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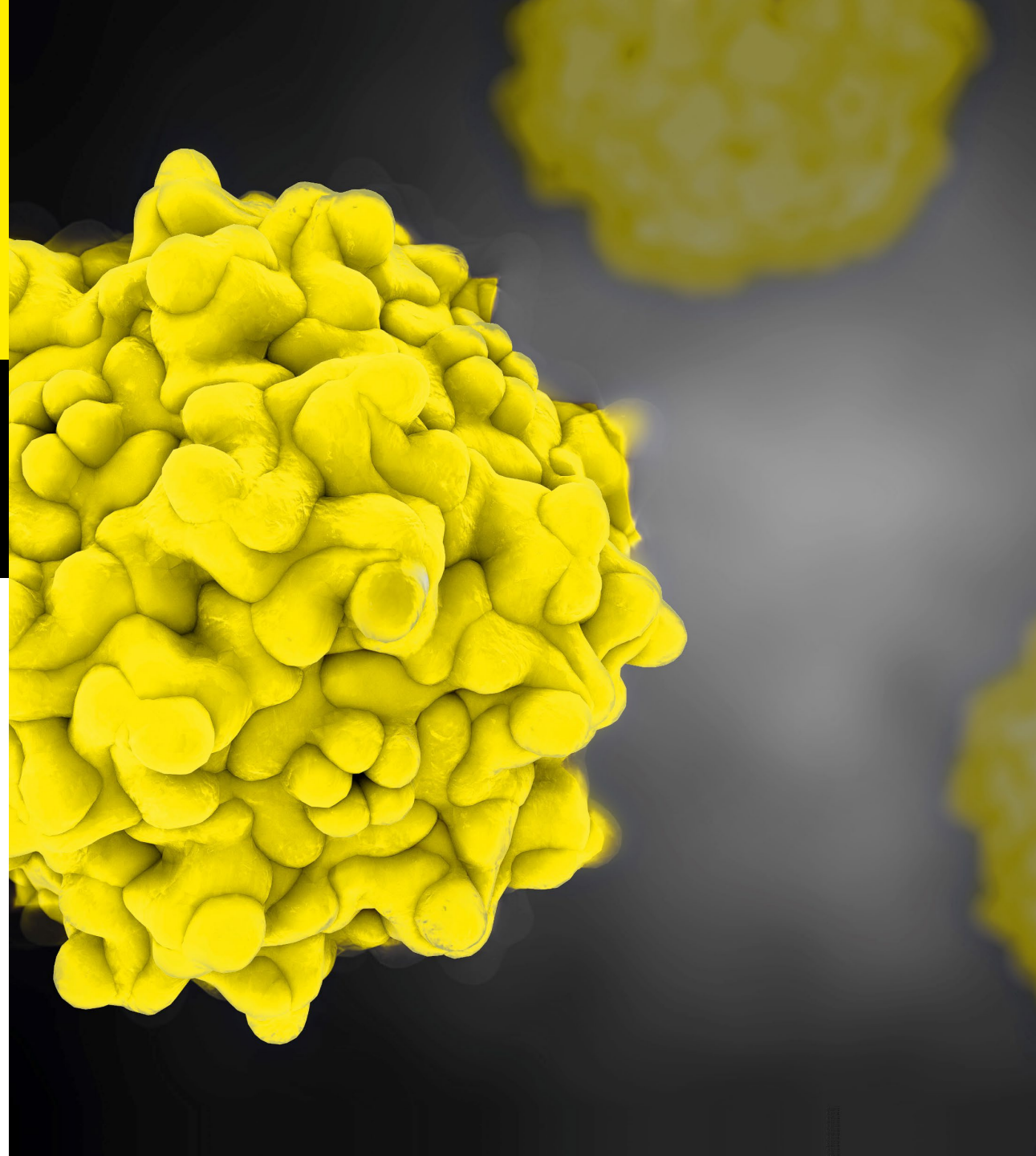
Simplifying Progress

