CASSS The Cell and Gene Therapy Products Symposium

JUNE 13TH, 2024

Balancing flexibility and standardization of AAV manufacturing processes

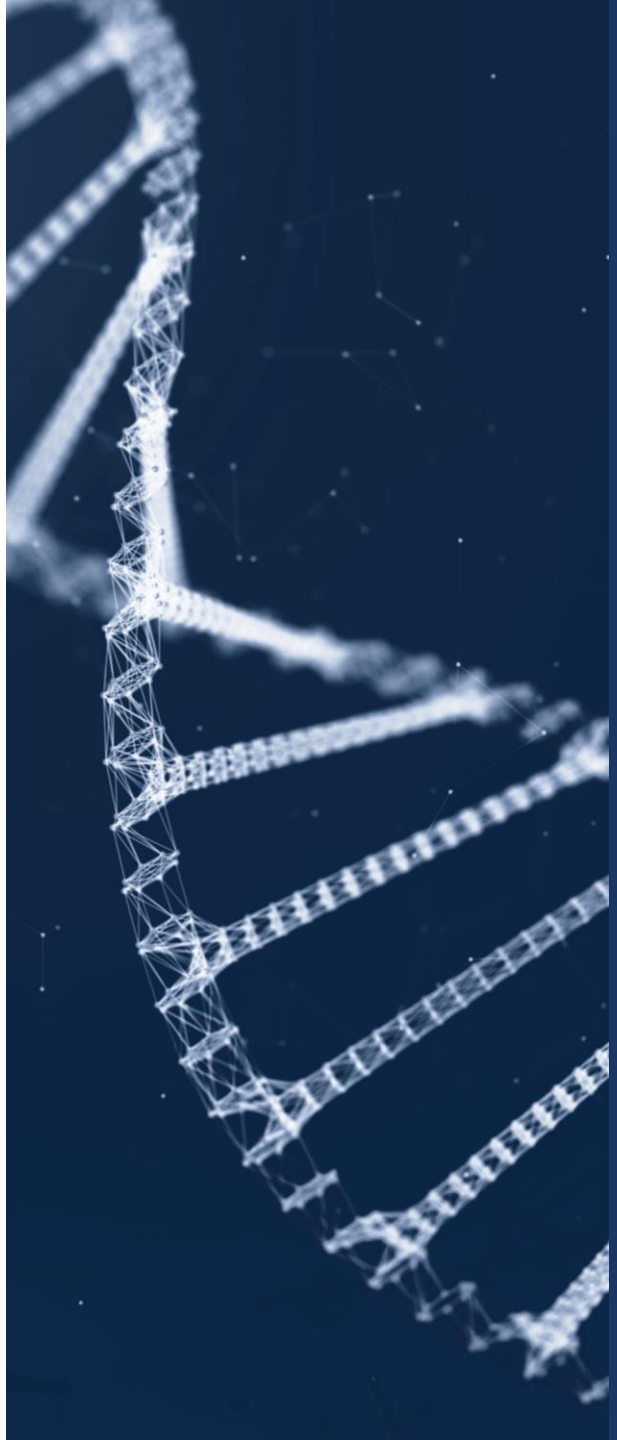
MERCEDES SEGURA GALLY

ELEVATE.BIO //BASECAMP

elevatebio®

Agenda

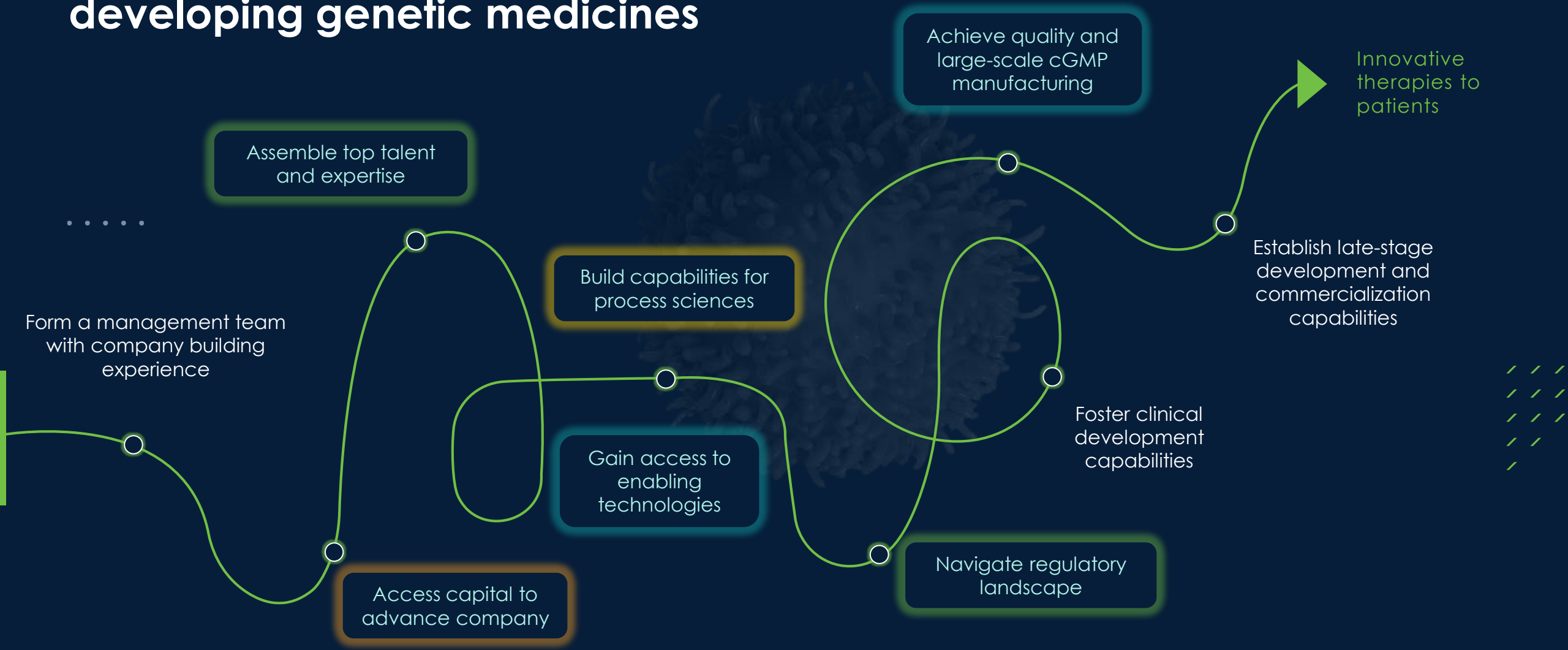
- Introduction to ElevateBio
- AAVs gene therapy landscape
- Unit Operation Standardization
  - Case study for Cell Lysis
- Unit Operation Optimization for new products
  - Case studies for USP Vector Genome Titer and Full capsid optimization
  - Case studies for DSP Vector Recovery and Full capsid optimization
- Final remarks



