Process Development, Technology Transfer, Clinical Manufacturing, and Process Characterization of AFTX-201, an Investigational New Medicine for the Treatment of BAG3-Associated Dilated Cardiomyopathy

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Disclosures

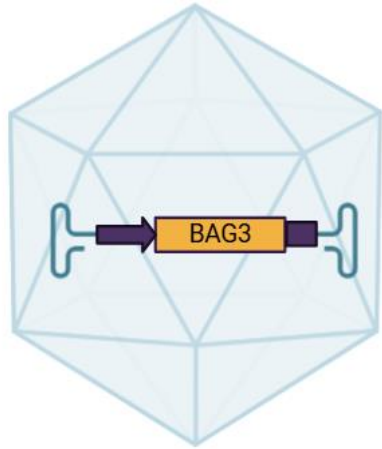
- I am an employee of Affinia Therapeutics

BAG3 DCM is a devastating disease

High unmet need

- ~76K patients in developed markets
- Present with heart failure phenotype
- Current treatments for symptoms only
- 22% require a heart transplant

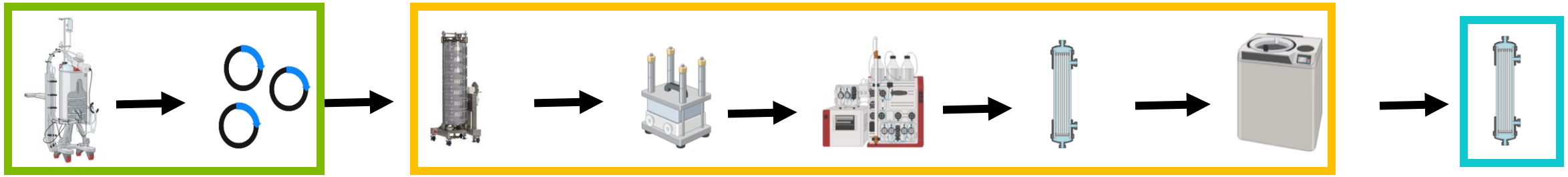
AFTX-201 has the potential to restore lost function in patients affected by BAG3 DCM



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

DeGroot, S. (2024, August 16). Facebook; accessed October 2, 2024.
Dominguez et al., JACC, 2018

Process flow diagram for AFTX-201



Use of Affinia's proprietary plasmid design resulted in high AAV yields

The purification process yielded a high-quality vector suitable for clinical use

The formulation for AFTX-201 resulted in product stability for at least 12 months

Harvest yields for AFTX-201 exceeding 6e15 vg/L are achievable using Affinia's plasmid system

	Tech Transfer Run (50L)	Demonstration Run (50L)	GMP Run (50L)
Harvest Yield (vg/L)	4e15	4.48e15	**6.07e15**
Total vg per 50L run (clarified lysate)	2e17	2.24e17	2.9e17
Total process yield	21.5%	24.7%	43%

High process yields and a low clinical dose enables ample manufacturing capacity at the 50L scale, making supply of thousands of doses readily achievable

We believe the manufacturing process is readily scalable to meet demand, if needed

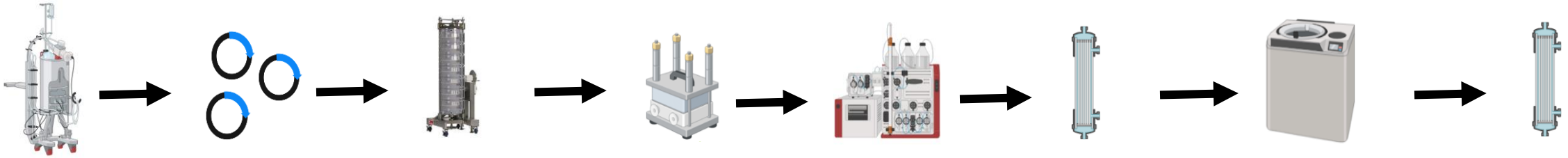
AFTX-201 process generates high-quality, clinical-ready vector