How CDMOs Can Digitalize Their Cell and Gene Therapy Processes

March 24th, 2021

Tiffany McLeod, Nitin Chopra, Julia Hupfeld



Agenda

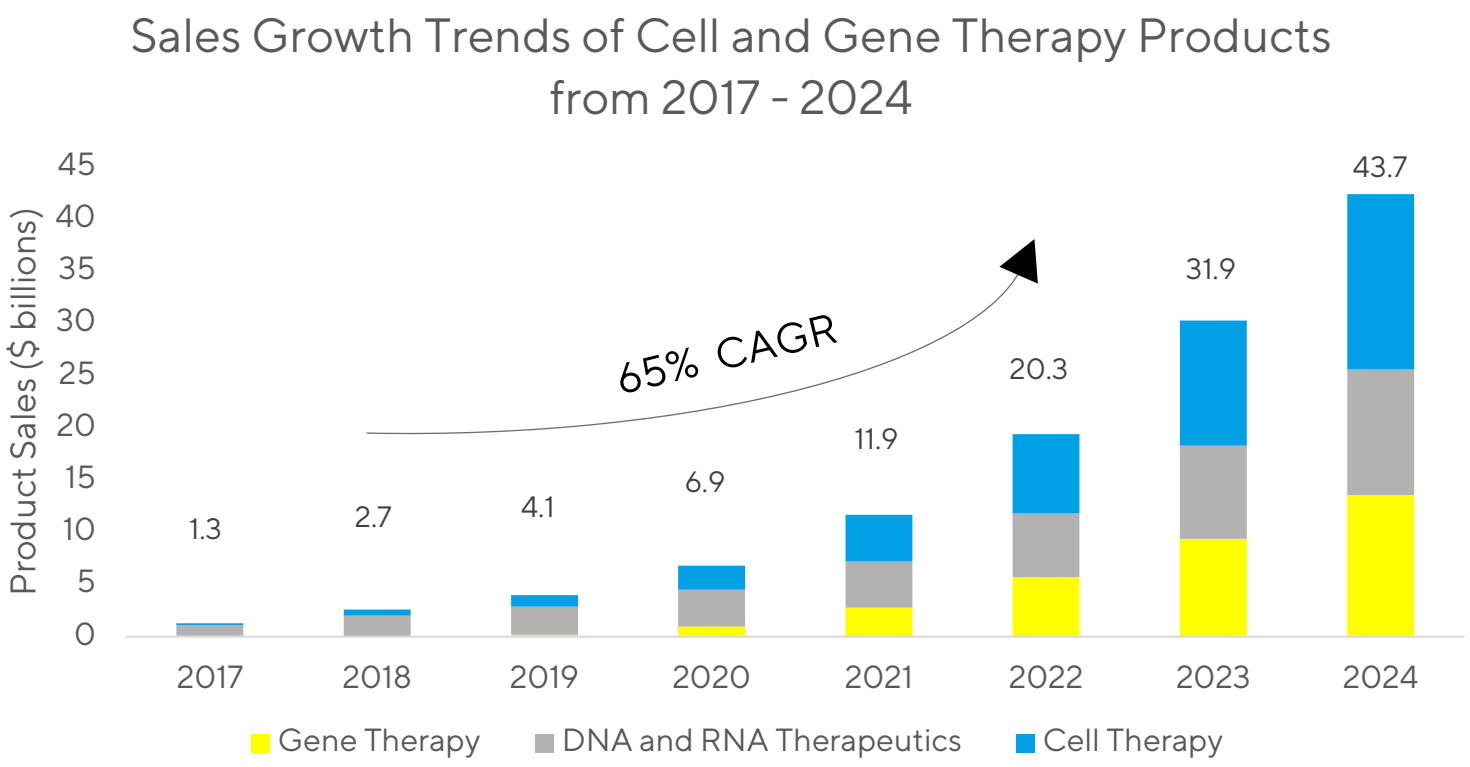
CDMO Market Overview CGT

Addressing CGT Challenges with Data Analytics

Areas Where CDMOs Can Add Value to CGT Processes

Q&A

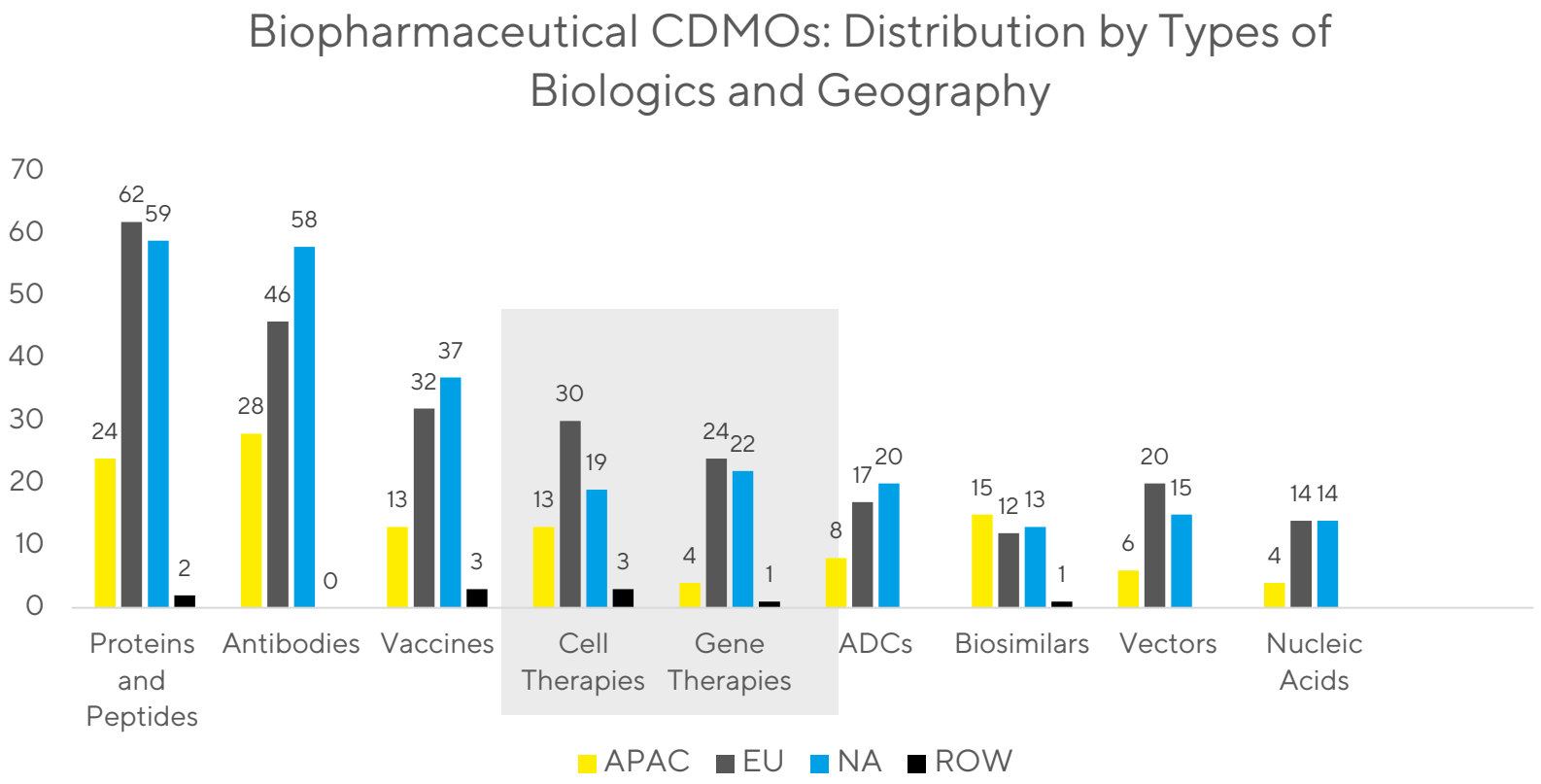
The Demand for Novel Biologics is Growing



Source: EvaluatePharma, March 2019

- With $1 Billion in 2017 to $44 billion in 2024, the CGT space will likely continue to be the most keenly watches pharma segments for business development
- To keep up with this growing global demand CGT developers will rely heavily on C(D)MOs for the outsourcing of both development and manufacturing activities

Current CDMO Market Landscape CGT Players

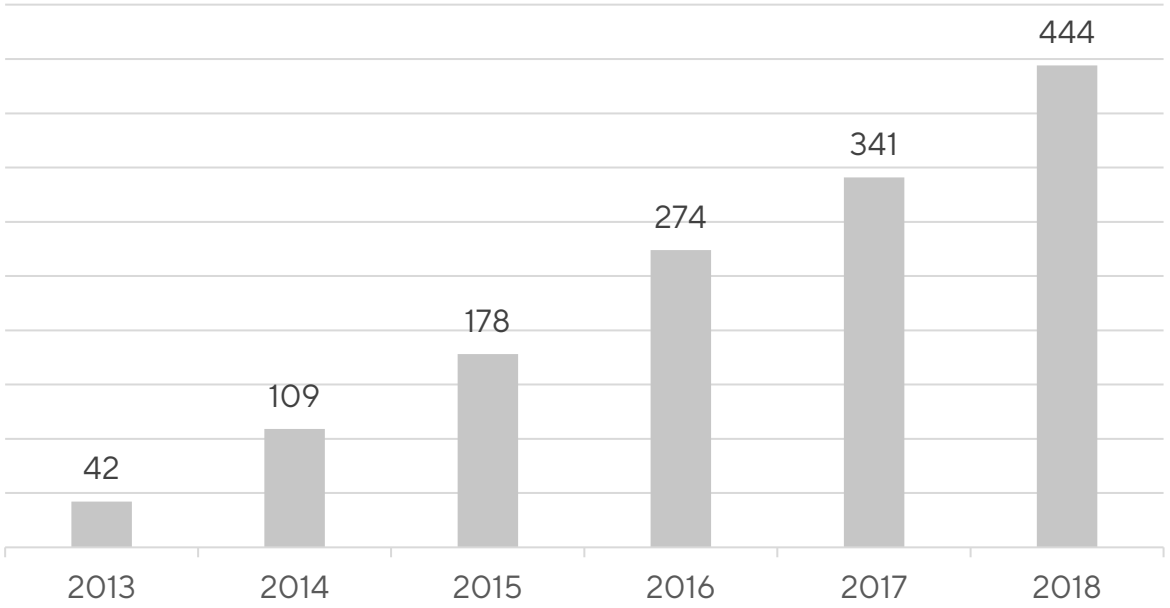


- Nearly 100 companies compete in the cell and gene global marketplace
- Majority of CGT CDMO operations covering lab and clinical scale operations (~70%)
- **Types of Cell Therapies** Handled by CDMOs include: Immune Cells (T-cell, NK, Dendritic, Tumor) and Stem Cells (Adult, hESCs, iPSCs)
- **Types of Viral Therapies** Handled by CDMOs include: Viral Vectors (AAV, Lentivirus, Adenovirus, Retrovirus) and Plasmid DNA