JUNE 13TH 2024

elevatebio®

Significant barriers to manufacturing and developing genetic medicines



Scaling the world's first integrated genetic medicine foundry to accelerate the design, development, and manufacturing of transformative therapies

life edit® an elevatebio company

elevatebio basecamp™

Innovative therapies to patients

Next-Gen Gene Editing

- BASE EDITORS
- RT EDITORS
- TYPE II/V NUCLEASES
- DIVERSE PAMS

Cell Engineering

- C-BASE EDITORS
- LENTIPEAK™ LENTIVIRAL
- CIRCRNA
- NON-VIRAL

Gene therapies and Delivery

- AAV
- LIPID NANOPARTICLE

Process Development

- PROCESS DESIGN
- OPTIMIZATION AND SCALE-UP
- PROCESS CHARACTERIZATION

Analytical Development and Quality

- ASSAY DEVELOPMENT
- QC RELEASE TESTING
- STABILITY
- QUALITY ASSURANCE AND VALIDATION

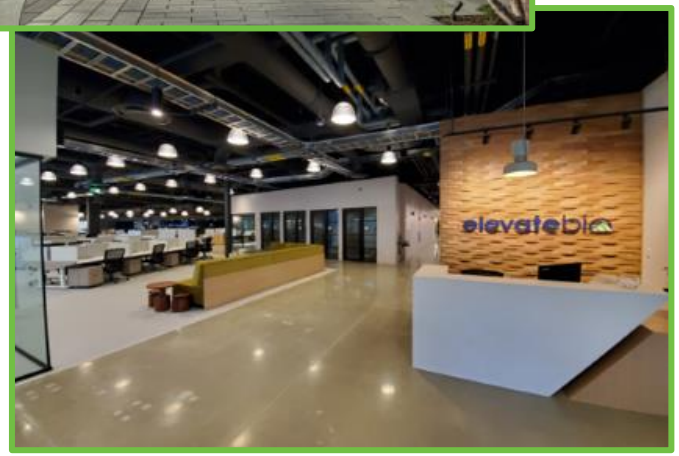
cGMP Manufacturing & Automation

- CELL THERAPIES (T CELL, B CELL, IPSC, HSC)
- VIRAL VECTORS (AAV, LENTIPEAK LVV)
- MRNA

BaseCamp Waltham is the center-of-innovation for pioneering the future of cell and gene therapies

140,000 SQUARE FEET					
R&D and QC Laboratories	cGMP Manufacturing	Offices	Utility Space	Warehouses	Support Spaces

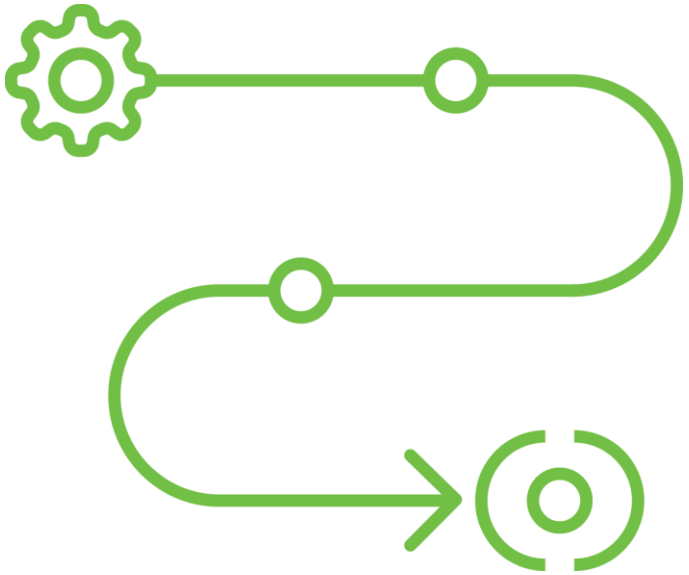
- **End-to-end capabilities** for process development and scaled manufacturing
- **Unmatched array** of viral vector and cell therapy capabilities, including design, construction, process characterization and validation
- **Quality assurance** and **quality control** laboratories with state-of-the art automation
- **Specialized, cell, gene and mRNA expertise** for clinical production and CMC regulatory
- **Commercial readiness** ongoing to support growing number of last stage clients



elevatebio basecamp

Full Spectrum Solution for Cell and Gene Therapy Manufacturing

PROCESS DEVELOPMENT



SCALE UP GMP MANUFACTURING

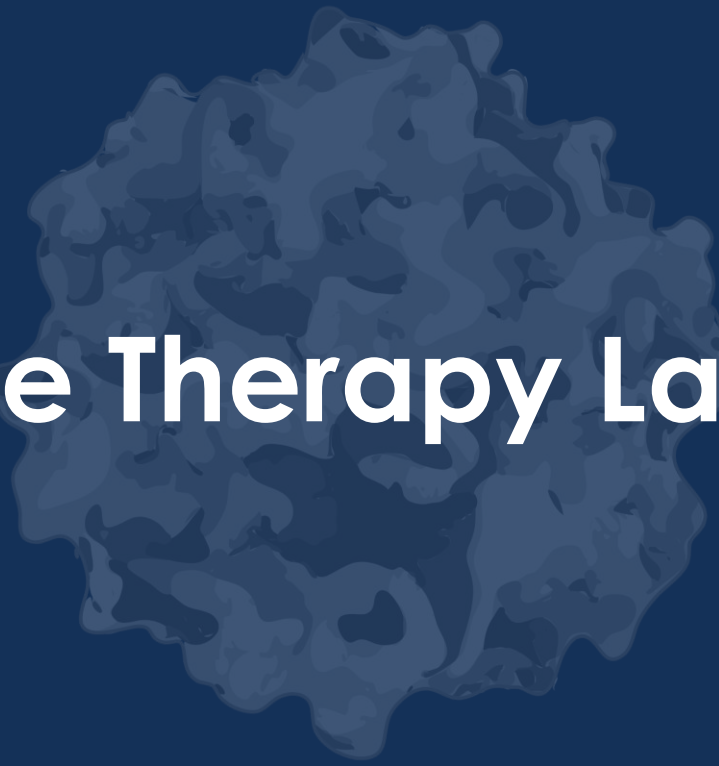
- Cell Therapy Process Development
- Viral Vector Process Development & Manufacturing
- Process Characterization
- Analytical Development

→

- GMP Scale Up
- Technology
- Regulatory Expertise
- QC Analytics and Release Testing

elevatebio basecamp™

AAV Gene Therapy Landscape



Gene therapy (r)evolution: AAV approvals around the world

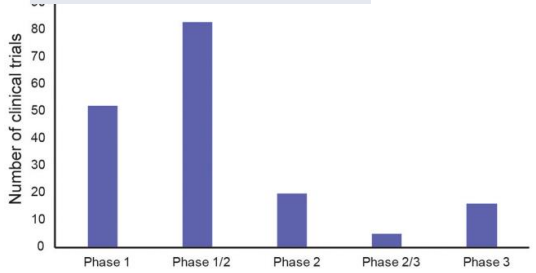
Product Name	Glybera®	Luxturna®	Zolgensma®	Uptaza®	Hemgenix®	Elevidys®	Roctavian®	BEQVEZ®
Generic Name	alipogene tiparvovec	voretigene neparvovec	onasemnogene abeparvovec	eladocagene exuparvovec	etranacogene dezaparvovec	delandistrogene moxeparvovec	valoctocogene roxaparvovec	fidanacogene elaparvovec
Company	Uniqure	Spark Therapeutics	Novartis	PTC Therapeutics	CSL Behring	Sarepta Therapeutics	BioMarin Pharmaceutical	Pfizer
Approvals	2012 (EC) (withdrawn)	2017 (FDA), 2018 (EC) and others (20+ countries)	2019 (FDA), 2020 (EC) and others 40+ countries	2021 (EC) UK, Israel	2022 (FDA) 2023 (EC) CA, UK, CH, AU	2023 (FDA)	2023 (FDA) 2022 (EC)	2024 (FDA)
Indication	Lipoprotein lipase (LPL) deficiency	Treatment of RPE65 mutation-associated retinal dystrophy	Treatment of spinal muscular atrophy (SMA) caused by mutations in the SMN1 gene	Aromatic L-amino acid decarboxylase (AADC) deficiency.	Treatment of adults with Hemophilia B	Treatment of Duchenne muscular dystrophy (DMD) with confirmed mutations in the DMD gene	Treatment of adults with severe Hemophilia A	Treatment of adults with moderate to severe Hemophilia B
Serotype	AAV2	AAV2	AAV9	AAV2	AAV5	AAVrh74	AAV5	AAVRh74var
Route of Administration	Intramuscular	Eye - subretinal injection	Intravenous (IV) infusion	Intracerebral (direct injection into the brain)	Intravenous (IV) infusion	Intravenous (IV) infusion	Intravenous (IV) infusion	Intravenous (IV) infusion
Recommended Dose	1 x 1012 gc/ kg body weight	1.5 x 10^11 vector genomes (vg)/eye	1.1 x 10^14 vector genomes per kilogram (vg/kg)	1.8 x 10^11 vg per hemisphere of the brain	2 x 10^13 vector genomes (vg) per kilogram of body weight	1.3 x 10^14 vector genomes per kilogram (vg/kg) of body weight	6 x 10^13 vector genomes (vg) per kilogram of body weight	2 x 10^13 vector genomes (vg) per kilogram of body weigh (pt with BMI <30 kg/m²