	Tech Transfer Run (50L)	Demonstration Run (50L)	GMP Run (50L)
Capsid Purity (% VP1/2/3)	98.3	100	100
hcDNA (ng per 1e13)	15.3	33	30
HCP (ng/mL)	BLOQ	BLOQ	BLOQ
Residual plasmid DNA (cp/mL)	4e11	5.75e11	3.04e11
Residual E1A DNA (cp/mL)	BLOQ	BLOQ	BLOQ
AUC (% Full / Partial / Empty)	95 / 3 / 1	95 / 2 / 2	96 / 2 / 2
Aggregation (% Monomer)	90.7	96.6	99.5
In vitro Potency (% Reference Standard)	187.9	86.0	92.3
Replication competent AAV	BLOQ	BLOQ	BLOQ

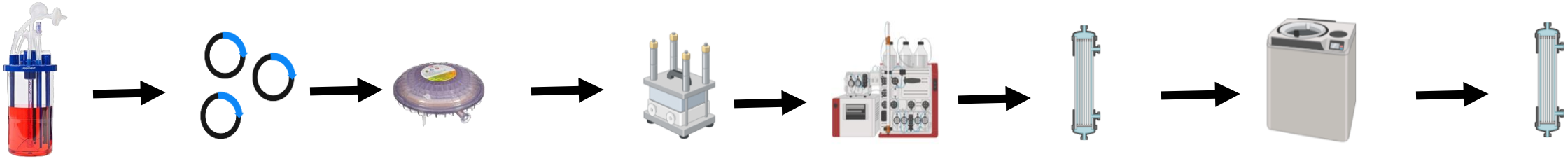
The AFTX-201 formulation has demonstrated stability through one year of storage at −80°C. Stability studies are ongoing, with an anticipated drug product shelf life of up to three years.

Establishing a representative scale-down model for the AFTX-201 process to support process validation

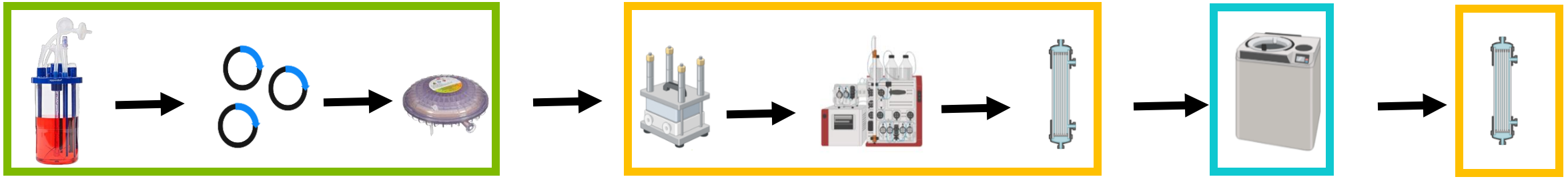
Clinical Process



Scale-Down Process



Establishing a late-stage representative scale-down model for the AFTX-201 process