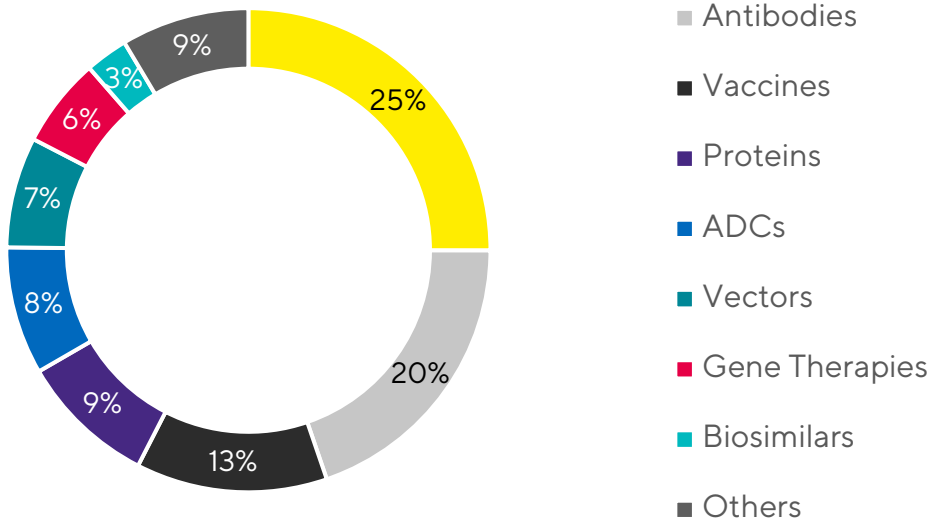
Source: Roots Analysis, 2019

Trend Towards Strategic Collaborations Between CDMOs and CGT Partners

Collaborations: Cumulative Trend by Year, 2013 -2018

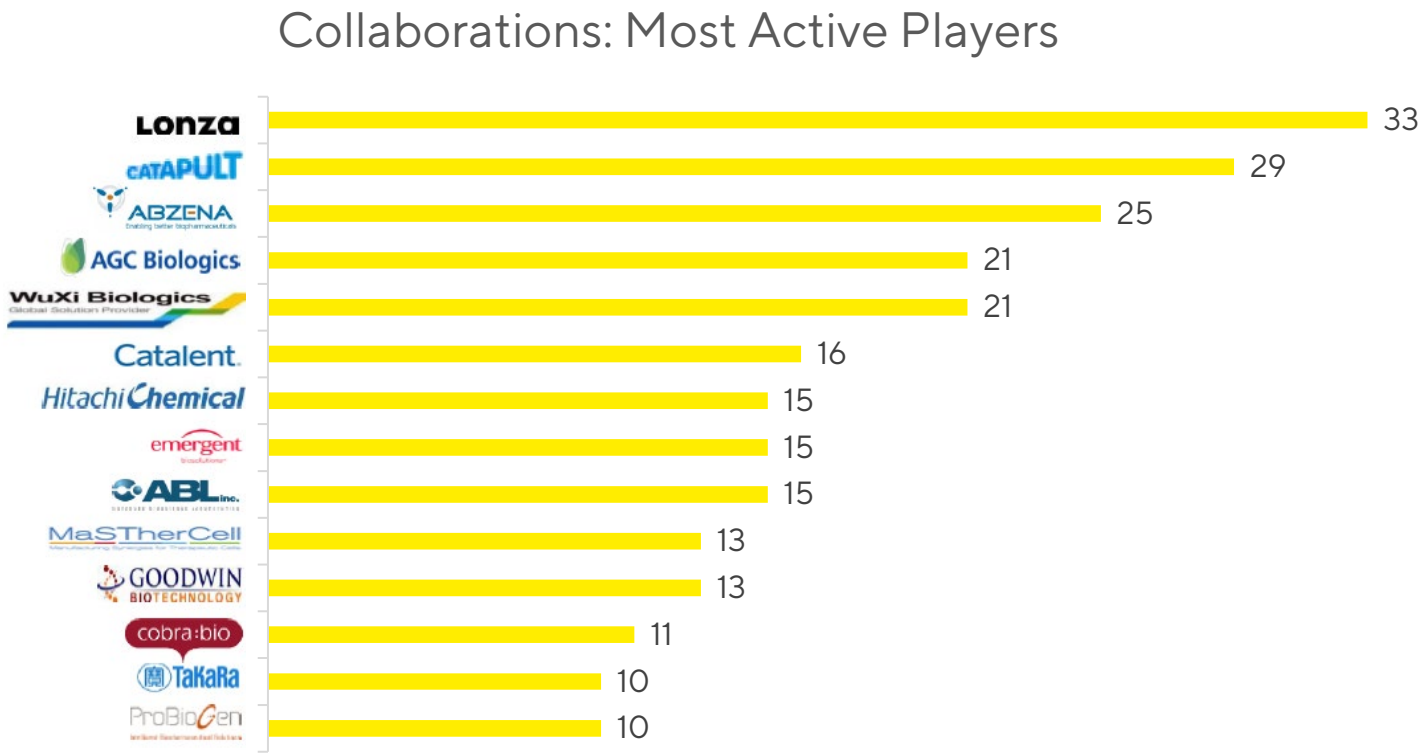


Collaborations: Distribution by Type of Biologics



Source: Roots Analysis, 2019

Cell & Gene Therapy CDMOs Represent Most Active Collaboration Players



- Lonza emerges as the most active in the biopharmaceutical manufacturing domain
- April 2018, Lonza opened a CGT manufacturing facility in Pearland, TX in order to cope with the rising demand from developers of cell and gene therapy products. This facility covers an area of 300,000 sq. ft, and claims to be the world's largest cell and gene therapy manufacturing facility.[1]
- Other prominent players include Catapult, Abzena, ACG Biologics, Wuxi Biologics, Catalent, Hitachi Chemical, Ermergent, ABL, MaSTherCell...

Source: Roots Analysis, 2019

Source[1]: ttps://www.lonza.com/about-lonza/media-center/news/Tensid/2018-04-10-12-00-English.aspx

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A Look Into the Future of CGT CDMOs

- The demand for CGT products will be exacerbated by accelerated regulatory approvals
- Phases of development are advancing so quickly so in order to be ready for commercialization, companies should be considering manufacturing at the beginning of development
- Portfolio breadth, including the ability to harness data, regulatory compliance, market presence, the ability to execute and implement, and cost will be used as key criteria's for CDMO selection

By 2025, the US FDA expects it will be approving 10 to 20 cell and gene therapy products a year.[1]

FDA U.S. FOOD & DRUG ADMINISTRATION

Outsourcing CGT operations will be a critical factor in keeping up with the growing global demand.

Source[1]: FDA, "Statement from FDA Commissioner Scott Gottlieb, M.D. and Peter Marks, M.D., Ph.D., director of the Center for Biologics Evaluation and Research on new policies to advance development of safe and effective cell and gene therapies," press release, January 15, 2019.

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How Outsourcing can Prove Beneficial for CGT Developers

Economic Benefits (i.e. Productivity, Efficiency, Time-to-market, and Quality Gains)

Make up for Shortage of Suitable Development and Manufacturing Facilities

Deliver Competencies for Developing, Scaling-up, and Manufacturing CGTs

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Addressing CGT Challenges with Data Analytics

Key Drivers for Viral Vector Manufacturing

Current Technology

- Transient transfection of DNA plasmids
  - In HEK293 cell line
  - SF9 baculovirus insect cell lines
- Adherent & suspension culture systems
- Serum supplemented cultures vs chemical defined medium
- Downstream needs to be adjusted to fit different serotypes

Pain Point

- Scale up issue with adherent platform
- No standard solution for downstream
- Empty vs full capsid separation
- Different process needs for different capsids
- Viral envelope protein toxic to host cells
- High COGS

Needs

Speed to Clinic | Market

Process Improvement

Flexible Processes

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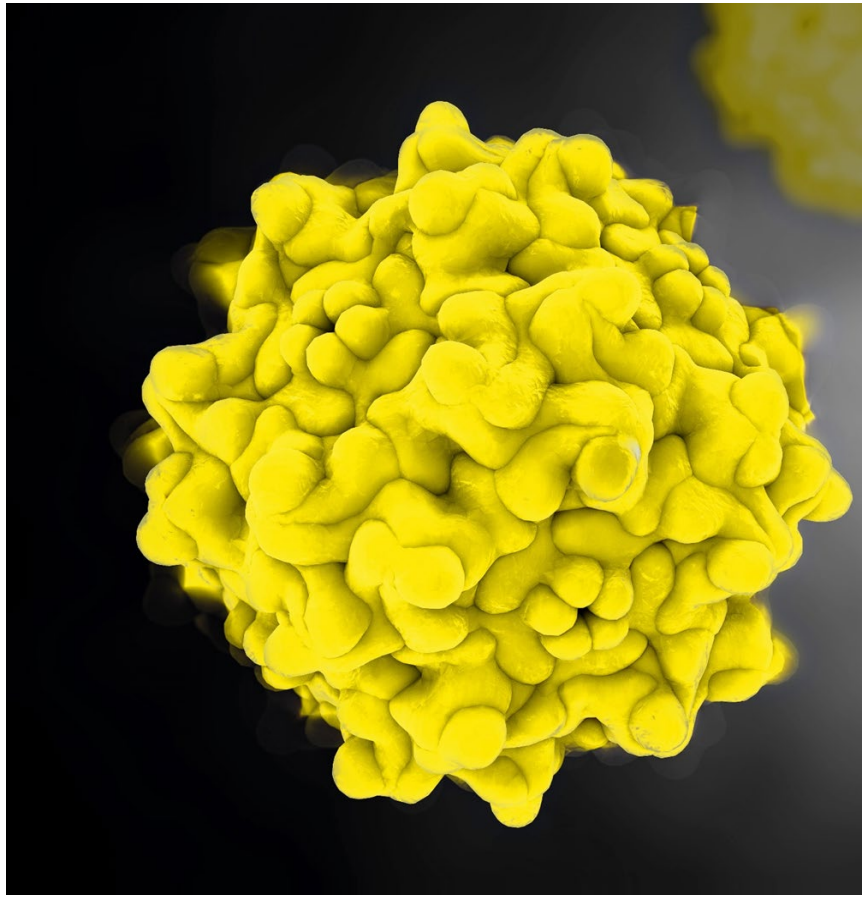
Increased Focus on Resilience Driving Biopharma 4.0

- Drug Discovery & Development
- Taking Product to Scale
- Facility Retrofitting or Design
- Foster Improvements in Manufacturing
- Supply Chain and Logistics



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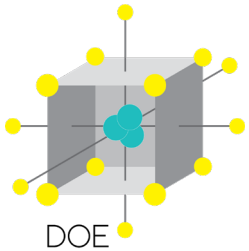
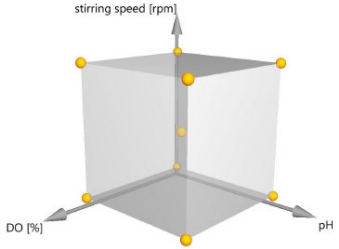
Addressing Viral Vector Challenges with Data Analytics: Process Development & Scale-up

