novel plasmid design to increase yields

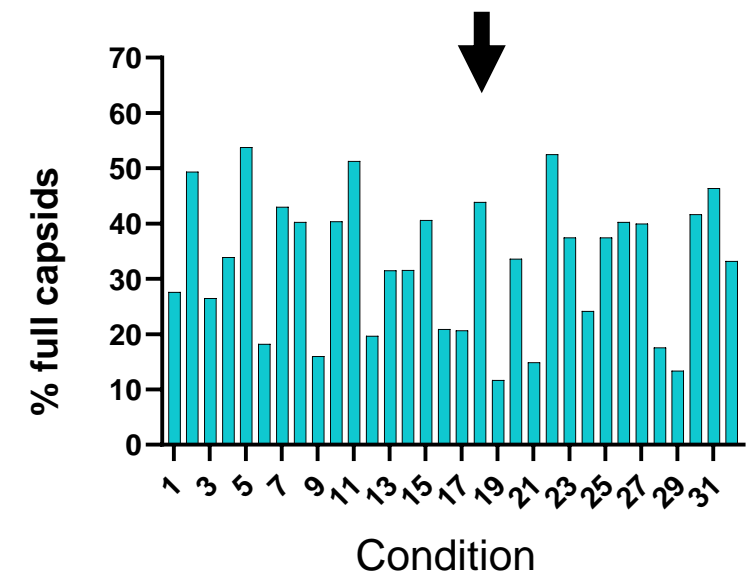
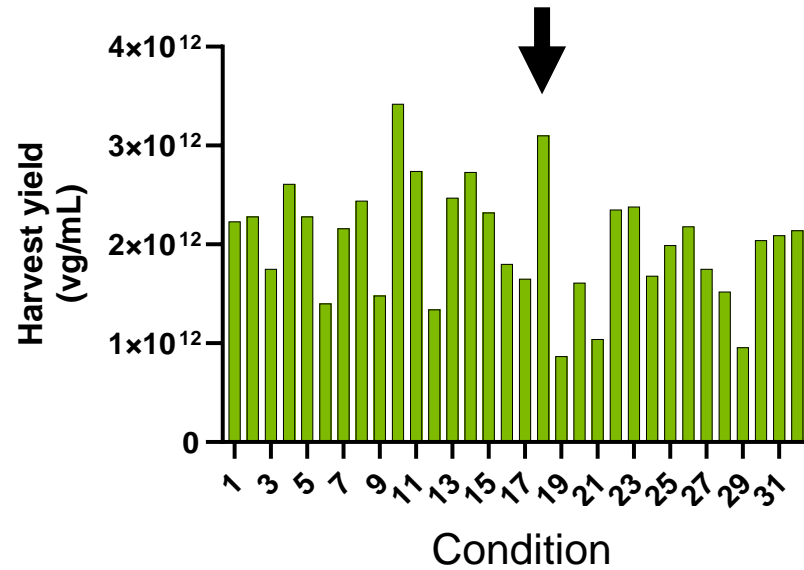
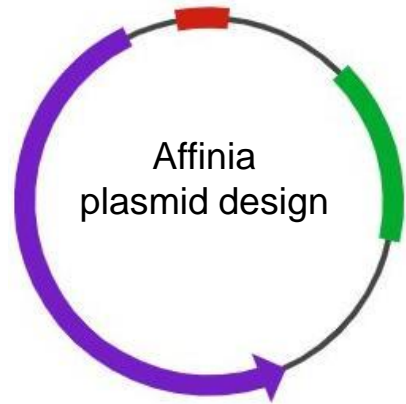
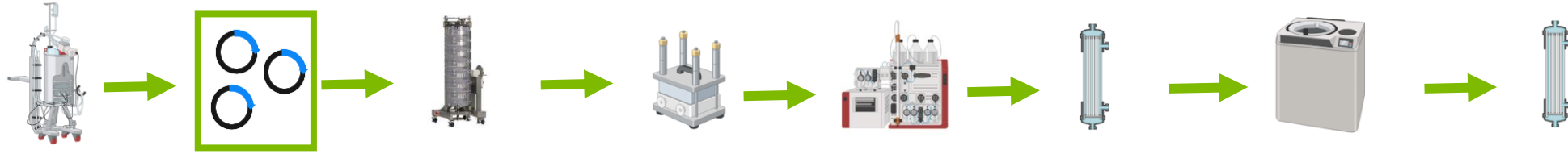
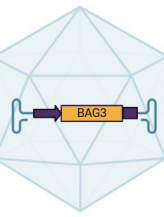
Explore multiple Affinity resins to ensure high performance

