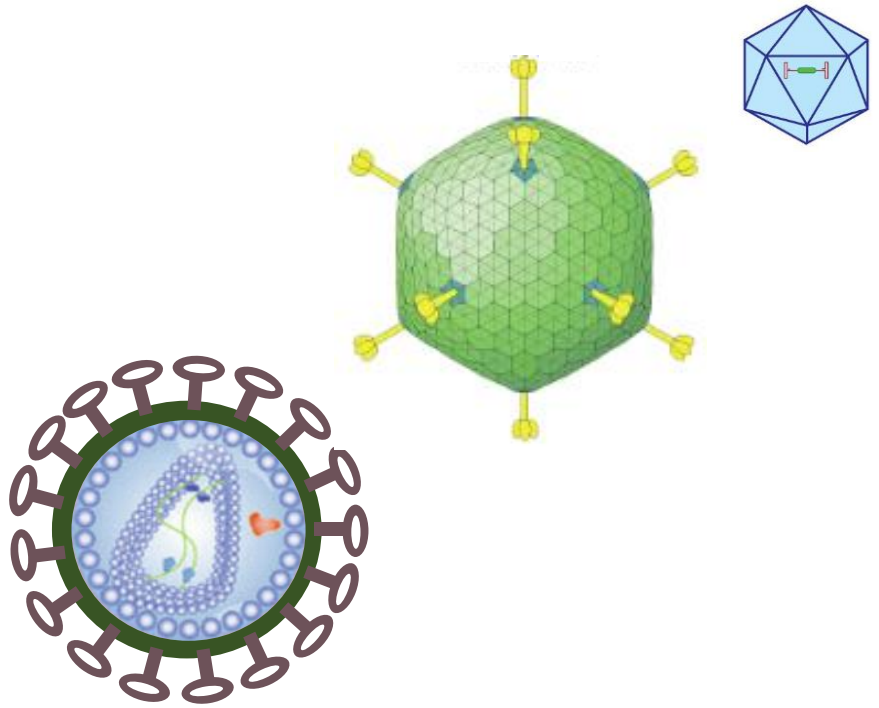
Integrating Process and Analytics

A Prerequisite to Streamlining the Production of Viral Vectors



AMITABHA DEB, Ph.D.
Founder and Principal Consultant,
CellGene Consulting LLC.

Outline

- CMC challenges in CGT Industry
- Case studies*: Manufacturability and Integration of Process & Analytics
  - Titer improvement: *Case Study #1*
  - Aggregation-free downstream process: *Case Study #2*
  - Process parameter screening (for microfiltration): *Case Study #3*
  - Optimization of TFF / Concentration of LVV: *Case Study #4*
  - HT screening of Chromatography resins: *Case Study #5*
  - Improving stability of LVV: *Case Study #6*
- Summary

*Lentiviral vectors for CAR T therapy; Some of the CMC challenges exist for AAVs & other vector types

Cell Gene Consulting : My Journey

Provide CMC guidance and Innovative Solutions for a Stable Supply of Viral Vectors

Clinical and Commercial development with CDMO & external collaborators:
Oxford BioMedica, AGC, Lonza, Catalent, Thermo Fisher, Aldevron

1990s
Cloning and Characterization of of JAK/STAT (IFN) & Cellular Antiviral Pathways (PKR)
Lab of BRG Williams, Ph.D.

1994 - 2001
Intervention of Antiviral Response pathways & Use of antisense Nucleotides for Therapeutic use
Amitabha Deb & BRG Williams.

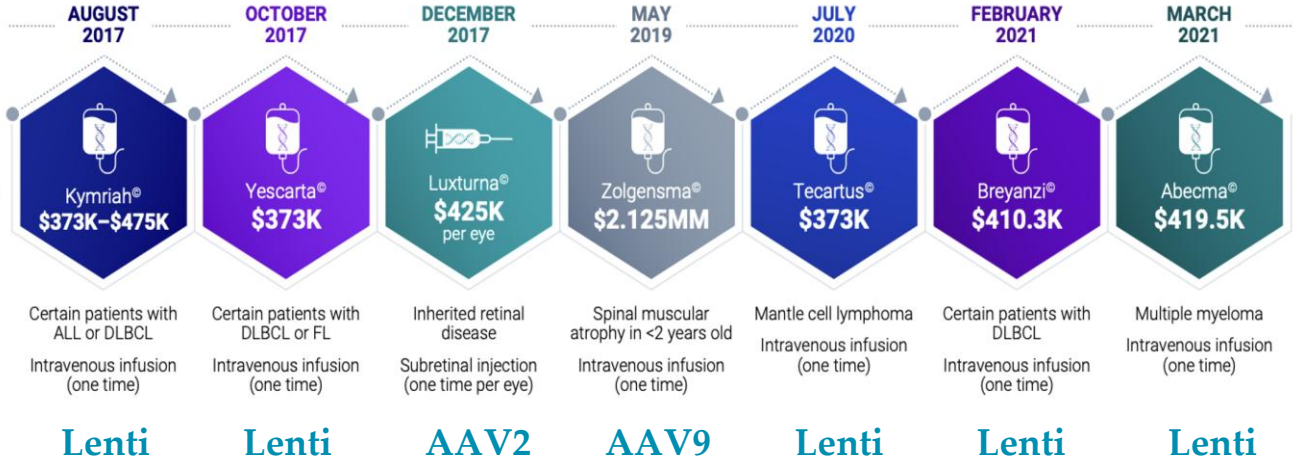
2001 - 2014
Develop Clinical & Commercial Processes for Biologics: Cosentyx, Zinplava, Rabishield, & AAV products.