Meta-analysis of AAV usage in clinical settings

Distribution of Adeno-Associated Virus (AAV) mediated gene therapy in clinical trials (n= 136 Trials)

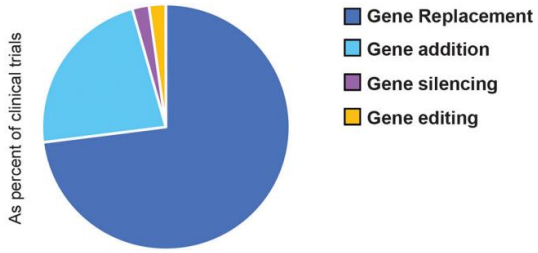
A

Clinical Phase status



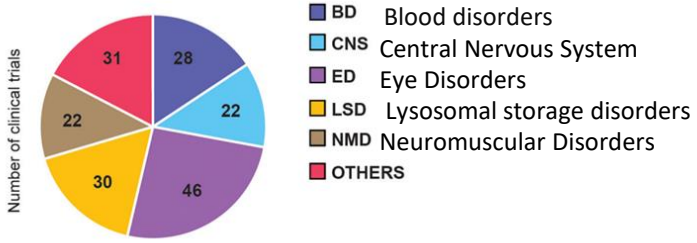
C

Gene Therapy approaches



Therapeutic area

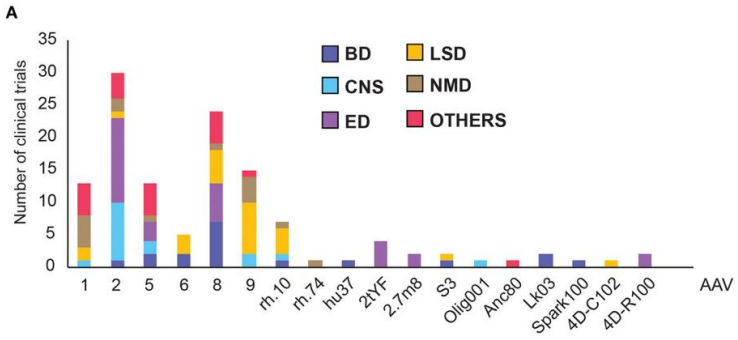
B



AAV capsid usage and frequency in clinical trials (n= 136 Trials)

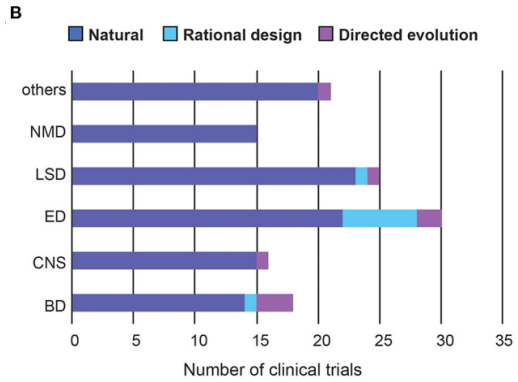
Overall AAV capsid type usage across all trials

A



Capsid design across therapeutic areas

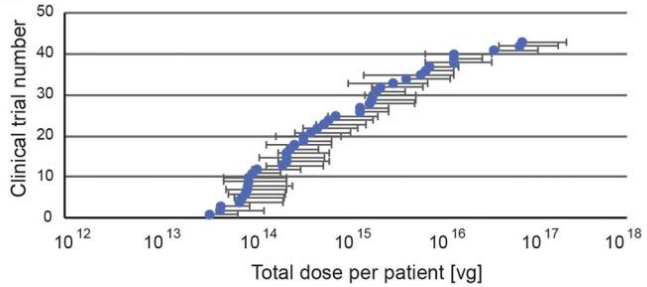
B



A summary of dosage regime of AAV administered in clinical trials (n= 136 Trials)

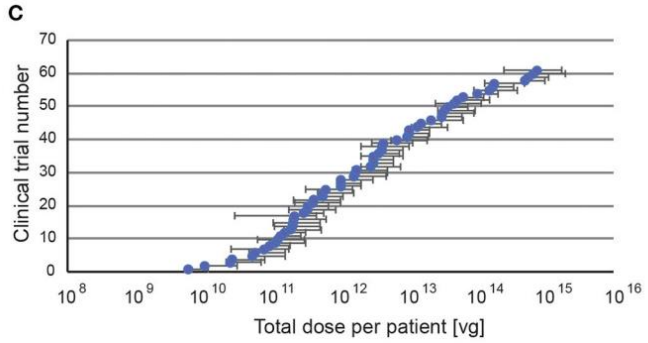
Range of doses in systemic administration

A



Range of doses in targeted administration

C



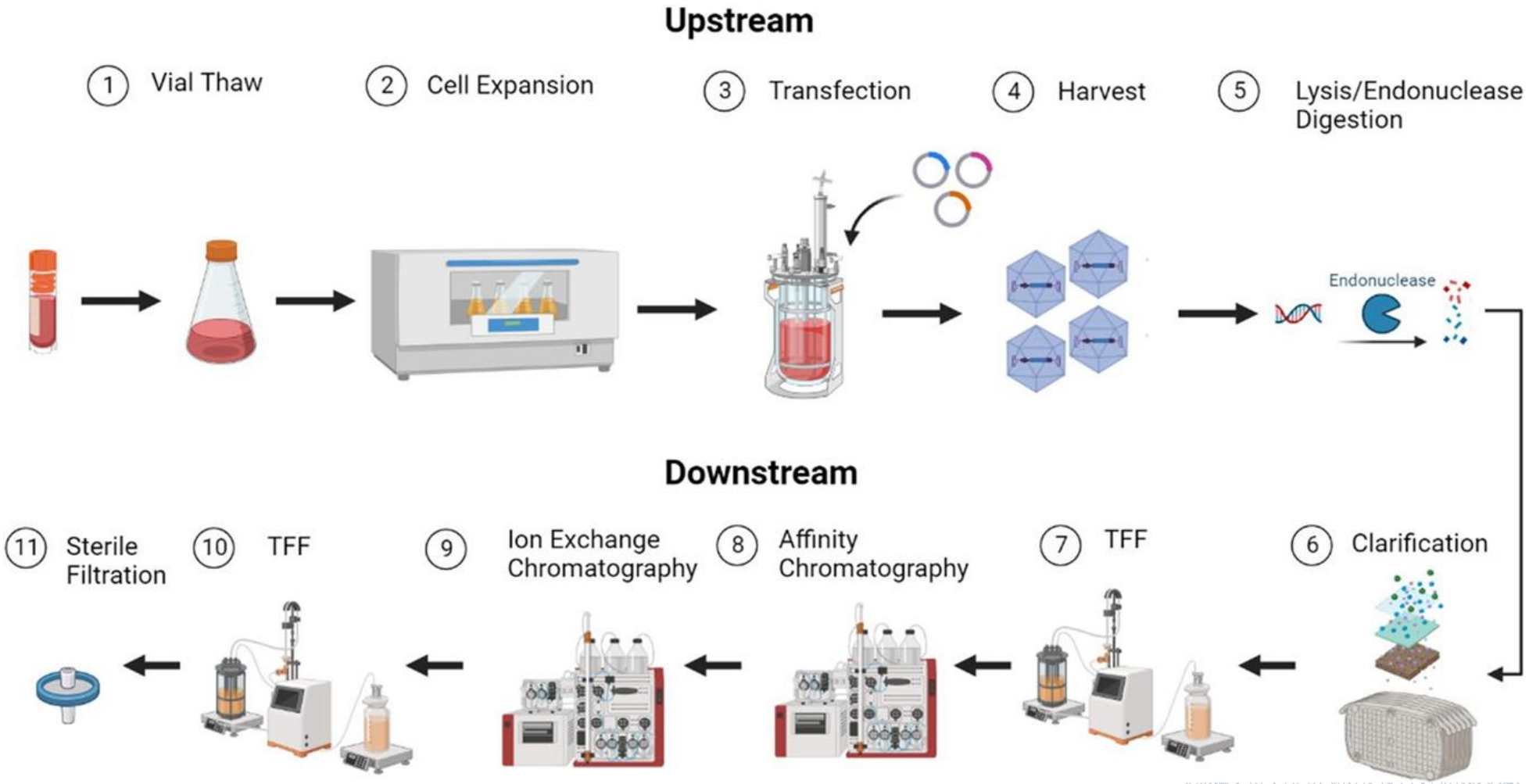
LITERATURE REFERENCE: Au HKE, Isalan M and Mielcarek M **(2022).** Front. Med. 8:809118.