Explore new depth filters to increase capacity

Using CsCl gradient to ensure >90% full capsids

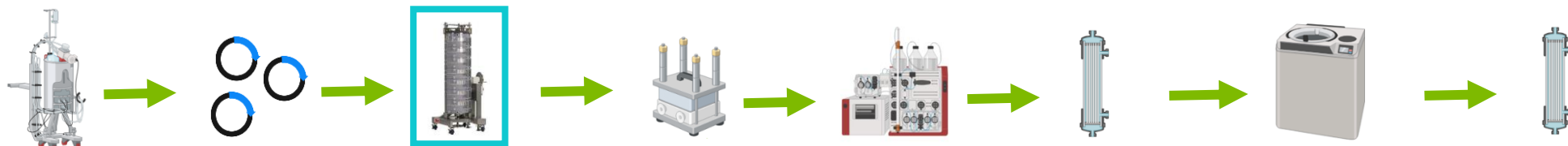
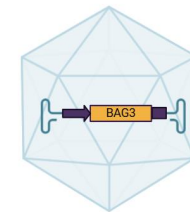


Use of Affinia plasmid design led to yields of $>3e15$ vg/L



We chose condition with highest yield to move forward since we were utilizing CsCl gradient for polishing

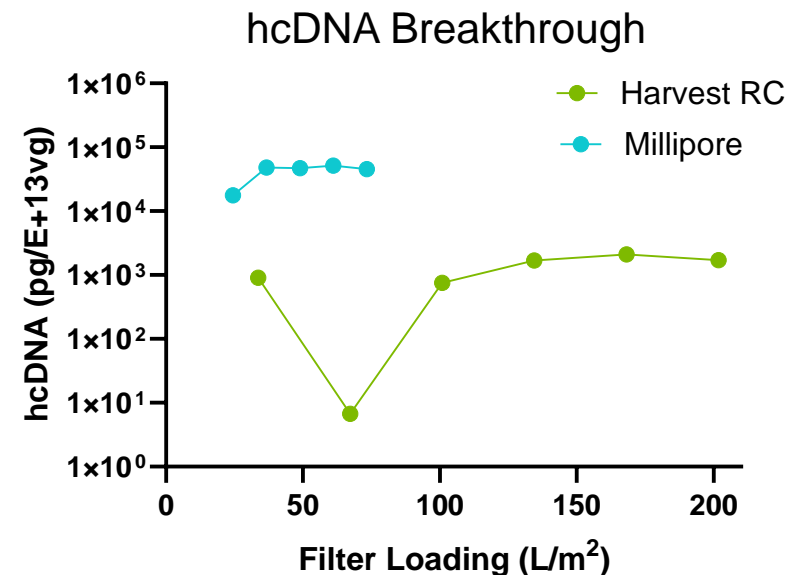
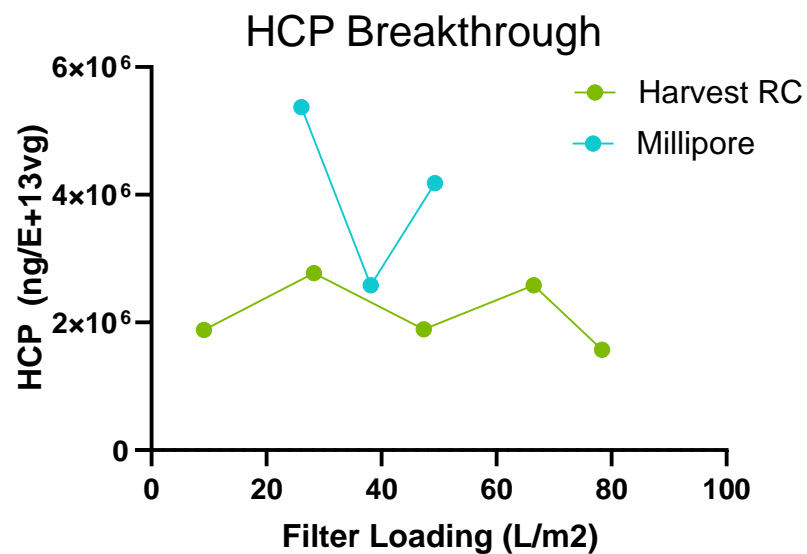
Harvest RC filters were optimal process choice