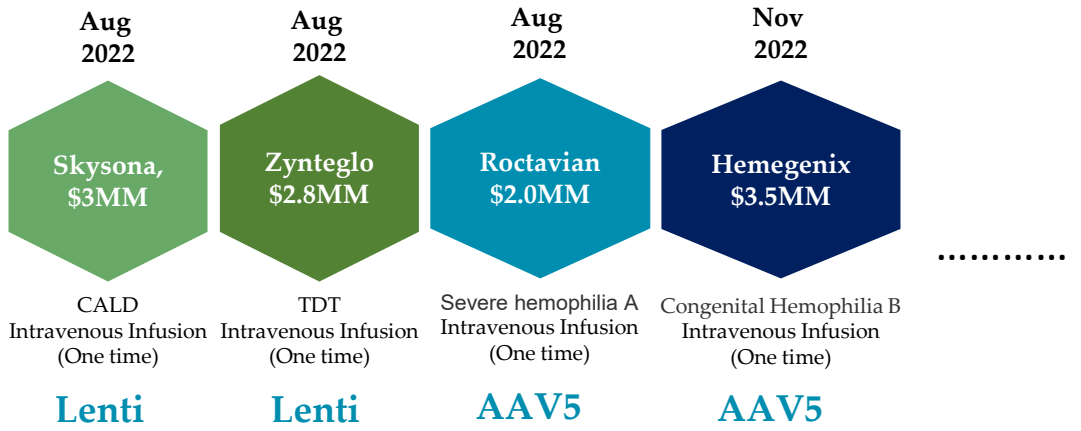
2014 - 2024
Lead Lentiviral Vector development for **Kymriah**, the first Gene therapy approved Product; **Pivotal patent** on LV characterization; Develop In vivo and ex-vivo LV programs for Phase I-III
Amitabha Deb & Eugene Nebelitsky

2019, CellGene Consulting
Provide CMC guidance to develop the next-Gen vector production:
A Single platform for biomanufacturing

Problem I: CGT Industry

A Need for Cost Reduction of Viral Vectors





Lenti: Ex-vivo Cell therapies
AAV: in vivo Gene Therapy

- ✓ Favorable Risk-benefit profile
- ✓ Highly Efficacious
- ✓ One-time Innovative Therapies
- ✓ Small patient indications, except Breyanzi & Abecma

Viral Vectors:

- Complex manufacture, high % of batch failures; challenging scale up
- Sourcing challenges of viral vectors impacting Cell therapy yield and quality

Expensive to Manufacture with a low margin of profit

Large COGs Sold and High Selling Prices

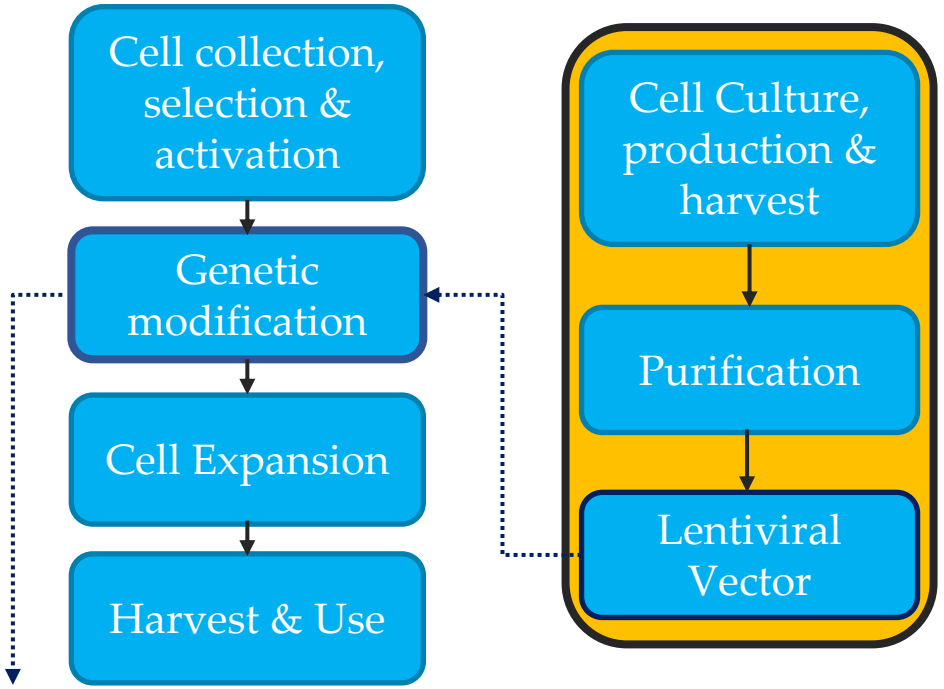
Market Dynamics Combined with Manufacturing Complexity

Profit on $475,000 Novartis cancer drug could be a while coming

By John Miller 5 MIN READ

ZURICH (Reuters) - Novartis's new gene-modifying cancer therapy's $475,000-per-patient sticker price has drawn fire from advocate groups calling for cheaper drugs, but analysts said the Swiss drugmaker could initially struggle to break even.

CAR-T therapy



Target ≤ 10% ⟷ Need for Scalable processes with high process yield & improved Quality ⟷ **25% - 30% ($45K - $70K)**

Vector COGs

Assuming $250K - $380K for per CAR T dose

Decentralized manufacturing, Unlocking CAR-T's potential
An innovative approach to bring therapies closer to patients
By Bonnot et. al. 2024