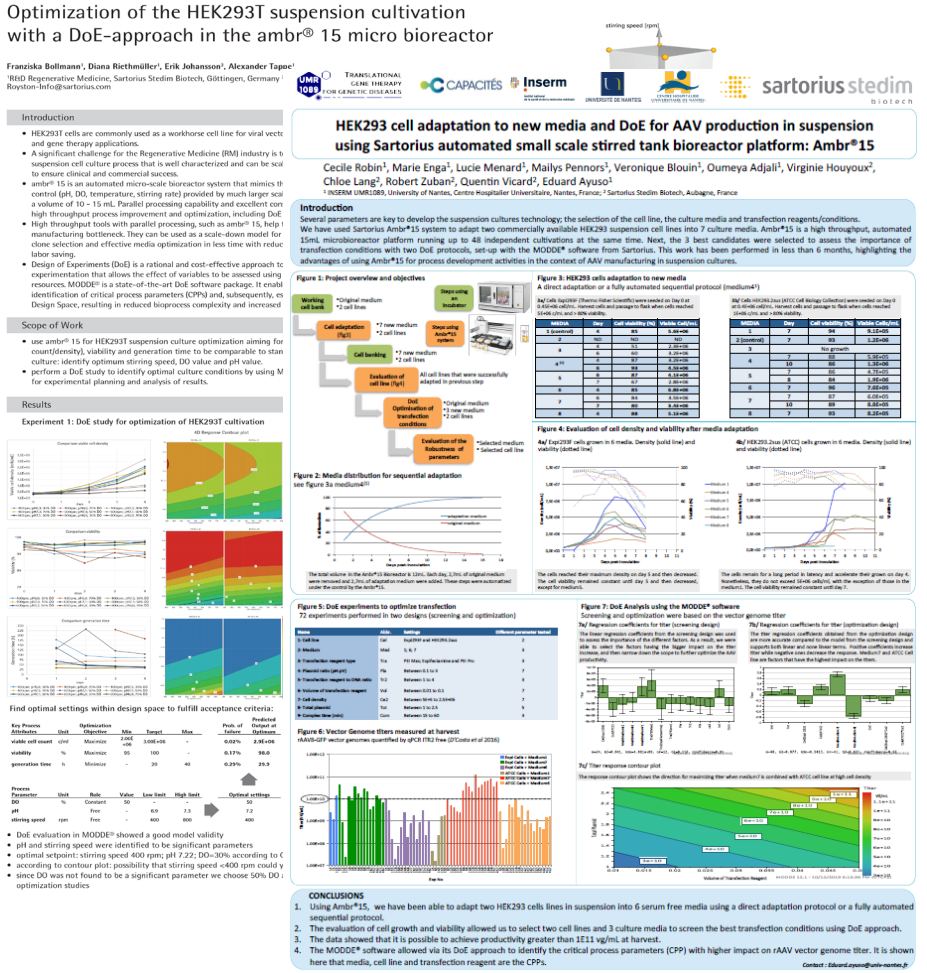
Rapid, High Throughput Process Improvement and Optimization

- Ambr 15® facilitates parallel processing capability and excellent consistency
- Micro-scale bioreactor system that mimics the features and process control of larger bioreactors
- Using MODDE® for DOE design and evaluation supports the high-throughput capabilities of Ambr 15® for rapid process parameter optimization



DOE
Design of Experiments





Optimization of the HEK293T suspension cultivation with a DoE-approach in the ambr® 15 micro bioreactor

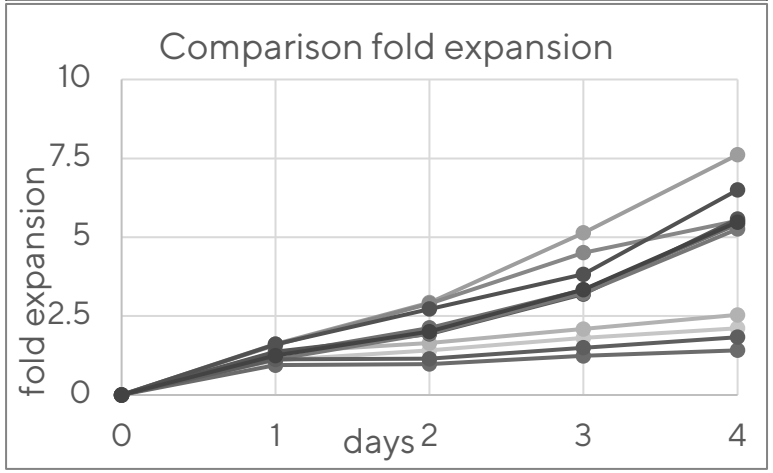
HEK293 cell adaptation to new media and DoE for AAV production in suspension using Sartorius automated small scale stirred tank bioreactor platform: Ambr®15

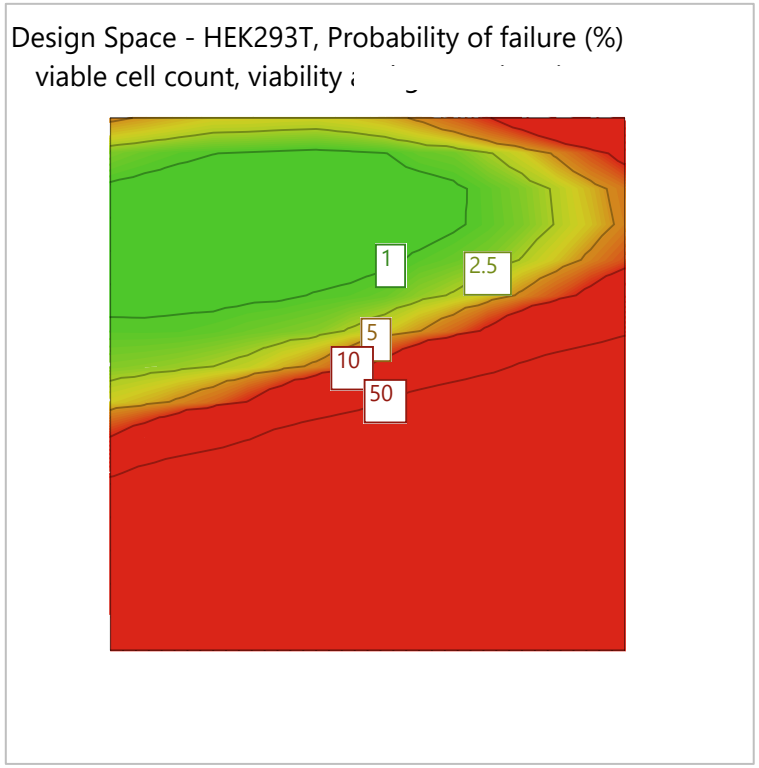
Optimization of HEK293T Culture Parameters with Ambr® 15 and DOE

Screen to get the right set point in a controlled manner comparing with shake flask

- pH and stirring speed were identified to be critical process parameters
- One experiment and cultivation over 5 days in the Ambr® 15 already leads to identification of optimal and robust HEK293T culture conditions via design space

Parameter	Range
Stirring speed	400 – 800 rpm
pH	6.9 – 7.3
DO	30 – 70 %
Responses	VCC, viability





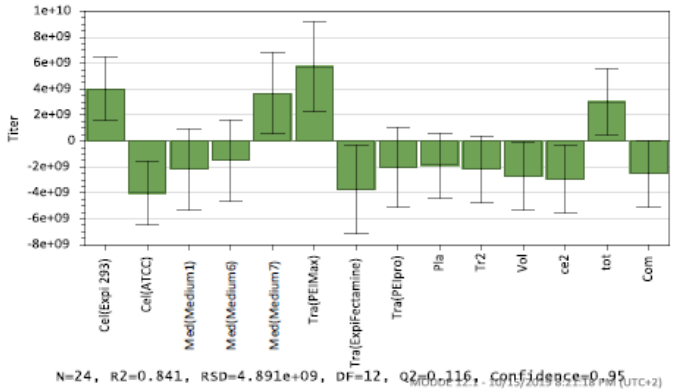
Watch the Recorded Webinar Here: https://register.gotowebinar.com/register/2024039674060989955

Optimization of HEK293 culture parameters with Ambr® 15 and DOE for AAV

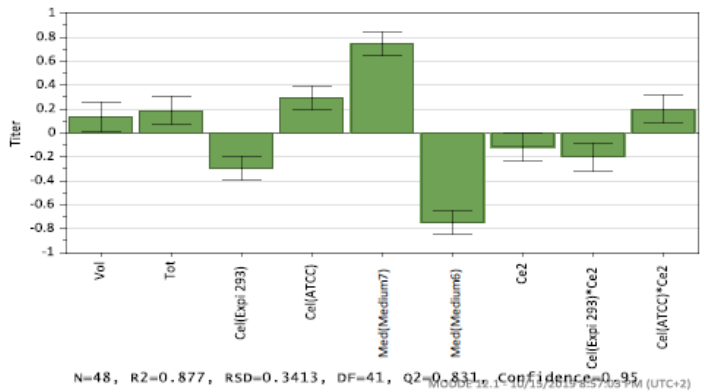
High Throughput Platform → DOE/MODDE® → Key Factors/ Parameters → Screening → Optimization

Parameters
Cell line
Medium
Transfection reagent type
Plasmid ration(pH:pV)
Transfection reagent to DNA ratio
Volume of transfection reagent
Cell density
Total plasmid
Complex time (min)

The linear regression coefficients from the screening design was used to assess the importance of the different factors. As a result, we were able to select the factors having the bigger impact on the titer increase, and then narrow down the scope to further optimize the AAV productivity.