Harvest RC plus STIC



Millipore plus STIC

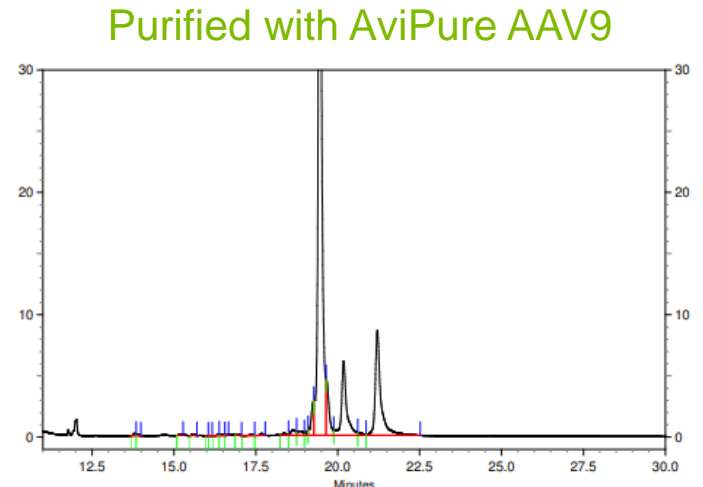
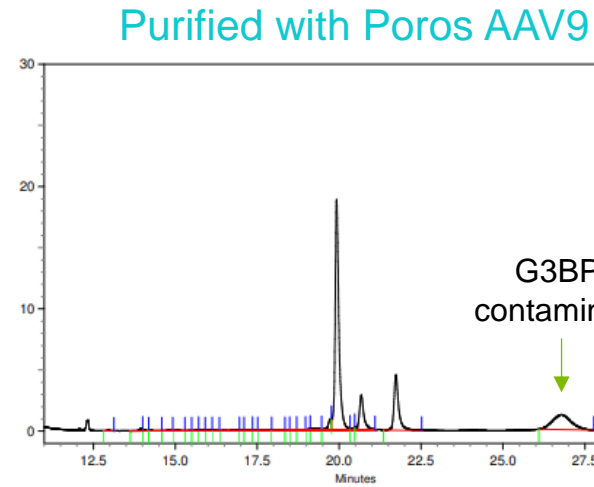
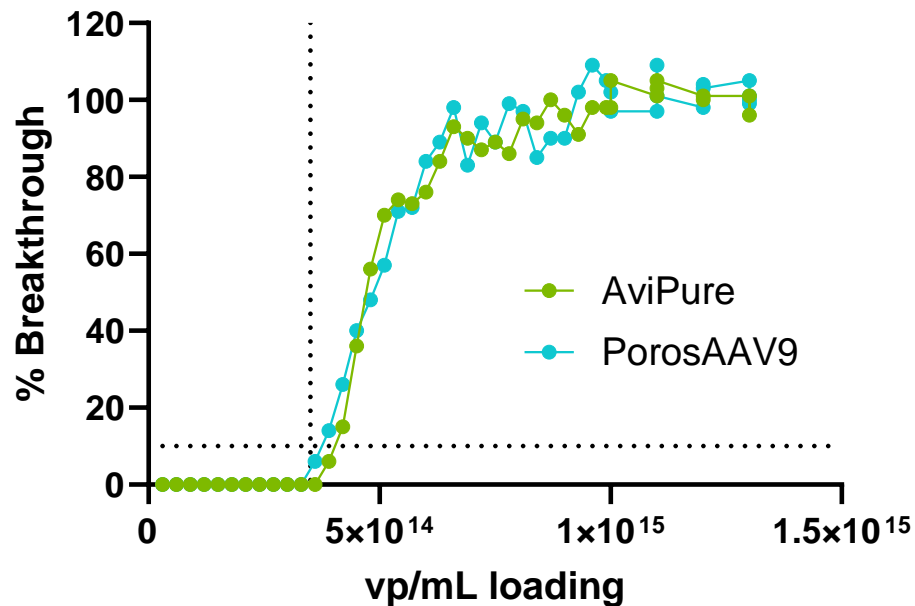
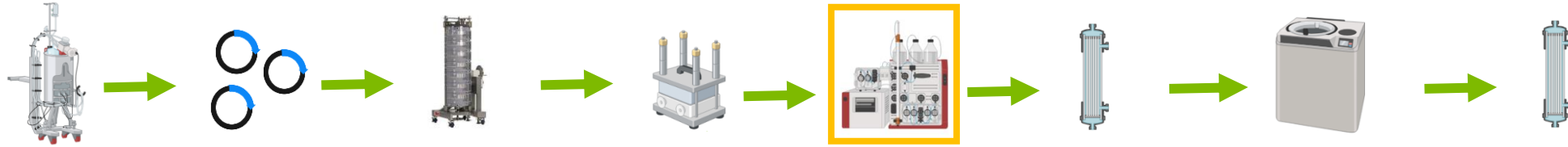
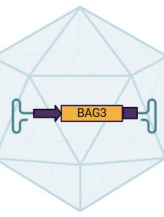