Problem II: CGT Industry

Increasing Demands for Gene Therapy Vectors

Market is not ready to address a high-dose/large patient/ in-vivo indications

100's of patients/year

50,000 of patients/year

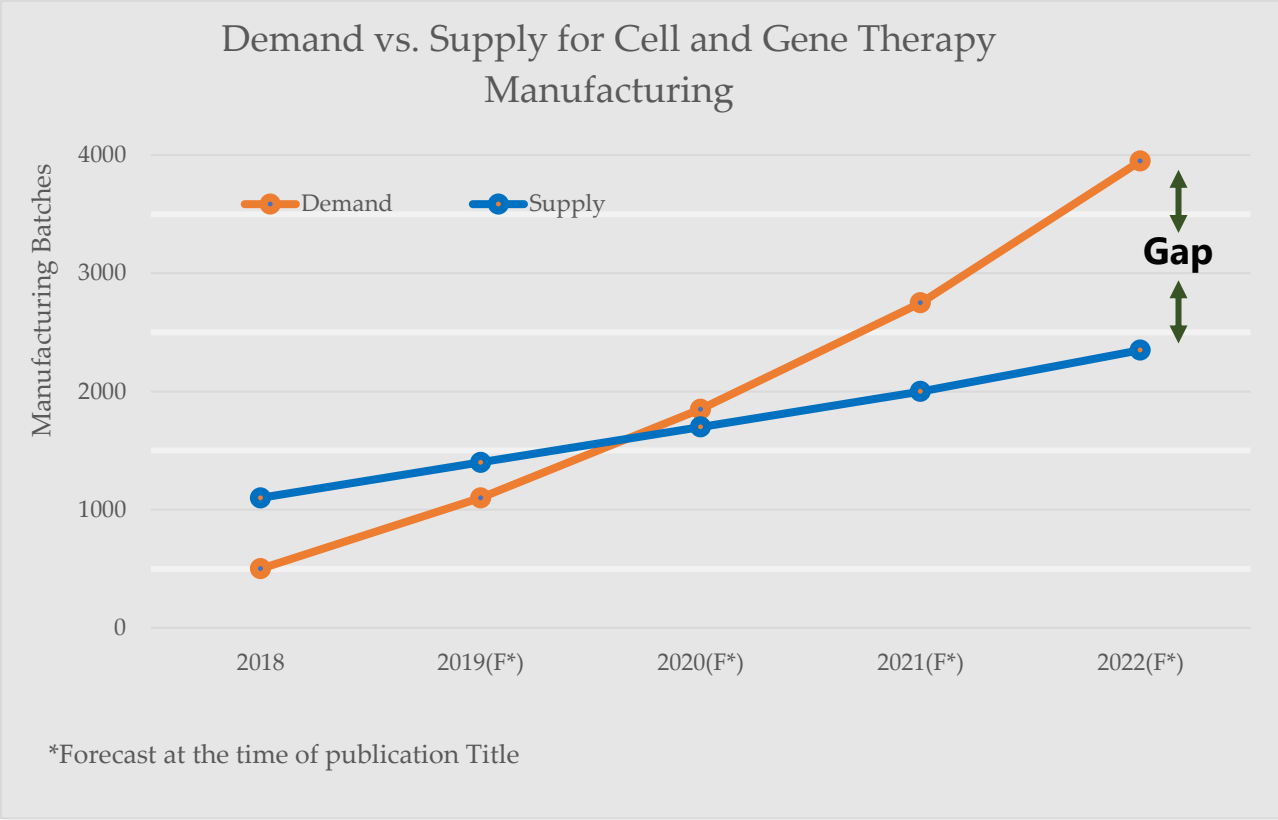
Lentiviral and gamma-retroviral vectors

Indication and dosage (AAVs)	Patients/ year	Vg*/ year	Cell culture vol./Year**
Retinal dystrophy Luxturna (1.5E11 vg**/eye)	50	1.5E13	**~ 0.3 L**
Spinal Muscular atrophy Zolgensma (1E14 vg/kg, 5 kg/patient)	1000	5.0E17	**~ 9000 L**
Hemophilia A (3E13 vg/kg, 40 kg/patient)	5000	6.0E18	**~ 100,000 L**
Duchenne muscular dystrophy (2E14 vg/kg, 40 kg/patient)	5000	4.0E19	**~ 700,000 L**
Solid tumor cancer (2E14 vg/kg, 80 kg/patient)	25000	4.0E+20	**~ 6,700,000 L**

*vg: Viral genome, ** Process assumptions, AAV: 2E14 vg/L virus titer in cell culture, 30% overall process yield

Problem III: CGT Industry

Gaps between Vector Supply and Demands



Adapted from BioProcess Int, Nov-Dec 2019, 17 (11-12)s

- Lack of platforms and solutions while struggling to catch up with material demands

Experienced CDMO's with the right capabilities are required who can accelerate drug development, streamline scale up, respond quickly to capacity changes, tap into drug and process development expertise, and more ….

Notes: We have not seen the same level or the rapid adoption and scale to support the widespread commercialization as reported in 2019

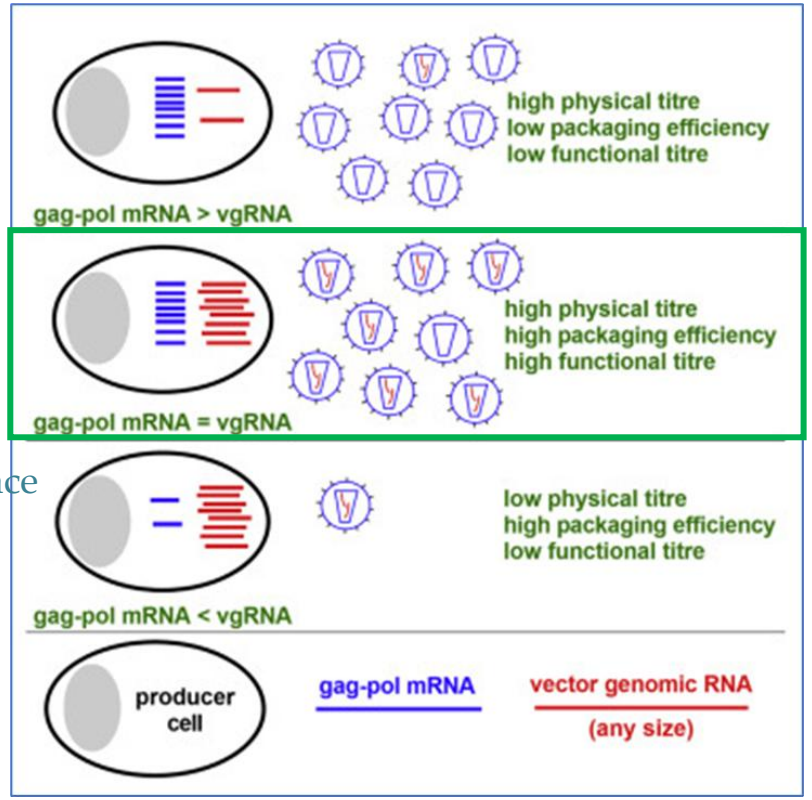
Rapid Optimization for optimal titer and product Quality