The titer regression coefficients obtained from the optimization design are more accurate compared to the model from the screening design and supports both linear and none linear terms. Positive coefficients increase titer while negative ones decrease the response. Medium7 and ATCC Cell line are factors that have the highest impact on the titers.



Source: HEK293 Cell adaptation to New Aedia and DOE for AAV Production in Suspension using Sartorius Automated Small Scale Stirred Tank Bioreactor Platform:Ambr®15
INSERM UMR1089, University of Nantes, Centre Hospitalier Universitaire, Nantes, France

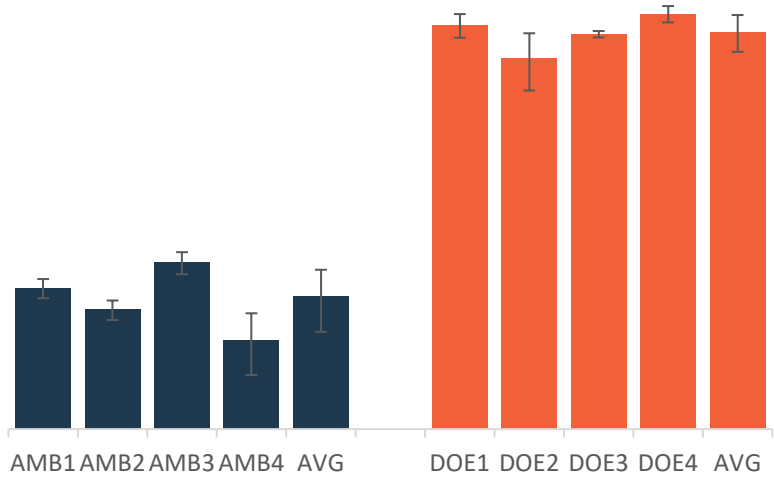
UMR 1089 TRANSLATIONAL GENE THERAPY FOR GENETIC DISEASES · CAPACITÉS · Inserm Institut national de la santé et de la recherche médicale · UNIVERSITÉ DE NANTES · CENTRE HOSPITALIER UNIVERSITAIRE DE NANTES · SARTORIUS

Case Study using Ambr® 15 and DOE for LVV Production Optimization

Rapid packaging and producer cell lines characterisation and process development for viral vector production in serum-free suspension culture.

Jakub Krakowiak, Qian Liu, Tom Payne

FACS Titre before and after 1st DOE optimisation



- **3 DOE** runs for optimization of **9** parameters
- These parameters incorporated media composition, supplements, additives, cell density, stirring speed, aeration rate/set-point, pH, and transfection-specific factors (DNA quantity, transfection reagent, and timing)
- **~10-fold** increase of overall infectious titre via DOE modelling

Source: Bioprocess UK conference poster 2018

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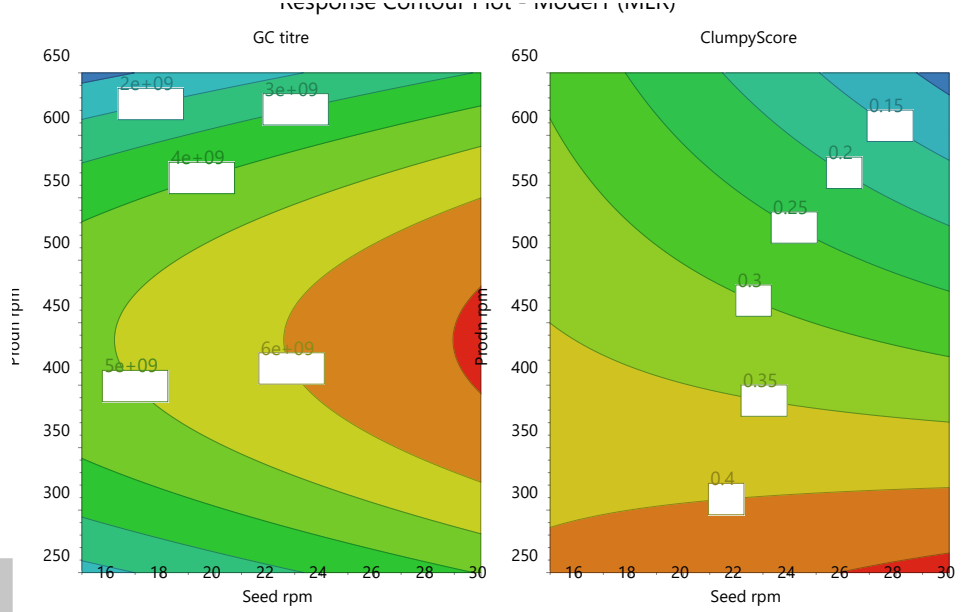
HEK293T expansion on Biostat® RM and seeding on Ambr® 250

Gyroscope Trial :

- RM 10L bag (5L working volume)
- BioPAT® Viamass
- Ambr ® 250 modular
- Combined MODDE® DOE and MVDA PCA analysis approach

Key learnings:

- Seed agitation speed may impact cell growth
- Insights for better tech transfer obtained
- Gene copy titre seems to be linked to rpm and seed
- Matching results between RM and Ambr® 250



Contour plot with best optimal zones for gene copy titre (left) and cell aggregation (right)

"MODDE ® was really useful to give a high level view of results" [1] Doug Marsh, Gyroscope Scientist

"Capacitance provides a real-time measure of cell growth. This give us a much more rapid feedback if something is wrong and offers the potential of a hands-off process operation" [1] Aline Hughson, Gyroscope Scientist

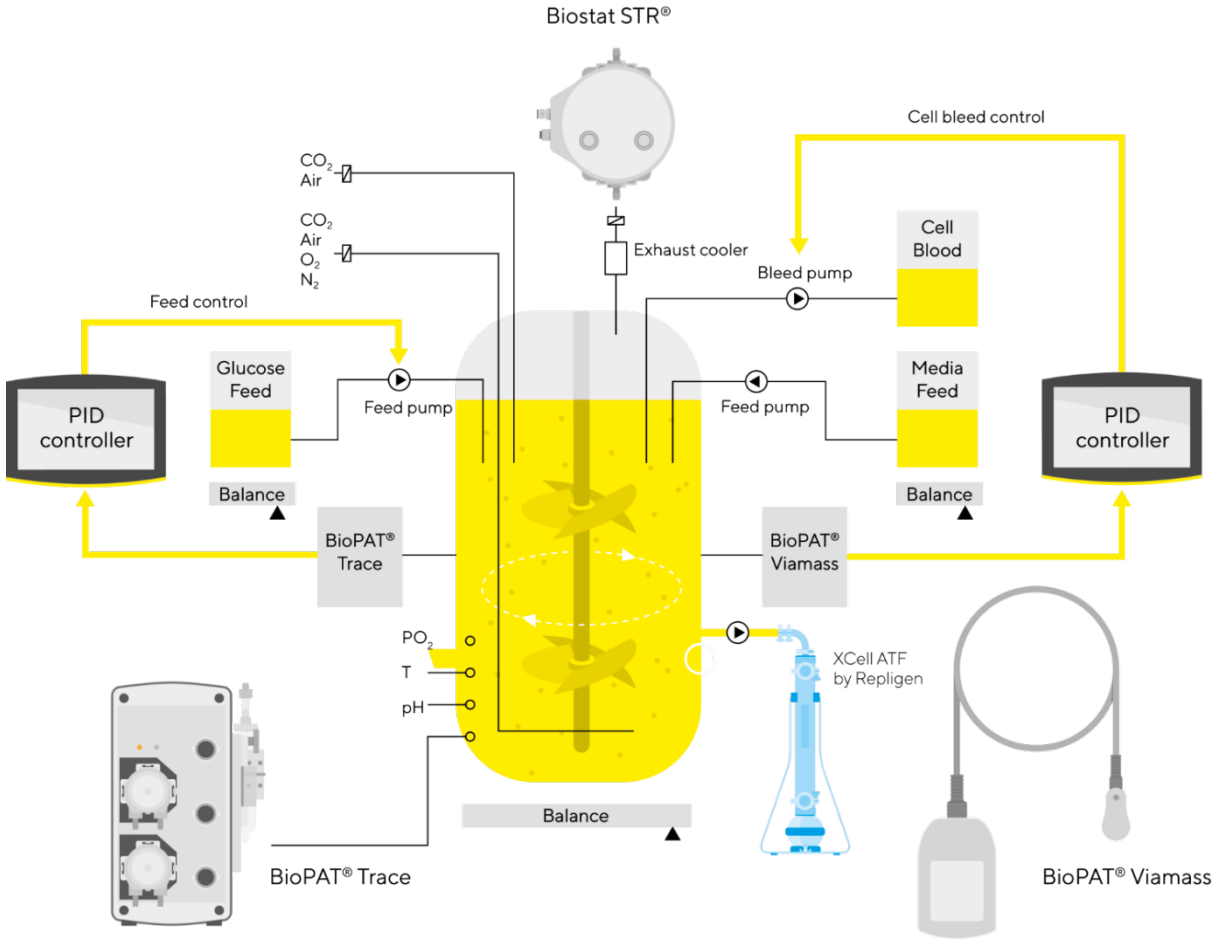
Ambr250	250	400	650 rpm	
50L SUB	90	150	240 rpm	
PPV	6	24	100	W/m^3