Note: Millipore filter was stopped due to rising pressure

Harvest RC filter better choice than Millipore DF

- Higher loading capacity
- Reduced HCP and hcDNA levels
- Cheaper at scale

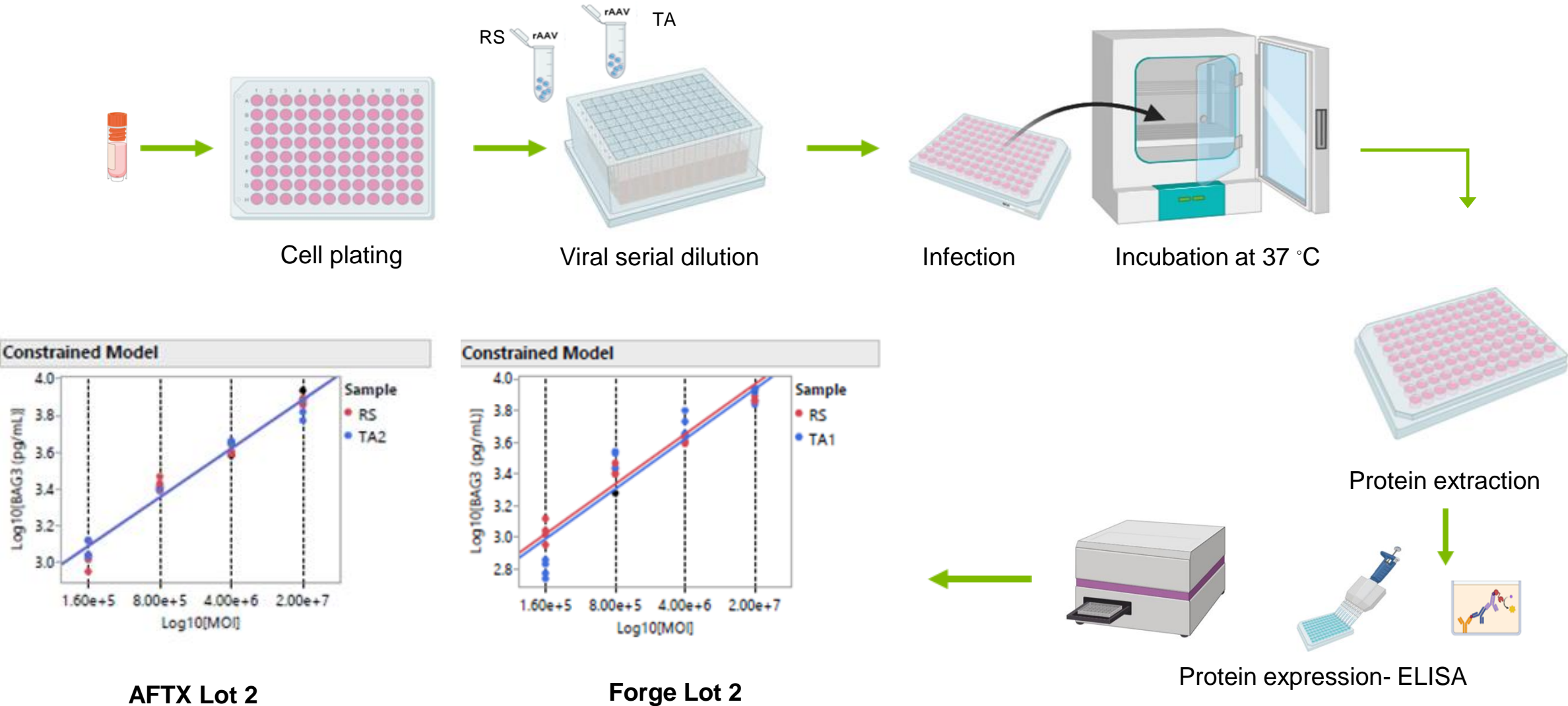
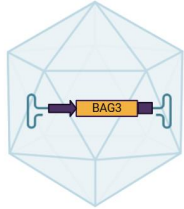
AviPure Affinity resin were the optimal process choice