Case Study #1, Upstream Process Development, LVV

- DOE approach: It can be extensive and time-consuming
- Alternative to DOE: Platform approach (Leveraged in this study)
  - **Preestablished Design Space from historical data reported from Clinical and Commercial LV programs**
    - GOI of different sizes, including GFP, CAR 19, and three other GOIs (Sizes <1 Kbp - 8 Kbp)
    - Packaging plasmids are different (Similar sizes; codon vs. non-codon optimized, In-house vs. CDMO provided)

  → Few plasmid ratios tested in an OFAT design from previously defined design space (next slide): Rapid screening and scale up

Notes: Plasmid ratios is just one of the upstream parameters that may require optimization. Other parameters such as cell lines, cell density at transfection, transfection reagents etc. can be optimized in separate experiments and kept constant as a part of platform development



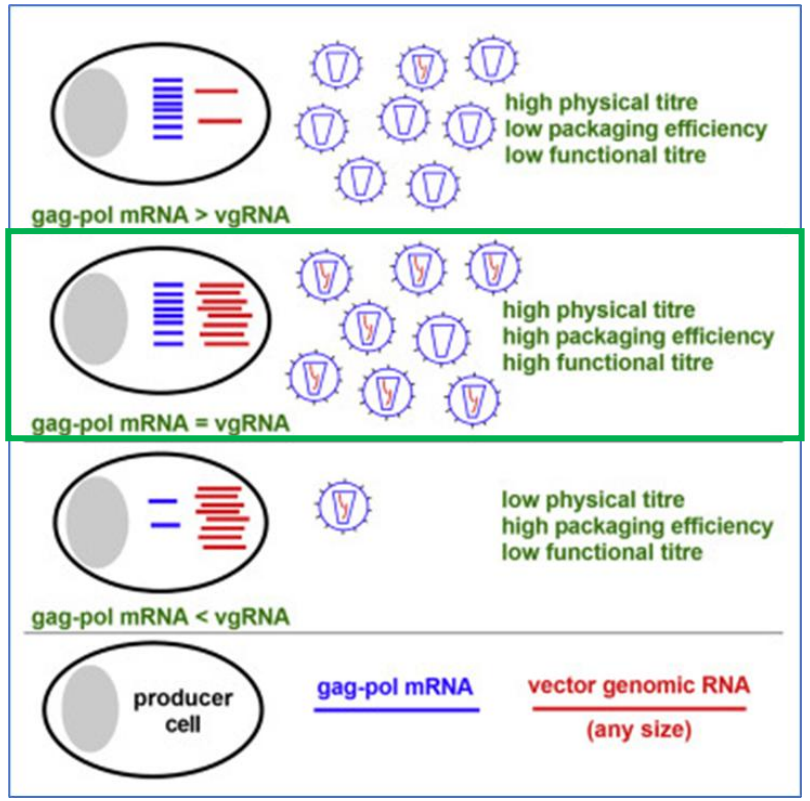
Optimal ratio of GOI and packaging components (Gag-pol, Rev, and VSVG)

- Develop your CMC (& regulatory) strategies and objectives early during preclinical development

Optimizing Plasmid Ratios to Increase Functional Titers

Rapid scale up from 30 ml to 2L for process confirmation; Case Study #1, Contd.

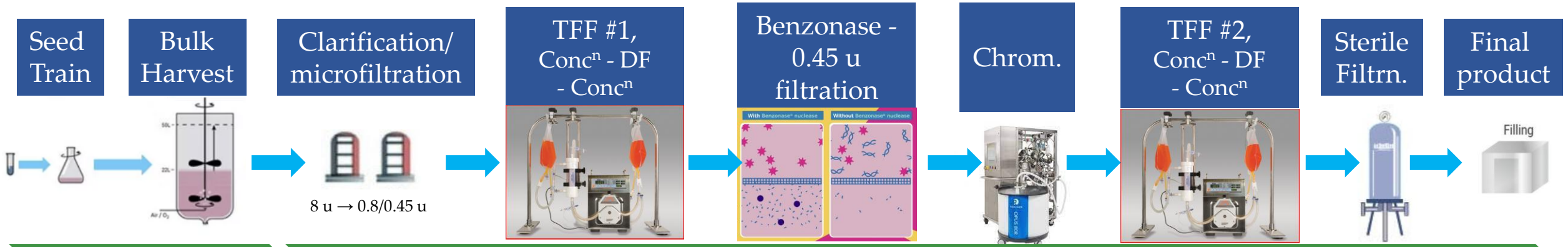
Arm	Plasmid Ratio Gag/Pol : REV : VSVG : GoI	ddPCR (TU/mL)	(Non-Infectious + Infectious) : Infectious
1	Diverse Plasmid ratios from preestablished design space	8.1E+06	1480 : 1
2		5.4E+06	2600 : 1
3		5.1E+06	4500 : 1
4		1.2E+07	1160 : 1
5		1.9E+07	1420 : 1
6		6.9E+06	2600 : 1
7		1.4E+07	1570 : 1
8	1 : 1 : 1 : 5	1.9E+07	200 : 1
9	Diverse Plasmid ratios from preestablished design space	1.7E+07	1650 : 1
10		1.2E+07	2400 : 1
11		1.1E+07	6800 : 1
12		7.7E+06	16800 : 1
13		1.6E+07	1940 : 1
14		1.1E+07	8100 : 1