How do you develop a platform process to manufacture AAVs?

Manufacturing process targets

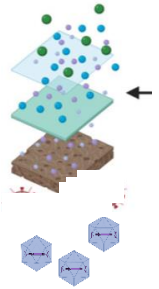
- **Robust manufacturing process**
  - Consistently provides high quality AAV material, suitable for clinical applications
    - Appropriate yields and vector genome titers
    - Eliminate or reduce to safe levels any process-related impurities
    - Produce minimal product-related impurities (e.g., empty capsids, truncated genomes, etc.)
- **Flexible manufacturing process for a broad spectrum of AAVs**
  - Quality AAV with various GOI constructs
  - Easily adapted for multiple AAV serotypes
- **Adaptable to generate material for various therapeutic indications**
  - Scalable to accommodate a range of clinical needs and therapeutic doses
  - Stable in various formulations according to the specific route of administration requirements
- **Fulfill global regulatory expectations**
  - Analytical testing and stability strategy
  - Process control strategy and validation
  - Raw material and starting material choices
  - Facility expectations

A scalable GMP-compliant AAV manufacturing process



Graph created by Biorender

ElevateBio AAV process: the building blocks

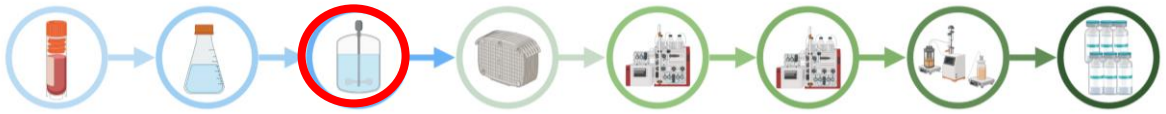
	DRUG SUBSTANCE MFG							DP MFG		
	UPSTREAM PROCESSING				DOWNSTREAM PROCESSING			FORMULATION	FILL&FINISH	
	Vial Thaw	Cell expansion	Transient Transfection	Nuclease + Lysis + Harvest	Clarification	Affinity Chromatography	AEX Chromatography	Tangential Flow Filtration	Sterile Filtration	Fill & Finish
										
Purpose	Establish and recover cell line	Cell culture expansion	AAV production	Cell lysis and DNA impurity removal pre- harvest	Cell debris and other impurity removal	AAV concentration and impurity removal	Full AAV capsid enrichment	Concentration and formulation	Sterile product	Fill into final container closure
Standard or Flexible	Standard Unit Operation	Standard Unit Operation	Optimization to maximize AAV yields and percent of full capsids	Standard Unit Operation*	Standard Unit Operation*	Optimization to maximize AAV yields and impurity removal	Optimization to maximize AAV yields and percent of full capsids	Final concentration and formulation are product specific	Standard Unit Operation*	Fill volume and container closure are product specific

elevatebio®

* Performance evaluation for standardized steps post-transfections is performed

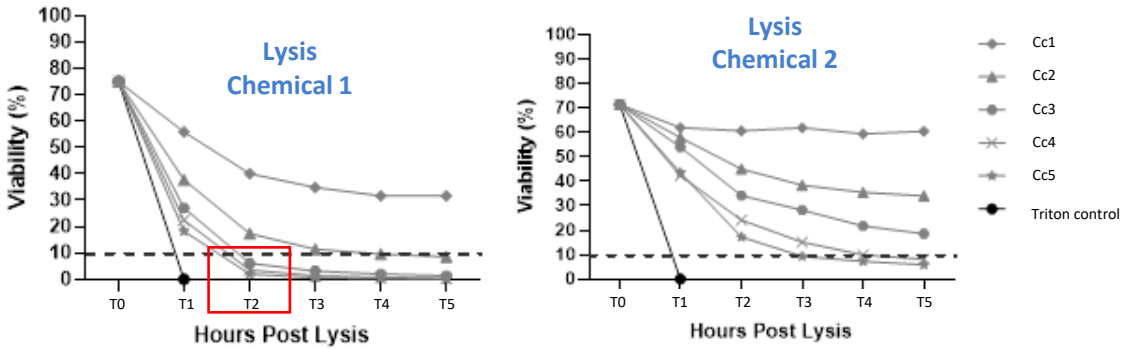
Standardizing cell lysis with non-triton chemicals for global supply

Selection of non-Triton X100 alternative in small scale cell lysis studies

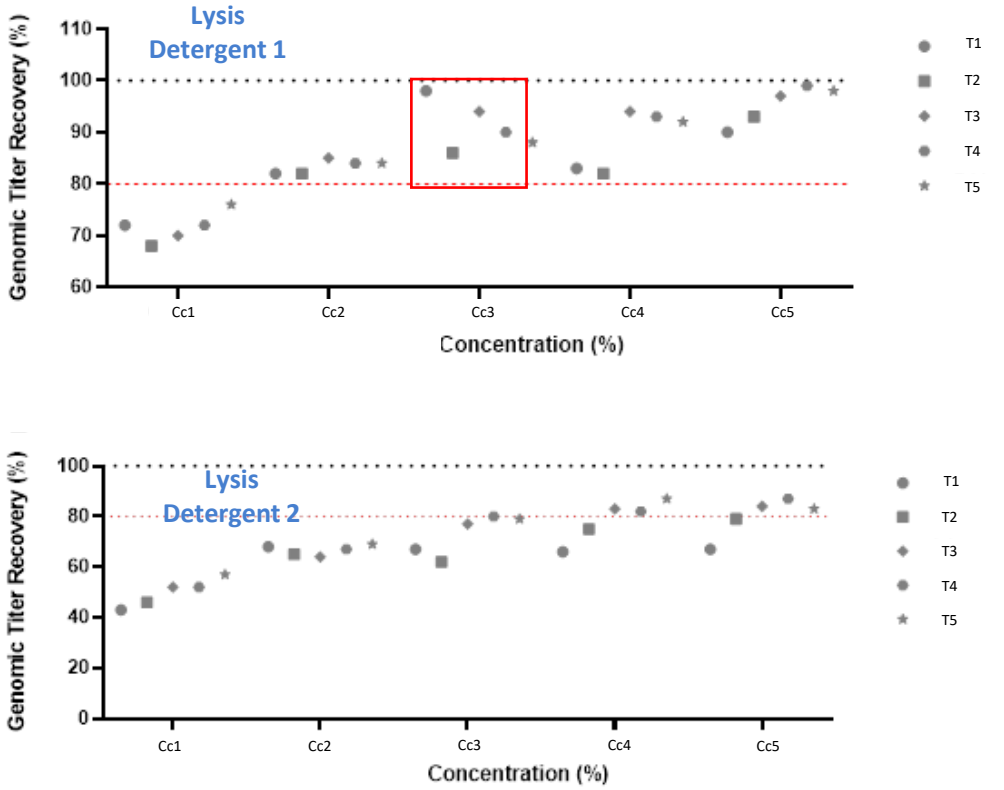


Lysis Reagent	Likelihood to be Banned in EU	Scalability	Version Available for GMP Manufacturing
Non-Triton Chemical 1	Unlikely	Scalable	Available
Non-Triton Chemical 2	Unlikely	Scalable	Available
Non-Triton Chemical 3	Unlikely	Scalable	Not Available
Non-Triton Chemical 4	Unlikely	Scalable	Not Available