Public Webinar Link: https://view6.workcast.net/ControlUsher.aspx?cpak=7731529367817173&pak=2296632979355964

GYROSCOPE VISION FOR LIFE SARTORIUS

Addressing Viral Vector Challenges with Data Analytics: Process Analytical Technologies (PAT)

Real-time Data for Outstanding Process Control with BioPAT® Toolbox



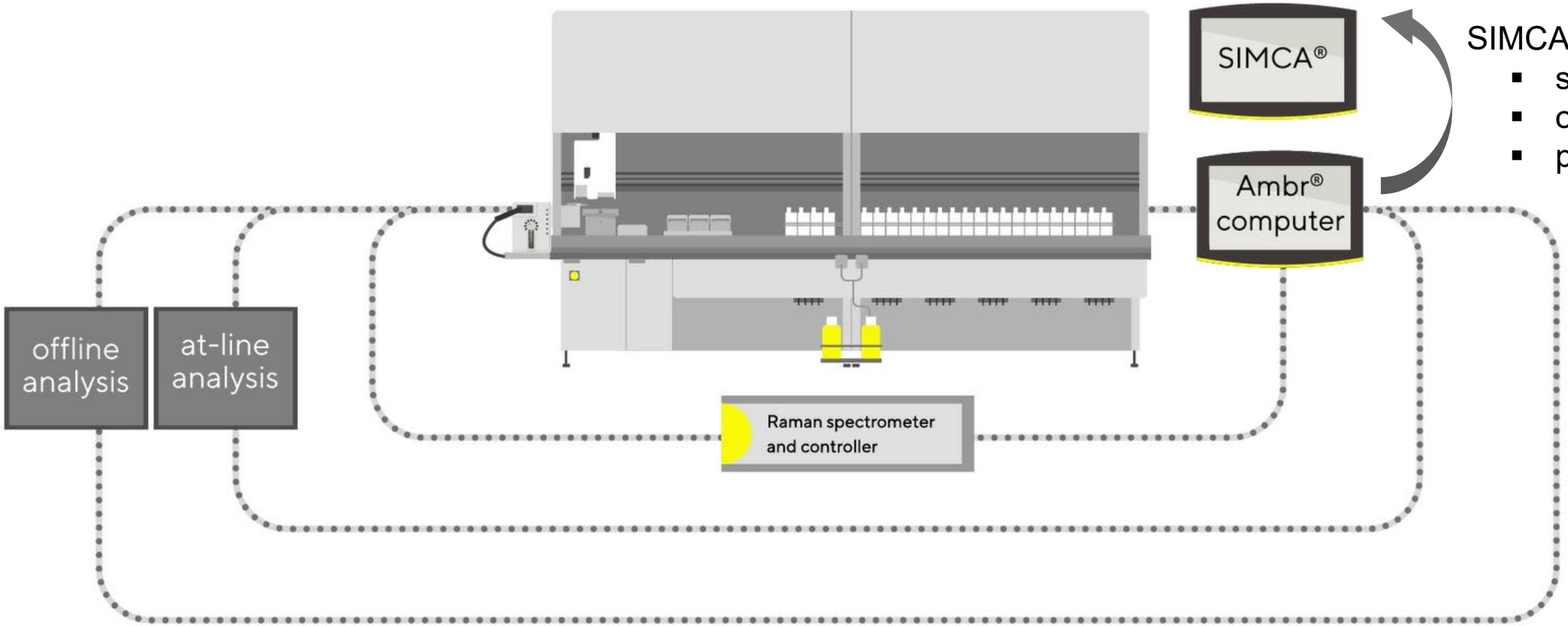
BioPAT® Spectro

- Enable Raman spectroscopy in high throughput process development
- Facilitate and improve the model building and data management process
- Full single-use integration and scalability for commercial manufacturing

With inline and online process analyzers, you can achieve:

- Robust processes
- High-quality and consistent production
- Process understanding of key production steps

BioPAT: Data Acquisition and Consolidation is Fully Automated in Ambr®

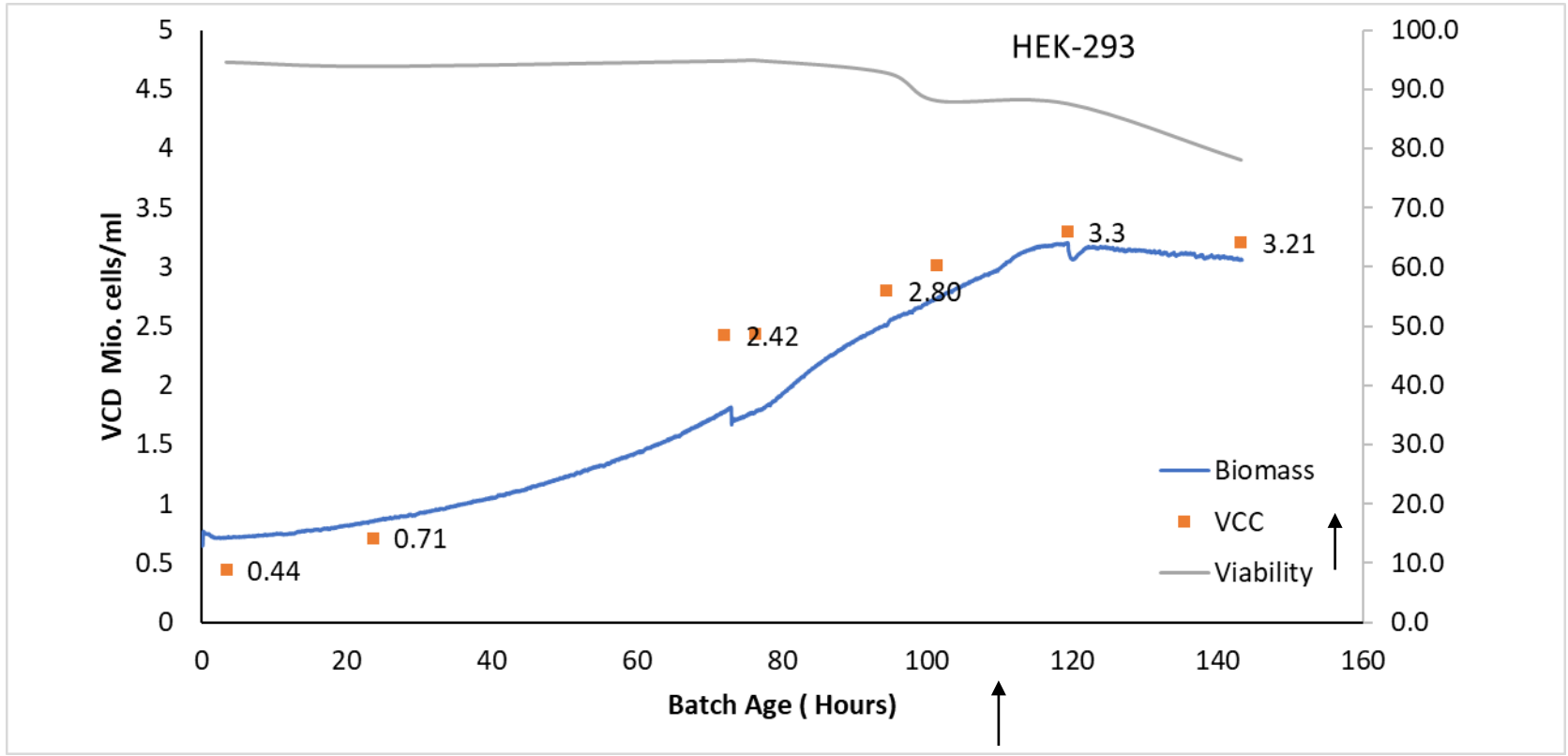


SIMCA®-ready data file
- spectral data
- offline data
- process data

After the run, a consolidated and contextualized data file can be exported from the Ambr® software, ready for model building in SIMCA®.