Optimal ratio of GOI and packaging components (Gag-pol, Rev, and VSVG)

Overall Process time & yield for LVV

Case Study #2: Aggregation-free purification steps improves process yield



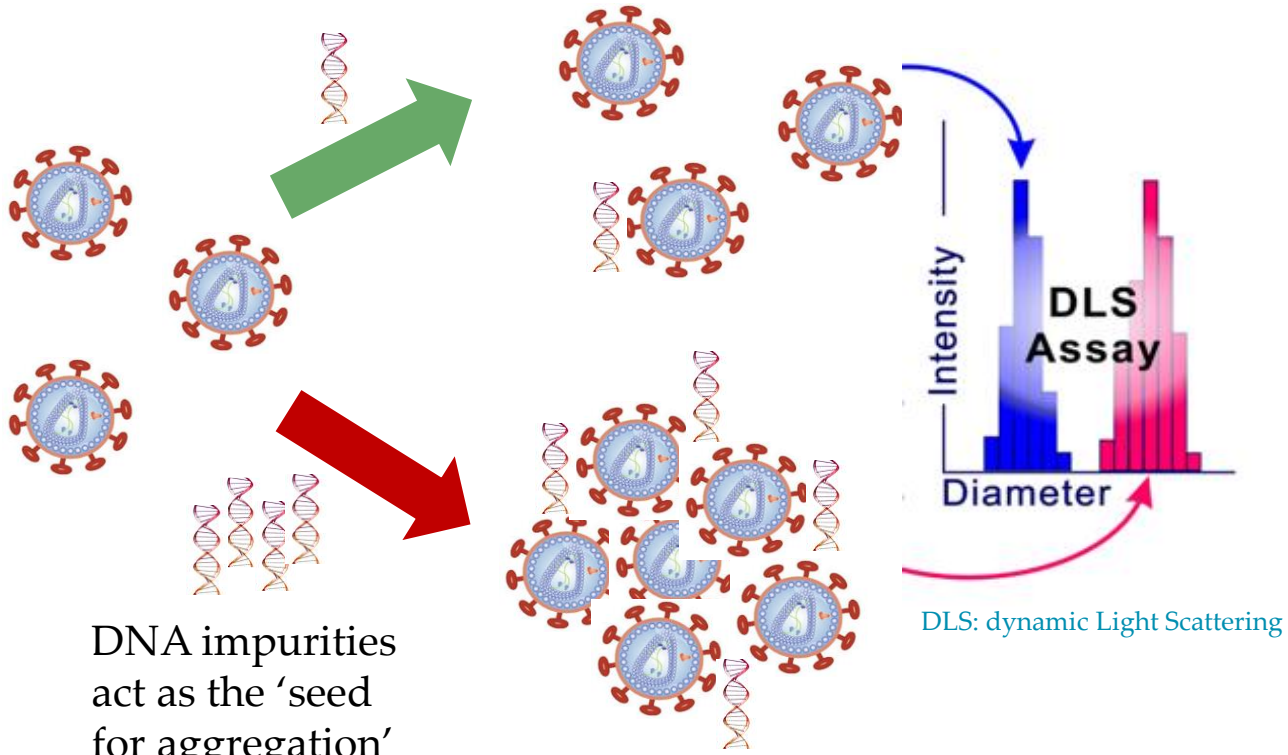
GFP - LV	Bulk treated	Clarified	TFF #1 (100 x concentration)	Benzonase (± filtration)	Chrom.	Sterile filtration	Post fill-finish
Titer (TU/mL)	1E+07	0.88E+07	0.79E+09	0.63E+09	0.44E+09	0.35E+09	0.28E+09
Overall yield (%)	100	88	90	80	70	80	80

Final yields are transgene dependent; A final concentration factor of x100 is desired to reach low - med E+09 TU/mL for the LV product.
Short process time due to instability of LV; TFF operation may need two different systems; exchange to LV stabilizing buffer (physiological pH & NaCl + excipients) is required during early steps to increase stability & minimize aggregation

Overall process yield of GFP-LVV: 28% with no aggregation

*5 - 20% with aggregated LV is a commonplace

LVV Aggregation is directly related to process performance



DNA impurities act as the 'seed for aggregation'
(* Reported for AAV and LV)

DLS: dynamic Light Scattering

- **LVV being lipid enveloped virus is sensitive to temp, shear, pressure, salt, pH etc. faced during biomanufacturing**
  - Formation of LVV aggregates

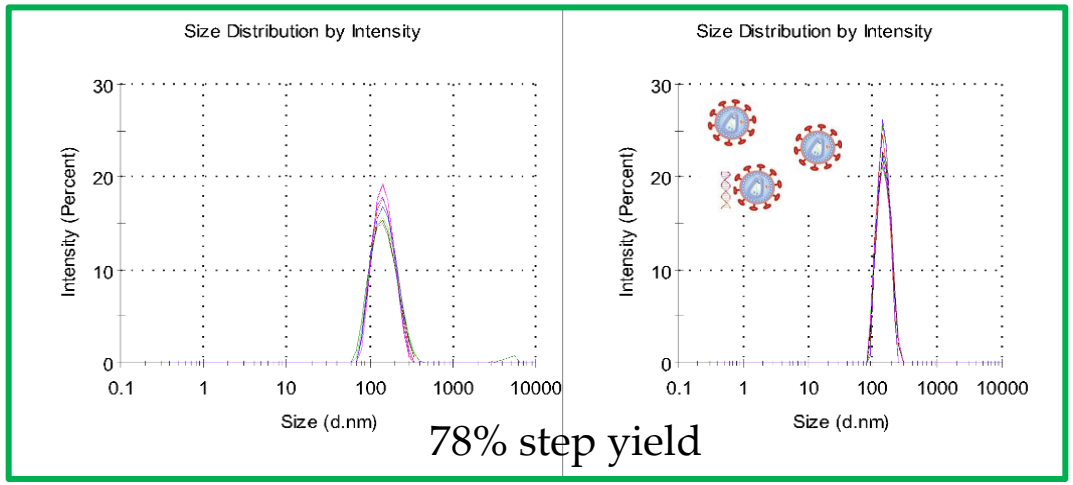
Problem:

- Fouling of the filtration steps
- Batch/Production failures: Loss of valuable Product & Increase in development time + COGs
- Impact of potency: Unpredictable

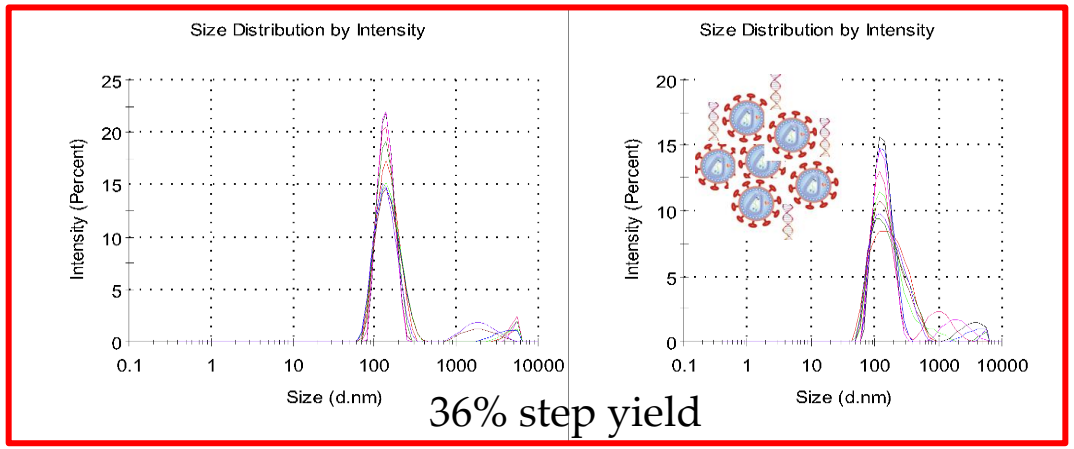
- Define your product and deep dive into your CMC program to identify gaps and risks
  - Interact with Health authorities to mitigate risks in your CMC program

LVV Aggregation is directly related to process performance

Case Study #2, Contd.



78% step yield



36% step yield

(10) International Publication Number
WO 2017/087861 Al

Amitabha Deb, et.al.
Kymriah-related patent,
Novartis Pharma

- No Aggregation of LV
- Hydrodynamic radius: ~ 100 nm
- Sterile Filtration: **Step recovery 78%**

- The patent only focused on Final formulation & DLS data for trend analysis

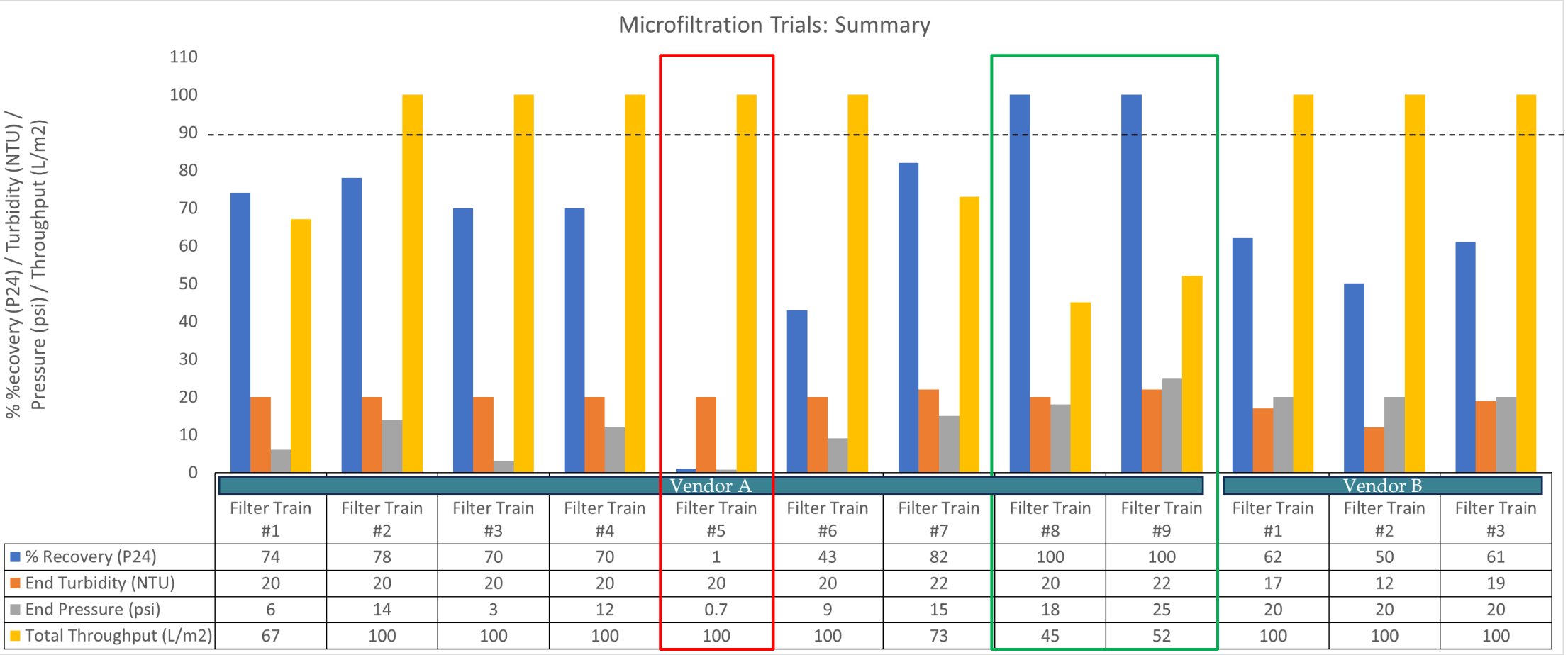
→ The scope of using DLS as a Process Analytical Technology has not been considered