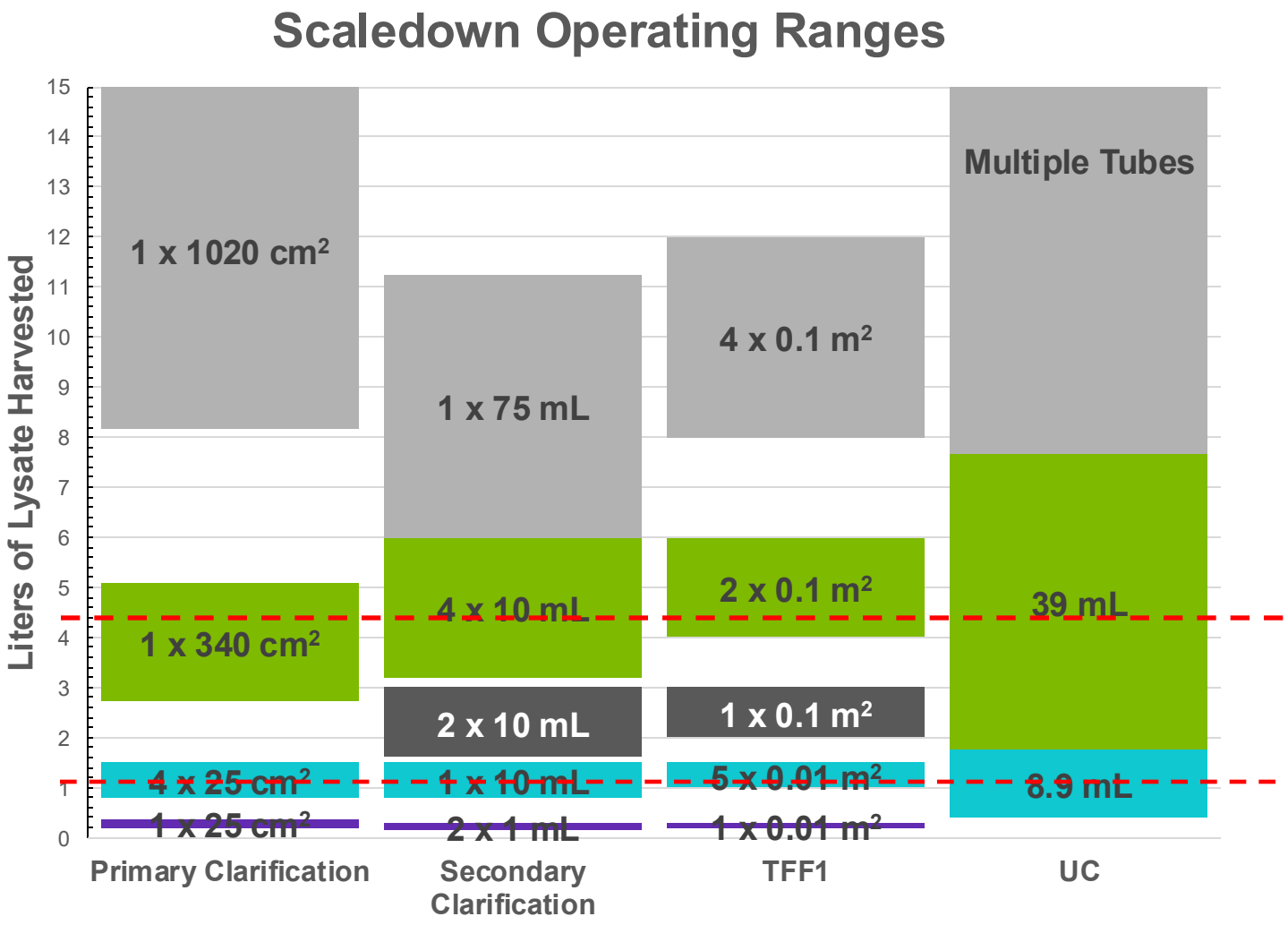


Identify a scale-down bioreactor model that replicates 50-L performance while remaining within midstream operating parameters

These processes can be scaled linearly

Develop a scale-down CsCl gradient model that reproduces manufacturing scale performance