Time-course lysis study: Cell Lysis



Time-course lysis study: AAV Yield

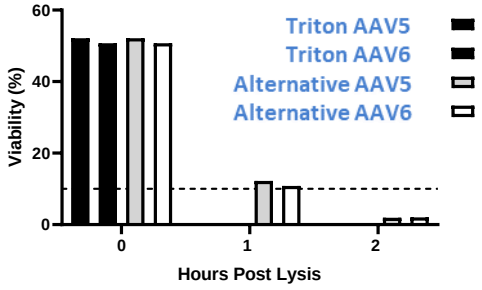


Standardizing cell lysis with non-triton chemicals for global supply

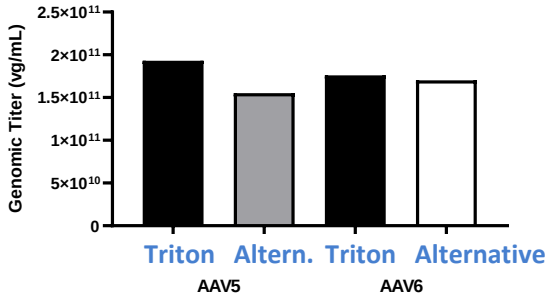
Evaluation of optimal lysis conditions using a process scale down model

Assessment of top lysis conditions for AAV5 and AAV6

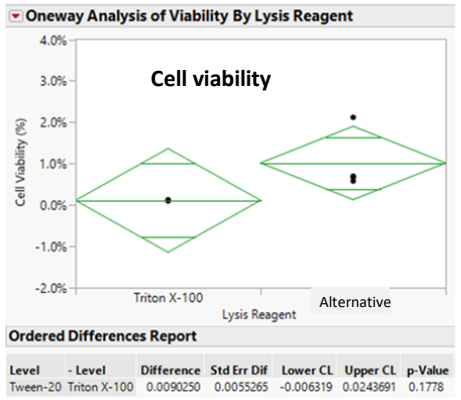
>98% cell lysis

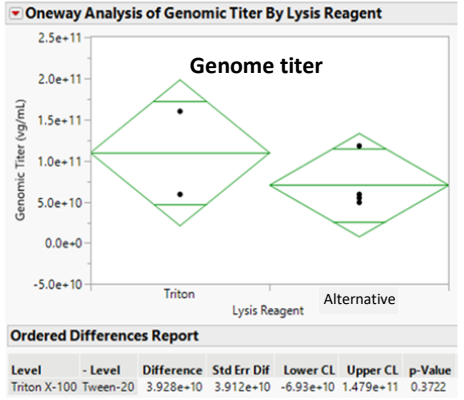


>80% Step yield



Comparison of Cell Viability and AAV Titer at 2L Scale

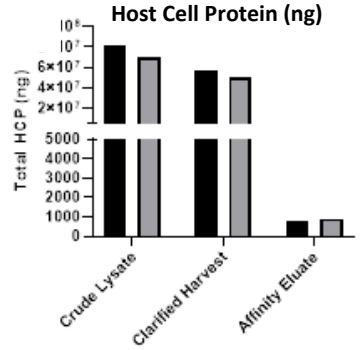


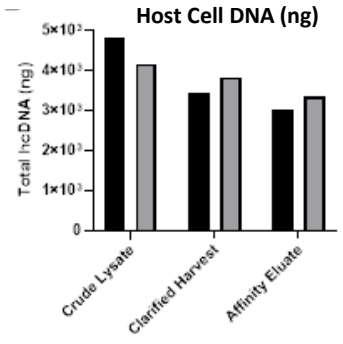


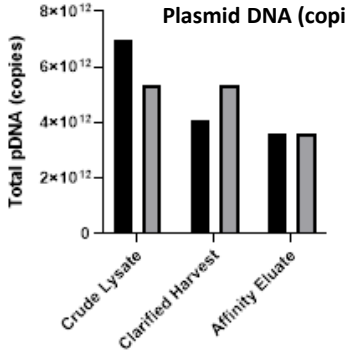
No significant difference with Triton Control

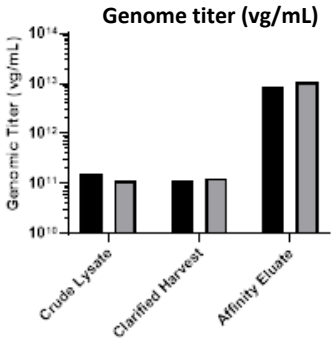
ANOVA p-value >0.05

Comparison of Impurities and Genomic Titers in In-Process Pools





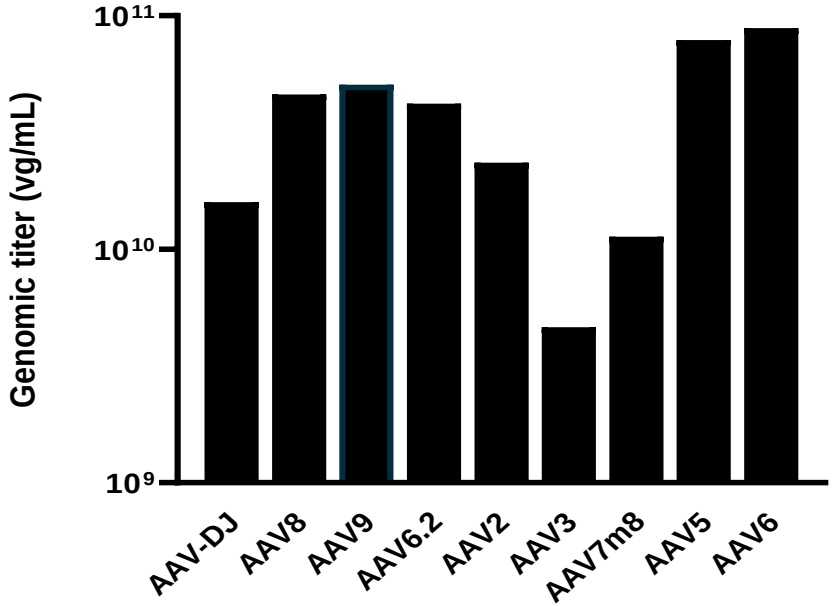




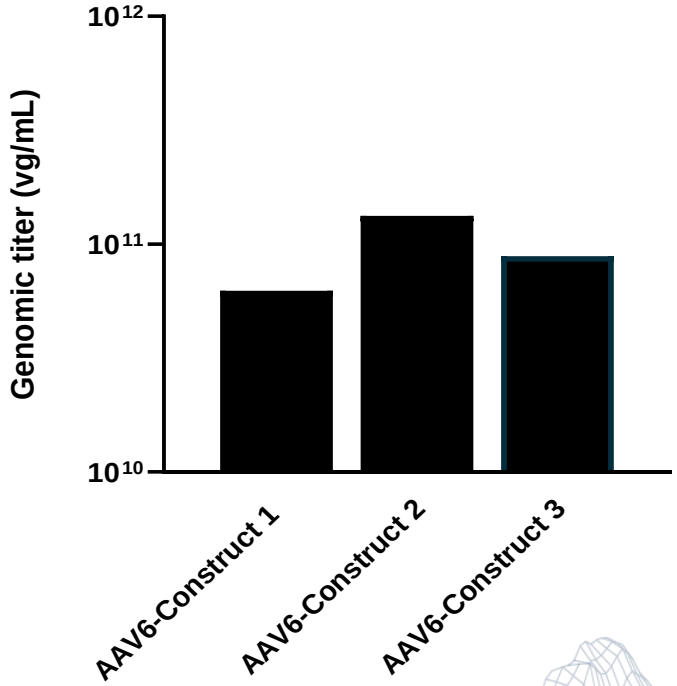
New detergent does not significantly impact impurity levels in subsequent downstream operations compared to base Triton-X based process