- Aggregating LVV, Filter fouling and experience high pressure during sterile filtration
- Sterile filtration: **Step recovery 36%**

(Overall process yield <10% resulting in production batch failure)

Multiple-parameter screening for microfiltration

Case Study #3



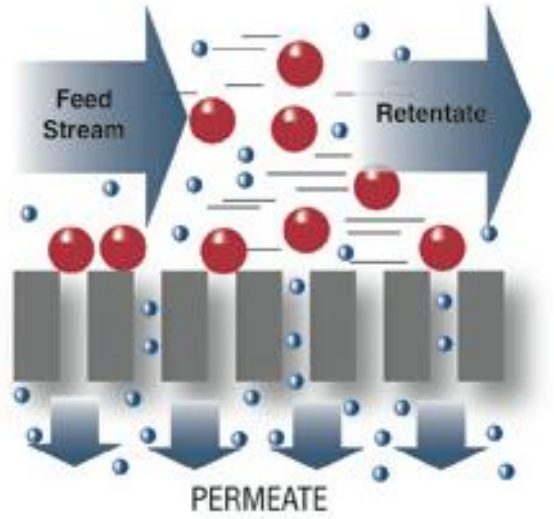
	Filter Train #1	Filter Train #2	Filter Train #3	Filter Train #4	Filter Train #5	Filter Train #6	Filter Train #7	Filter Train #8	Filter Train #9	Filter Train #1	Filter Train #2	Filter Train #3
% Recovery (P24)	74	78	70	70	1	43	82	100	100	62	50	61
End Turbidity (NTU)	20	20	20	20	20	20	22	20	22	17	12	19
End Pressure (psi)	6	14	3	12	0.7	9	15	18	25	20	20	20
Total Throughput (L/m2)	67	100	100	100	100	100	73	45	52	100	100	100

- Rapid microscale process development using surrogate analytical outputs
- Filter #8 and #9 tested in limited scale-up with diverse GOI (GFP and CAR19)

Tangential Flow Filtration Development

Case Study #4

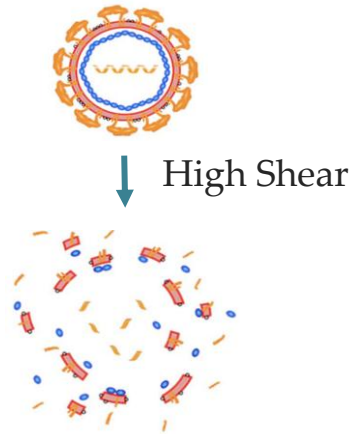
- **Shear Rate (s^{-1})**
  - ≥ 4000 s^{-1} provides sweeping action across the membrane
- **Transmembrane Pressure (TMP)**
  - TMP = Driving force; drives small MW particles into Permeate
- **Opportunity to:**
  - Concentrate & diafilter into a stabilizing buffer
  - Remove host cell impurities due to 500 kDa MWCO membrane
- **Caution:**
  - High shear rates negatively affect LVs



Tangential (Cross) Flow Filtration

Determining the 'edges of failure' rapidly for TFF

Case Study #4, Contd.



Question:

Can we concentrate 100x concentration with high recovery & impurity clearance?

- Sample: Transient Transfection, Benzonase-treated Harvest 100 U/mL, 0.45 µm clarified
- Shear rates: 3000-5000 s^{-1}
- Hollow Fiber: 500 kDa MWCO, mPES
- TMP: 5 psi
- **Procedure: 10x UF → 7x DF → 10x UF**
- Desired processing time: < 5 hrs.
- Target concentration: low-mid E9 TU/mL

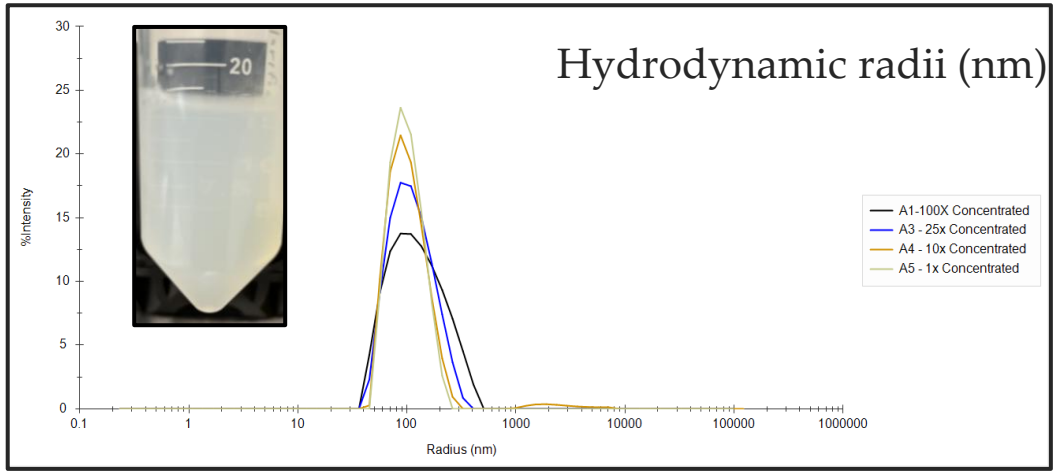
Shear rate (sec^{-1})	% Recovery (Infectious Titer)	% HCP Recovery (BCA Assay)	% DNA Recovery (Picogreen Assay)
3000	74	ND	ND
4000	90	~ 5	~ 11
4500	95	~ 5	~ 11
5000	79	ND	ND

- **Does 100x concentration induce LV aggregation?**
- **Changing stabilizing buffer/pre-formulation early in the process is essential**