Industrial partner: BioPAT® Viamass

Real Time AAV Phase Monitoring and Enhanced Decision Making

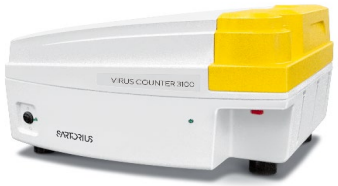


Location: Collegeville PA
200L STR Pilot Scale
AAV process
Integration of SU Viamass

Added Value:

1. Monitors cell growth throughout entire process
2. Help determine optimal transfection density/time
3. Help determine optimal time for cell harvest
4. Help determine optimal time for media refeeding
5. Easy to use

Analytics



Virus Counter® 3100

- The Virus Counter 3100 can be used to assess the viral particle Titer.

Octet

- Direct detection of virus particles on sensor; no secondary binding steps
- Automated set up and walk away
- Actual assay time on Octet system: 5 min to 60 min, depending on instrument model
- General method for all AAV serotypes – capture molecule (such as Heparin or anti-AAV antibody) can be loaded on SAX or anti-HIS or NTA biosensor to customize assay for any serotype

iQue

- Infectious titer is the more relevant lentivirus titer: measure functional viral particles (particles of interest) by transducing cells and measuring transgene | reporter gene expression
- iQue flow cytometer is the ideal instrument for infectious titer determination
- Forecyt software : designed to process whole plates of data, creating a simplified, interactive workflow where all the analysis, visualization and interpretation tools are integrated together

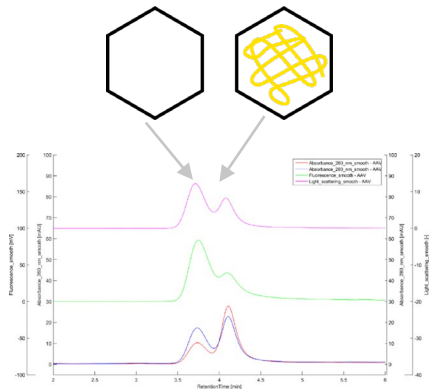
HPLC Analytics using CIMac ™ Monolith Columns

Fingerprinting Methods	▪ Information about the chromatographic profile of your sample (purity/impurity)
	▪ No exact quantitative data
	▪ Use for decision making and process optimization:
	  ▪ When to stop fermentation
	  ▪ When to stop collection of chromatography sample | component cuts
	  ▪ Is gradient type correct?

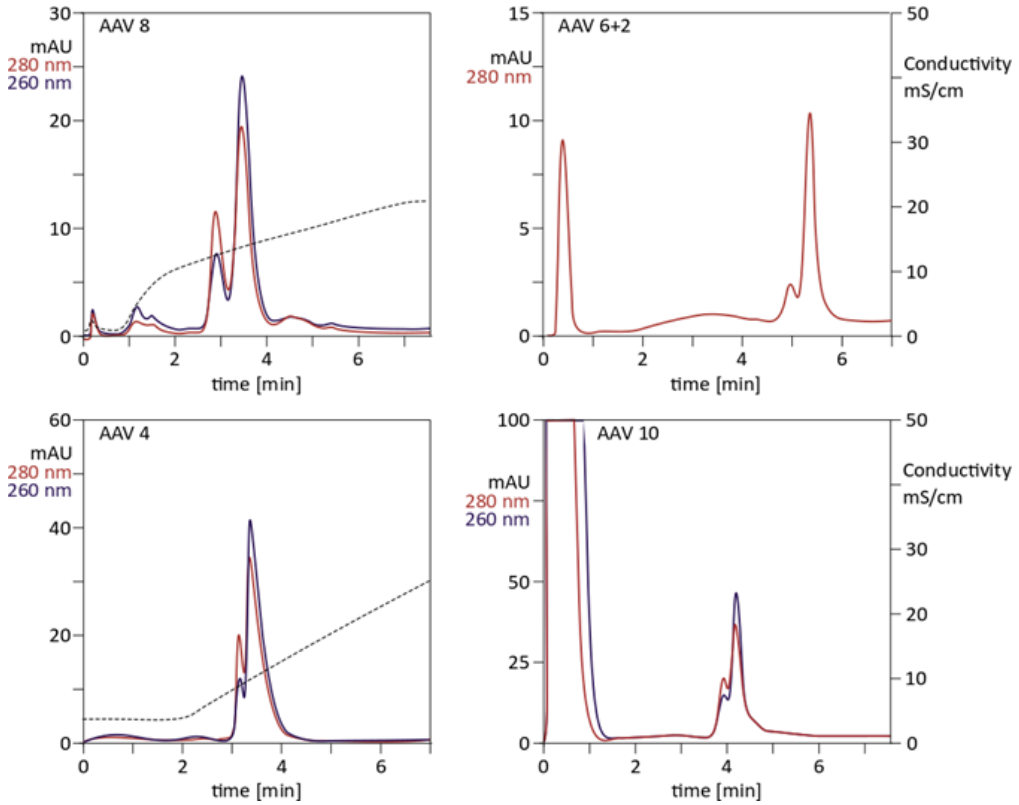
Quantification Methods	▪ Purified biomolecule preparations - standards with a known concentration:
	  ▪ Linear range
	  ▪ LOQ and LOD
	  ▪ Repeatability, reproducibility

Fast Platform Analytics with PATfix™: E/F Analytics

CIMac™ AAV for rapid analytical separationof full and empty capsids.

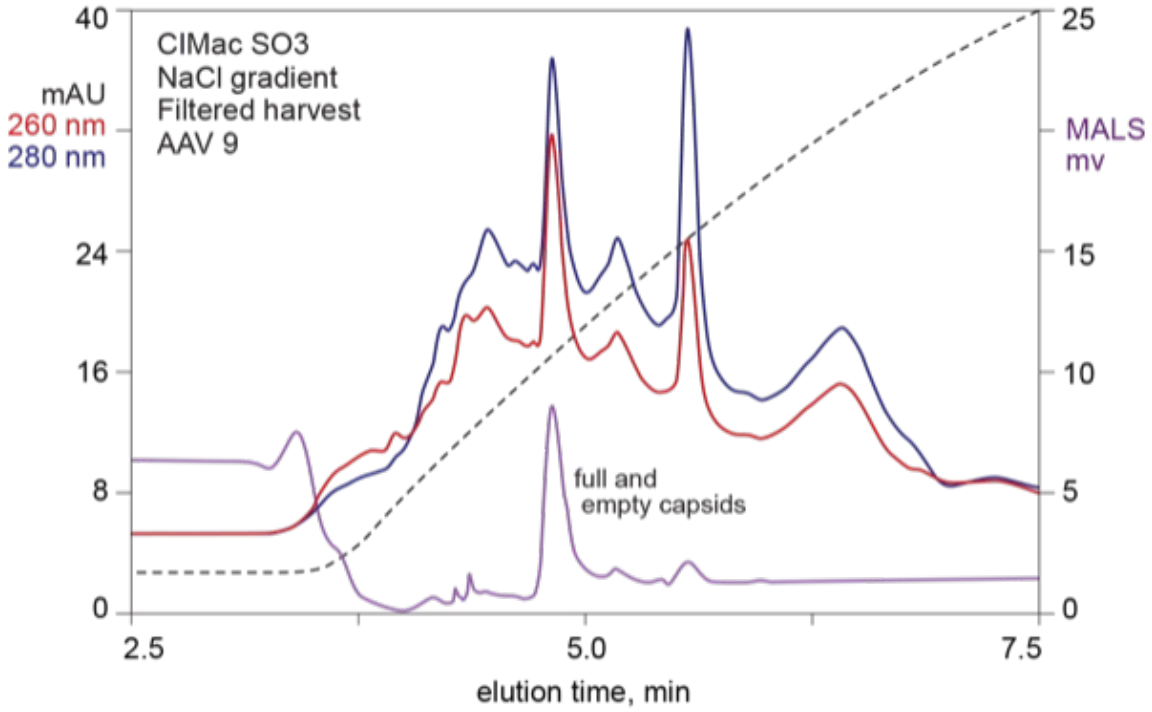


- Separation between empty and full AAV capsids is transferable between majority of AAV serotypes with minimal method modification.
- CIMac AAV enables hight throughput analytics of the samples. CIMac AAV column can be also used for initial process optimization.