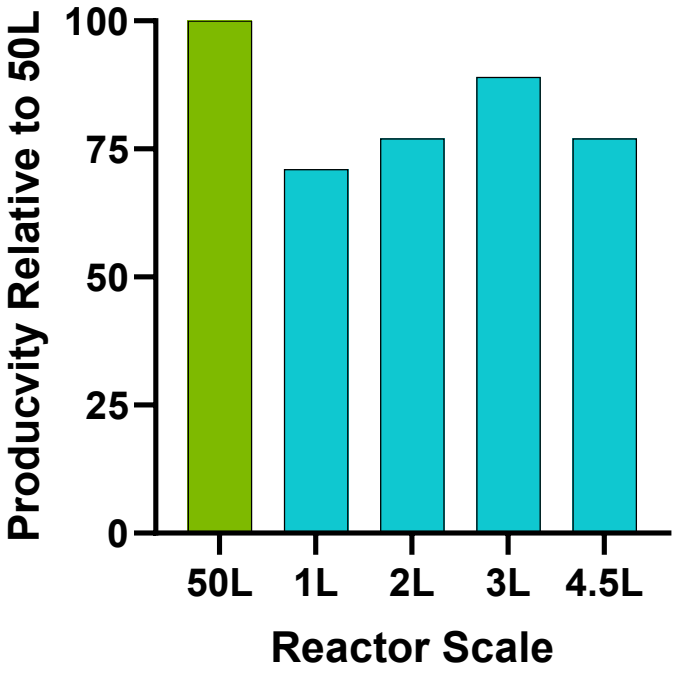
Clarification filter sizing is the primary driver of midstream and bioreactor scaling



4.4L Scale:
Provides material for testing multiple downstream conditions / more precise downstream parameter evaluation

1L Scale:
Allows efficient representative processing to DP of upstream experiments.

AFTX-201 upstream scales efficiently across reactor sizes



All the reactor scales performed similarly with a slight decrease observed compared to 50L scale

Continued development work is underway to increase small scale yields to match 50L scale

Critical parameters for a representative CsCl UC scale-down model

- To properly scale UC, the following factors must be considered:
  - TFF2 Load material concentration (analogous to tube loading)
  - K-factor (separation efficiency)
  - Spin time (must be adjusted if K-factors differ)
- Workflow
  - Identify smaller tubes (and a compatible rotor)
  - Calculate the r-min, r-max, and k-factor for the tube-rotor combination
  - Calculate the equivalent spin time to the at-scale process
  - Assess % full capsids after band pulling from each tube size