AAV upstream yield across different constructs and serotypes

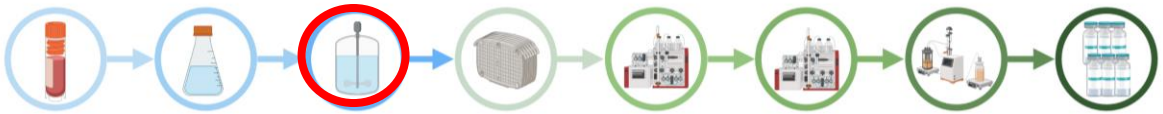
Harvest Lysate Genomic Titer for various AAV Serotypes



Harvest Lysate Genomic Titer for various AAV6 GOI Constructs



A range of harvest titers for various serotypes and GOI plasmid constructs



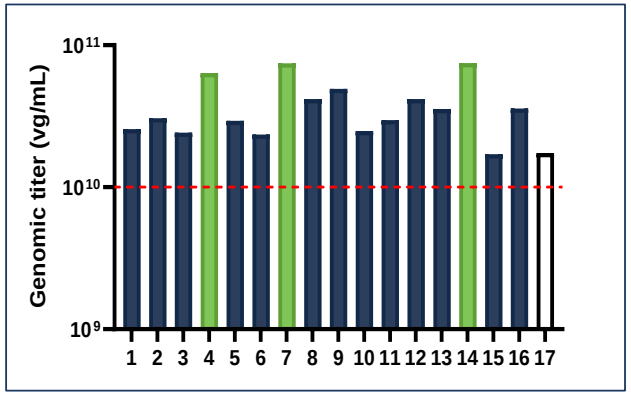
elevatebio®

DoE optimization of AAV upstream titer

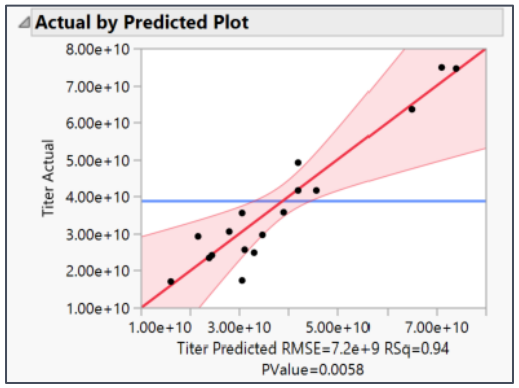
Transfection parameters

(DoE process inputs)

DNA/cell
Cell Density
DNA/Reagent Ratio

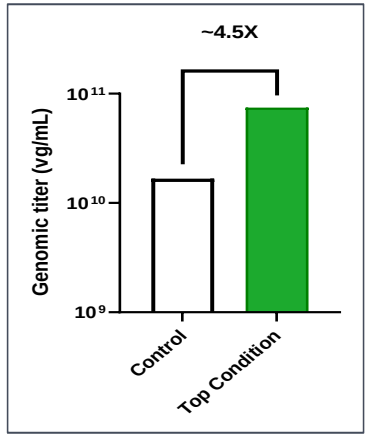


AAV6 transfection optimization

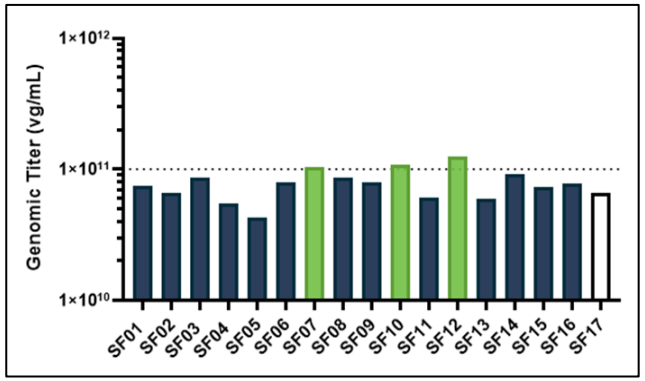


Predictive Model

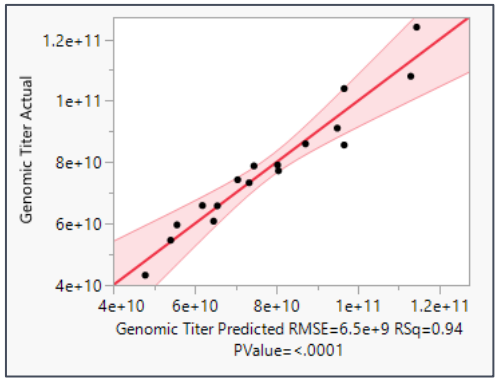
AAV upstream titer optimization



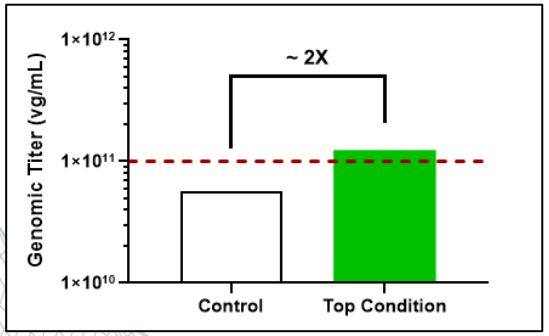
~4.5X titer improvement



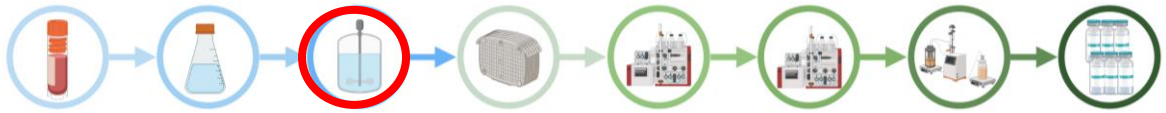
AAV5 transfection optimization



Predictive Model



~2X titer improvement



elevatebio®

Evaluation of %full capsids generated in the upstream process

Analytical challenge

AAV yield

Shake flask

VG Titer (ddPCR)

Cannot assess Empty/Full ratios in lysate

Shake flask + HTP affinity

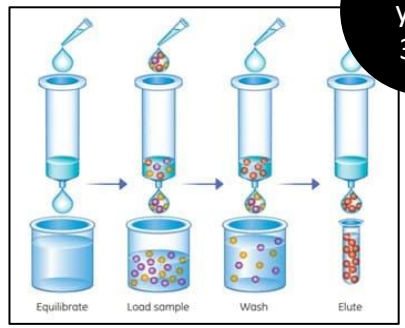
VG Titer (ddPCR) + **Empty/full ratio** (UV/Vis spectroscopy)

STR + AKTA affinity

VG Titer (ddPCR) + **Empty/full ratio** (UV/Vis spectroscopy) + **Empty & full %** (AUC)

High Throughput Sample concentration

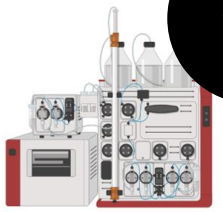
AAV yield 37%



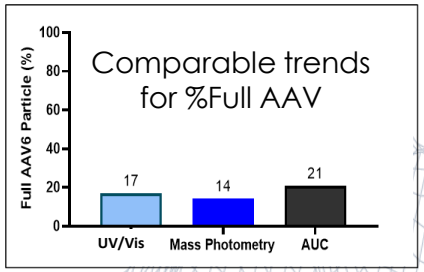
High-throughput batch affinity purification for AAV enrichment