Use of Multiple Detectors: Total AAV Characterization in Clarified Harvest

- AAVs scatter light more intensively compared to other smaller species in complex mixtures.
- MALS detector DOEs not pick up signal from smaller components, thus increasing signal specificity
- Straightforward estimation of total AAV titer directly in clarified harvest is possible
- No tedious sample preparation



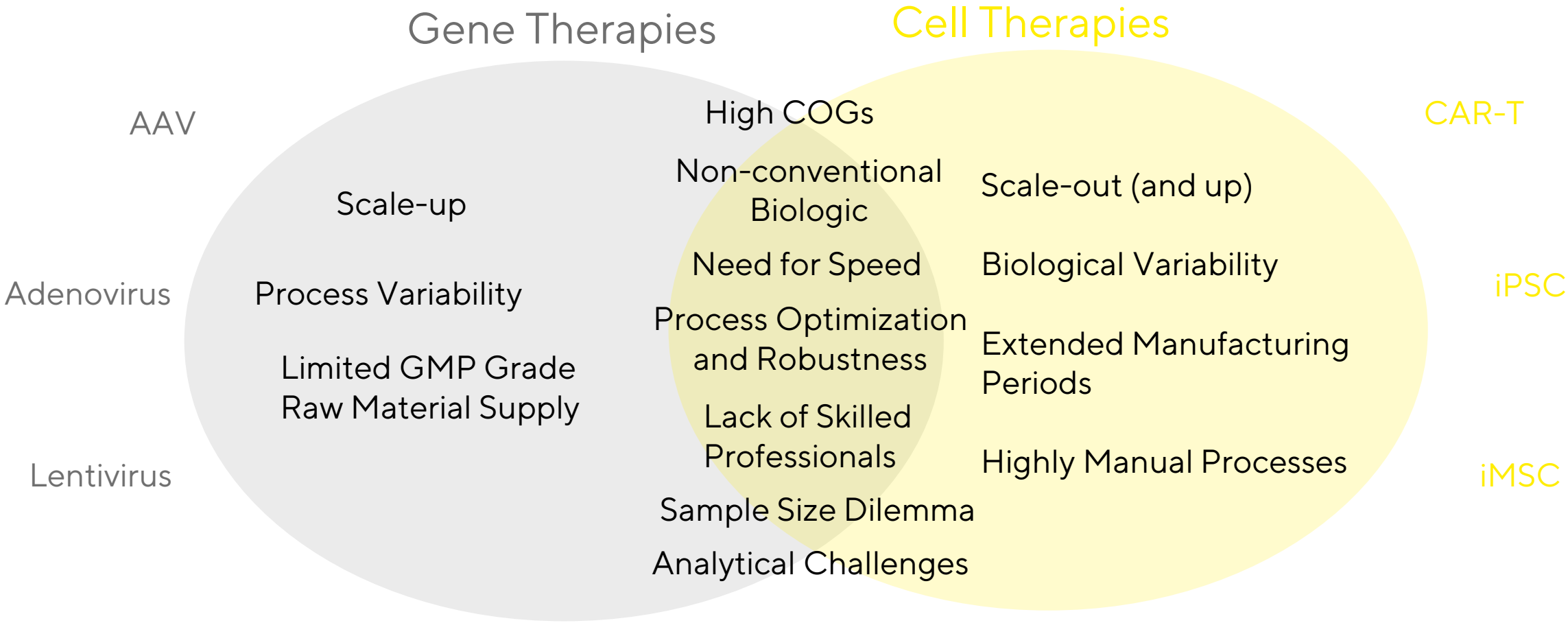
Increased Focus on Resilience Driving Biopharma 4.0

- Drug Discovery & Development
- Taking Product to Scale
- Facility Retrofitting or Design
- Foster Improvements in Manufacturing
- Supply chain and Logistics

Equipment	PAT	Data Analytics
 Ambr® 15	 BioPAT Trace	 MODDE® DOE
 Ambr® 250 HT	 BioPAT® Viamass	 SIMCA® MVDA
 Benchtop Glass Bioreactor, RM Bioreactor		
 Biostat® STR Bioreactor	 BioPAT® Spectro	 SIMCA®-online

Areas Where CDMOs Can Add Value to CGT Processes

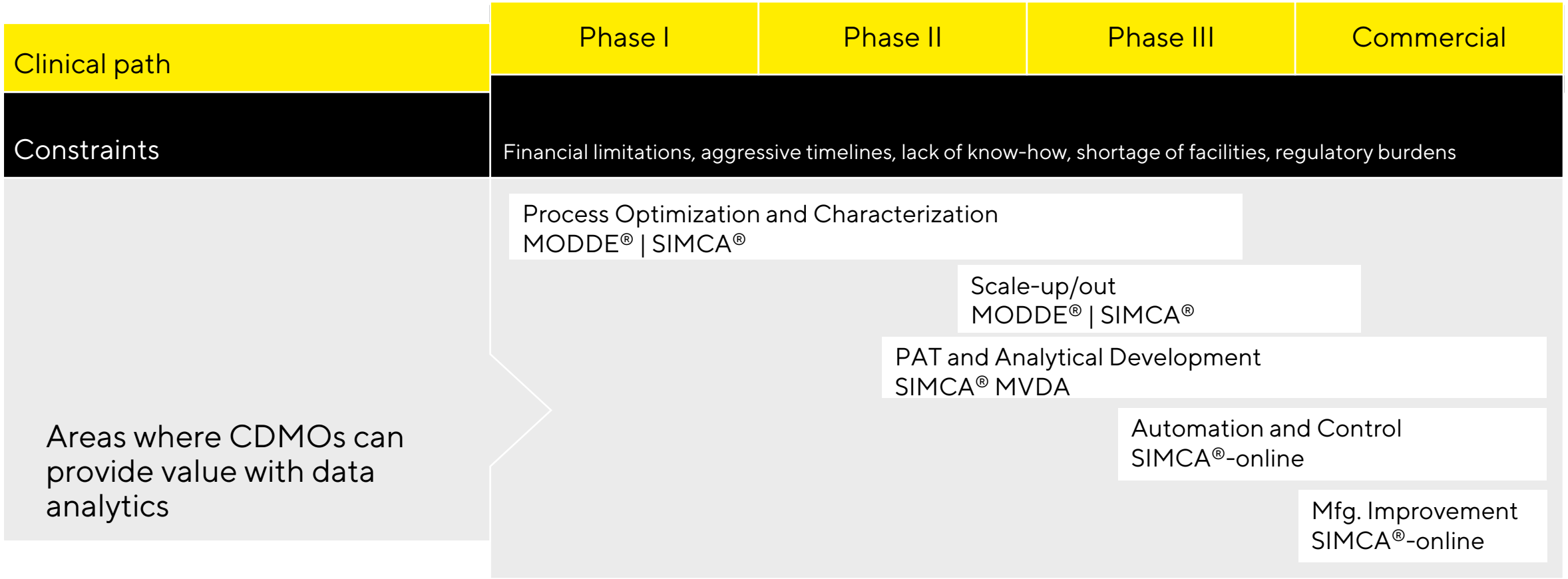
Cell and Gene Therapy Companies Have Common Challenges



Cell and Gene Therapy Facility of the Future

	Process Development	Manufacturing
Opportunities	▪ Optimization of processes ▪ Reducing COGs ▪ Scale-up/out	▪ Raw Material Testing ▪ End-to-end Monitoring ▪ Adaptive Control ▪ Rapid Product Release
Challenges	▪ Knowledge ▪ Validation	▪ Data capture, processing, and interpretation ▪ Systems
Solutions	▪ Increase PAT integration ▪ DOE and MVDA	▪ Automation ▪ MVDA, RT-MVDA (real-time), MPC (Model Predictive Control)

Where Can CDMOs Provide Value?



Thank you!

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Want to Learn More About the Use of Data Analytics at CDMOs?
https://landing.umetrics.com/en/cdmo