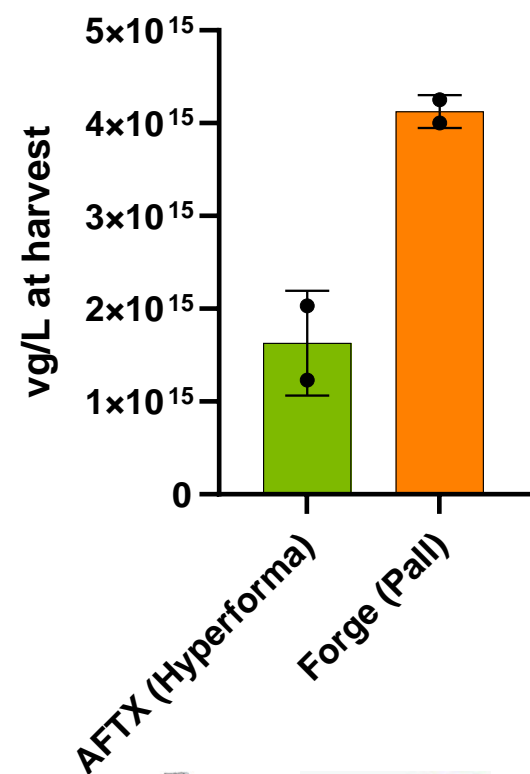
Avipure resin better choice than Poros AAV9

- Similar binding capacity
- Similar yield (>85%)
- Reduced G3BP contaminant
- Easily cleaned and reusable

Potency assay for AFTX-201 developed to help support process development



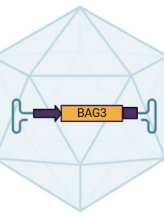
Process for AFTX-201 was successfully tech transferred to Forge Biologics



	AFTX Lot 1	AFTX Lot 2	Forge Lot 1	Forge Lot 2
hcDNA	5.17e4 pg / 1e13	Pending	9.13e3 pg / 1e13	2.9e4 pg / 1e13
HCP	BLOQ	BLOQ	BLOQ	BLOQ
% Full / Partial / Empty by AUC	95 / 4 / 1	94 / 4 / 2	95 / 3 / 1	96 / 2 / 2
Aggregation	99.3	97.6	90.73	96.56
rcAAV	BLOQ	BLOQ	BLOQ	BLOQ
Relative potency	ND	Passed	ND	Passed



Conclusions



- Developed a robust process for a novel capsid that achieves high yield and quality in support of Phase 1/2 clinical trials
- Attain yields of $>4e15$ vg/L at scale with the AFTX-201 process
- Ensure excellent critical quality attributes (CQAs)
- Reduce manufacturing costs



50L Bioreactor
~ $2e17$ total vg



Drug Product
~ $5e16$ total vg



~60 doses
 $8e14$ total vg
(80kg @ $1e13$ vg/kg)



~30K/dose

Acknowledgments



■ Process Science Team

- Esther Aribilola
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