Benefits
Small sample volume
Speed
High-throughput

AAV yield 43%



AKTA affinity chromatography (comparable yield)



elevatebio

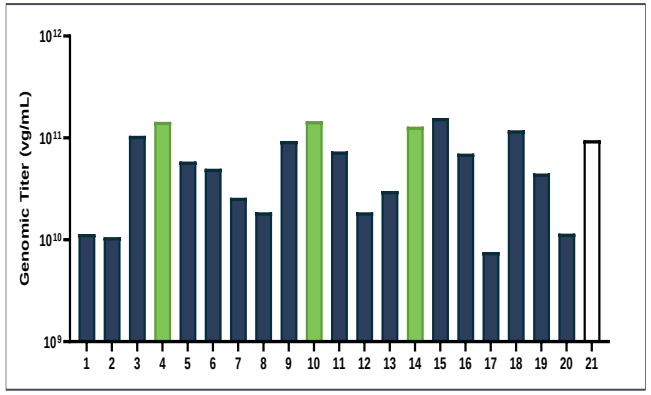
DoE optimization of AAV upstream full%

Upstream %Full optimization

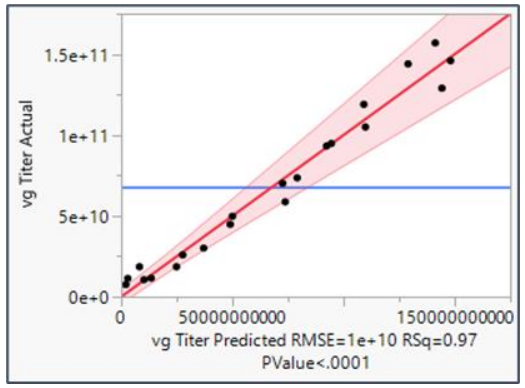
Transfection parameter

(DoE process input)

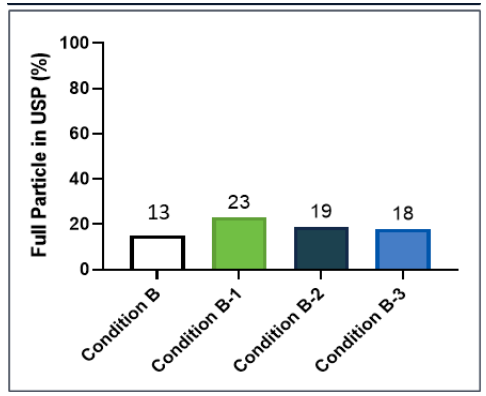
3- Plasmid ratios
- RepCap
- Helper
- GOI



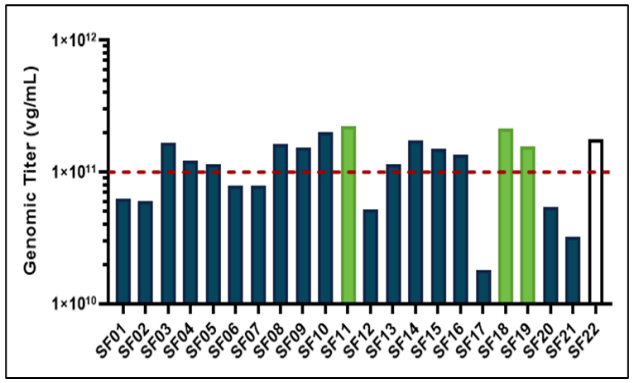
AAV6 transfection optimization



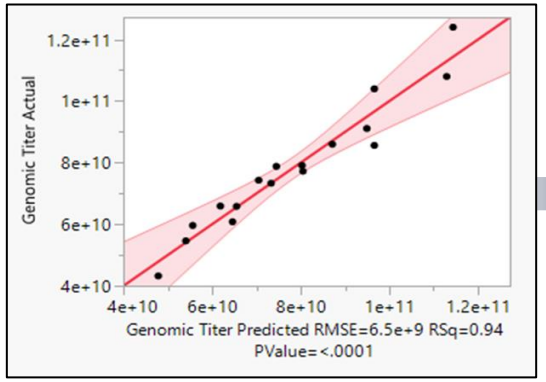
Predictive Model



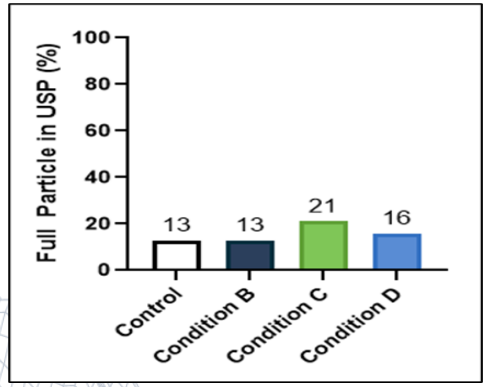
~2X % Fulls improvement



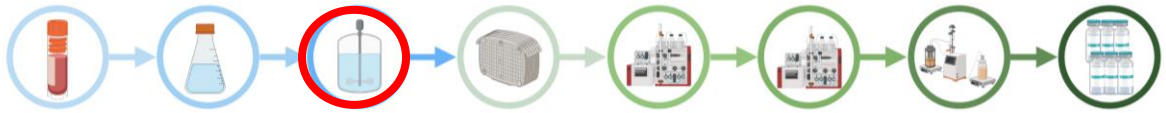
AAV5 transfection optimization



Predictive Model



~ 2X Fulls improvement



elevatebio®

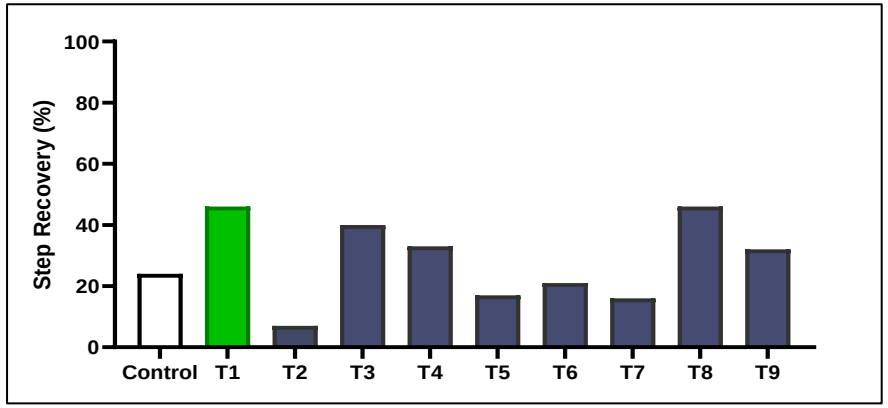
DoE optimization of AAV AEX downstream process

Chromatography parameters

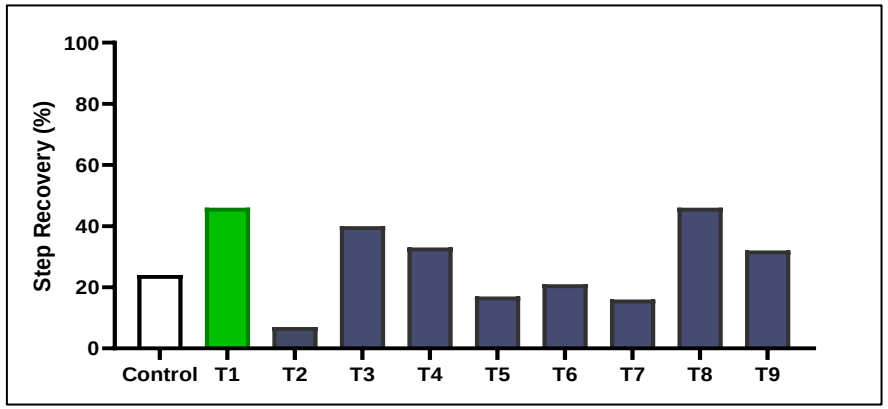
(DoE process inputs)