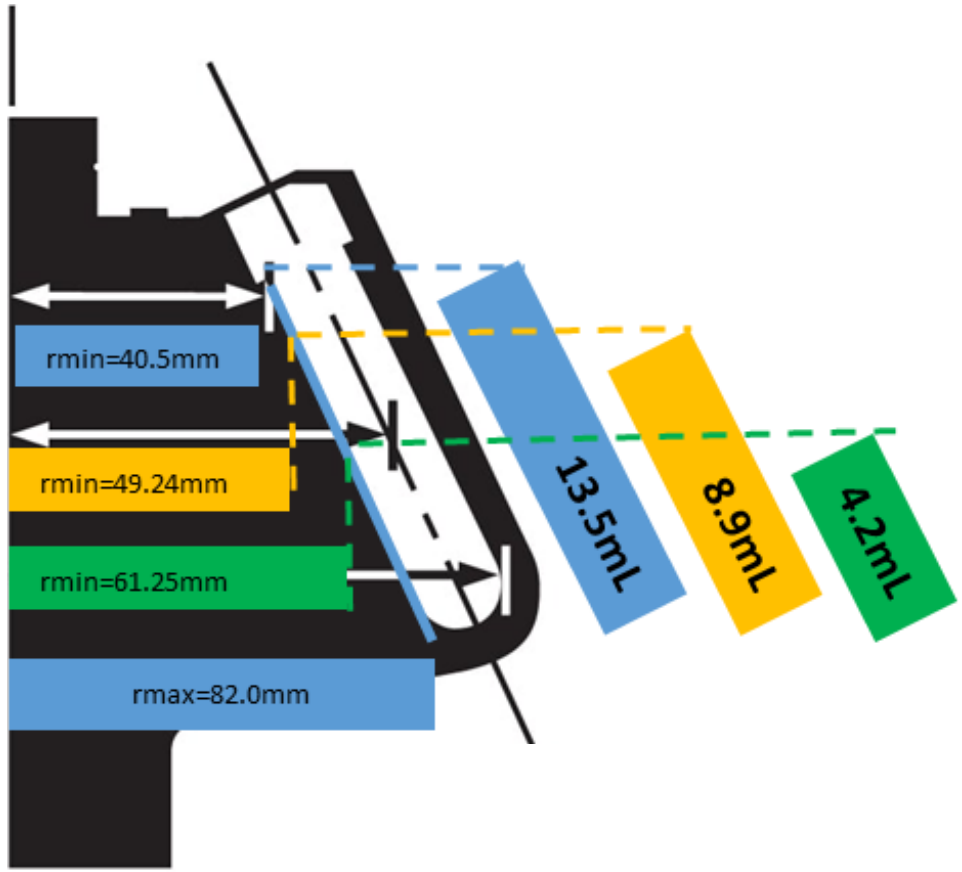
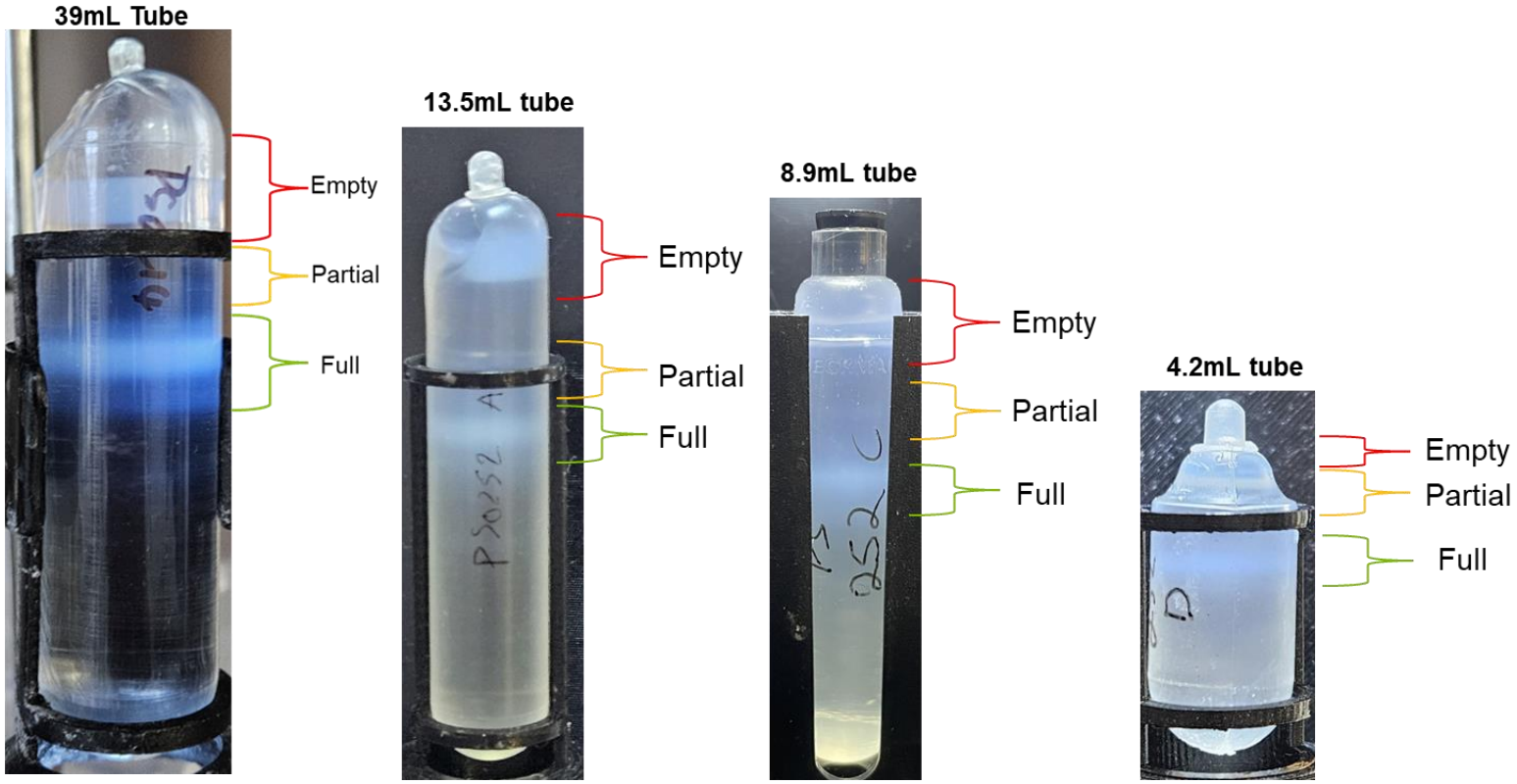
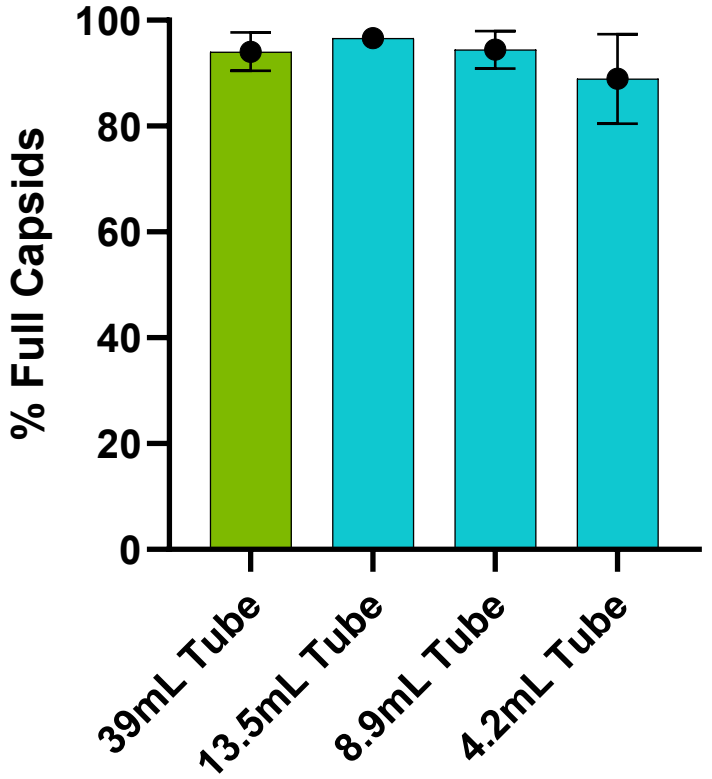