DoE 1 Elution
Salt type
pH
Supplements

DoE 2 Binding
pH
Conductivity

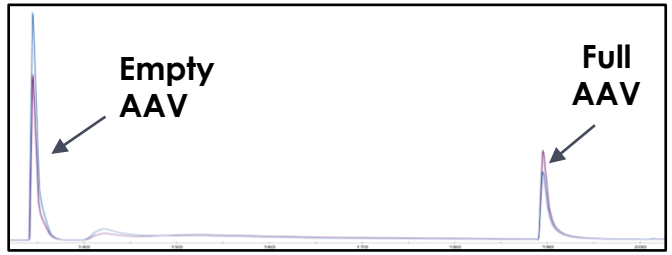


AAV5 DoE 1 Elution parameter optimization



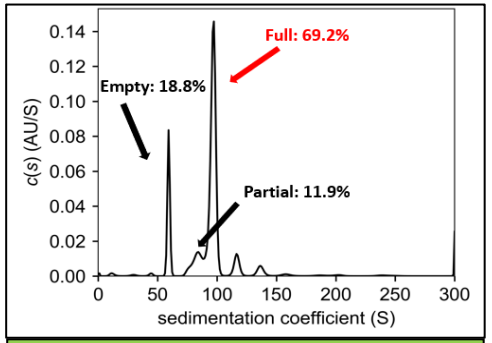
AAV5 DoE 2 Loading adjustment

Downstream AEX %Full optimization



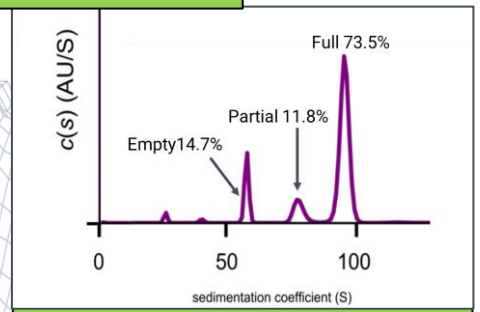
AAV5 - AEX 2-step elution profile

AAV5 AUC



Full content 69.2%

AAV6 AUC

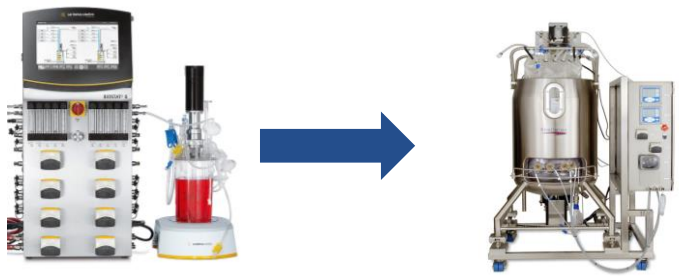


Full content 73.5%

elevatebio

Demonstrated end-to-end scalability

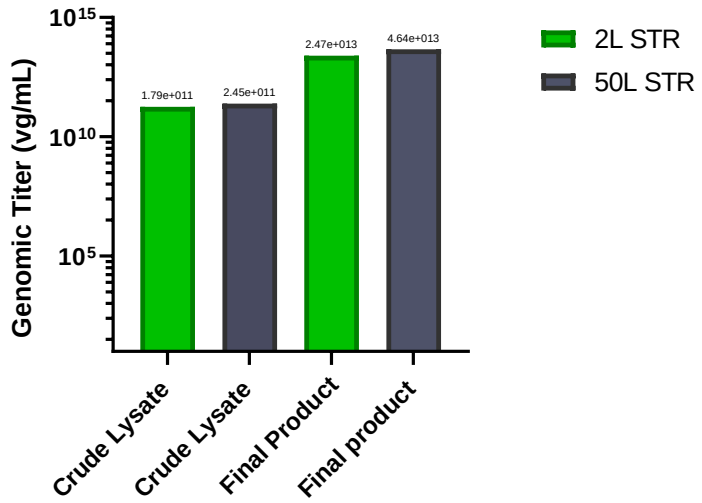
UPSTREAM SCALE UP



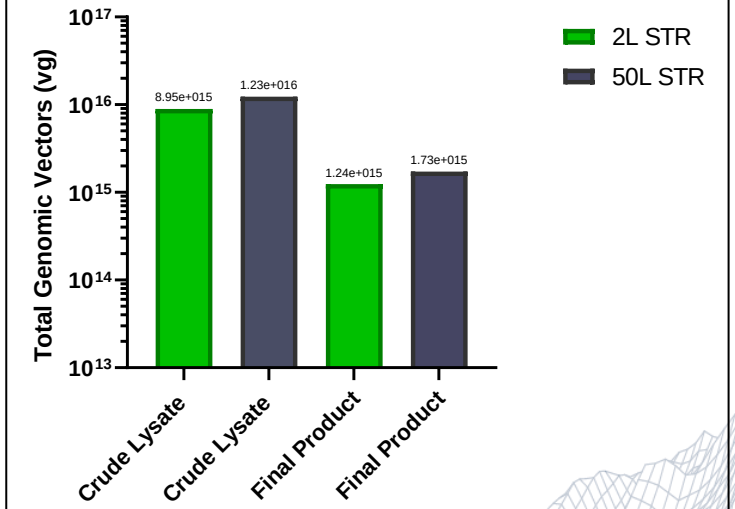
DOWNSTREAM SCALE UP

Basecamp's 50L STR

Genome titer



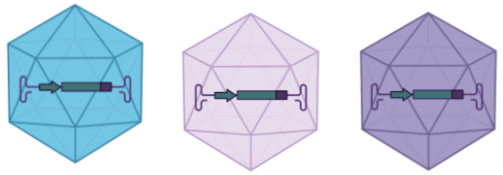
Total VG/batch



Highlights for the AAV process developed

- Developed a serum-free cell suspension process based on transient transfection
  - HEK 293 GMP cell bank
  - Proprietary 3-plasmid system
  - Single-use technologies and raw materials suited for GMP manufacturing
  - Aligned PD with MFG capabilities (as well as AD and QC)
- Developed an AAV assay panel to support PD and QC
  - In-process, characterization, stability and release testing
- Successfully demonstrated end-to-end operations up to 50 L (for both upstream and downstream steps)
- Established experimental approach for process optimization that speeds up new AAV product introduction
- Looking into alternative avenues to further accelerate timelines from construct nomination to IND
  - E. g. machine learning to reduce genome truncation and predictive modelling
  - E. g. Predictive modeling based on data base to predict best conditions and reduce lab work

What is a platform process?



Definition:

A platform process is a production process that can be used to manufacture a group of related products in a defined production system.

A platform process can include:

- ✓ A standard set of media, buffers, purification resins, transfection reagents, and other consumables
- ✓ A suspension-based cell line suitable for GMP manufacturing (e. g. GMP cell bank)
- ✓ A plasmid system for transient transfection (e.g. proprietary plasmid backbones)
- ✓ A comprehensive set of in-process analytics
- ✓ A simplified supply chain
- ✓ Standardized documentation
- ✓ Release and characterization analytics
- ✓ A simplified supply chain

X Some process parameters will need optimization for best product quality results **– Platform or not a platform?**

Acknowledgments

Process Development

- Bojiao Yin
- Azam Hassaninasab
- Jay Cai
- Theresa Dao
- Daniel Kennedy
- Nicholas Redmond
- Chyan-Jang Lee
- Jimmy Xin
- Briana Orlando
- Lily Tran

Analytical Development

- Deb Bhattacharya
- Mike Giffin
- Omar Matalka
- Miranda Williams
- Christine Beaudry
- Casey Kimber
- Arianna Spooner
- Chase Waxman
- Emily Sinclair

Viral Vector Engineering

- Stacie Seidel
- Lisa Santry
- Amira Rghei
- Richard Decker

Thank you for your attention!

elevatebio®