Image modified from Beckman Website

Equivalent banding pattern observed across tube sizes





Consistently pulling only the full band is more difficult in 4.2mL tube; so that size won't be used for scale-down purposes

AFTX-201 scale-down process generates similar quality vector to clinical process

	GMP Run	Scale Down 1	Scale Down 2	Scale Down 3
Capsid Purity (% VP1/2/3)	100	95.8	94.6	95.3
hcDNA (ng per 1e13)	30	18.4	33.1	41.1
HCP (ng/mL)	BLOQ	BLOQ	BLOQ	BLOQ
Residual plasmid DNA (cp/mL)	3.04e11	6.83e11	7.98e11	9.65e11
Mass Photometry (% Full / Partial / Empty / HMW)	96 / 2 / 2	95.3 / 3.2 / 2.6	94.5 / 1.5 / 4	93.6 / 3.1 / 3
Aggregation (% Monomer)	99.5	99.74	99.61	99.73
In vitro Potency (% Reference Standard)	92.3%	94.1	72.4	80.3

Conclusions

AFTX-201 clinical manufacturing

- A scalable, high-yield AAV manufacturing process was successfully developed and transferred to Forge Biologics for GMP production of AFTX-201
- First GMP run achieved exceptional harvest titers (> 6e15 vg/L), exceeding typical industry benchmarks
- The 50L scale can enable supply for thousands of patients, with scalability available as demand grows

Scale-down model development

- Representative scale-down models have been established for both bioreactor production and CsCl ultracentrifugation
- Bench-scale models show strong concordance with the 50L process for performance and key quality attributes
- These predictive models enable efficient process characterization and support continued clinical advancement

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