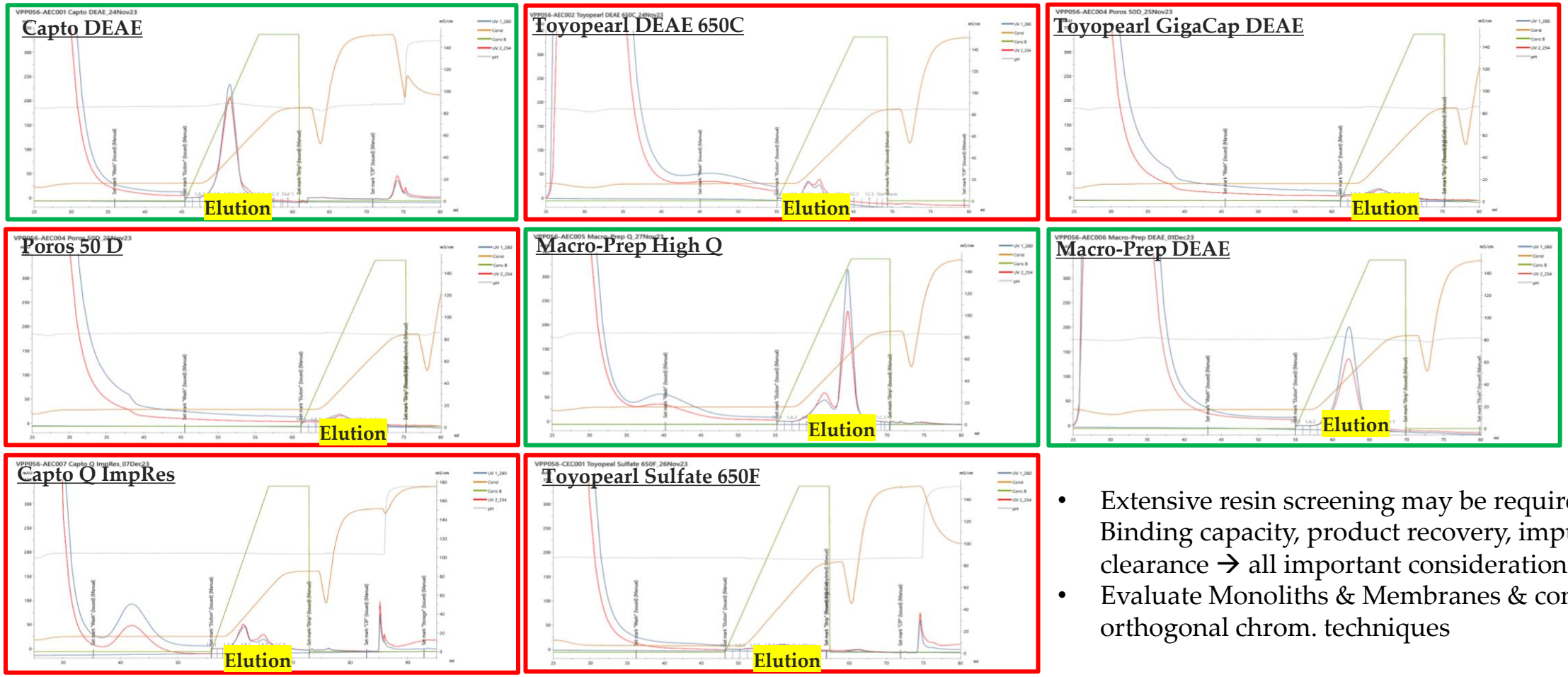
100x Concentration does not lead to LVV Aggregation

Case Study #4, Contd.

Concentration Factor	Hydrodynamic Radius (nm), LV-GFP	% Polydispersity (10 acquisitions)
1x Concentrate	102.6	21.0
10x Concentrate	105.6	25.6
25x Concentrate	99.1	19.7
50x Concentrate	96.8	20.4
100x Concentrate	101.9	26.4



Address Process attributes and DSP capability gaps early during preclinical development: Case Study #5

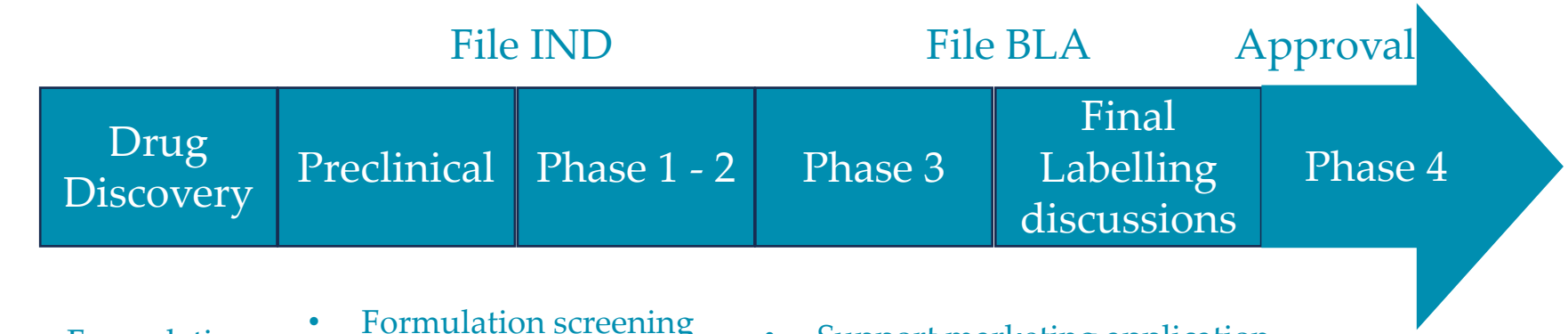


- Extensive resin screening may be required. Binding capacity, product recovery, impurity clearance → all important considerations
- Evaluate Monoliths & Membranes & consider orthogonal chrom. techniques

Develop modular steps screening to enable a 'platform' approach → Product and Impurity mapping

Testing LVV stability/aggregation throughout the lifecycle of the product

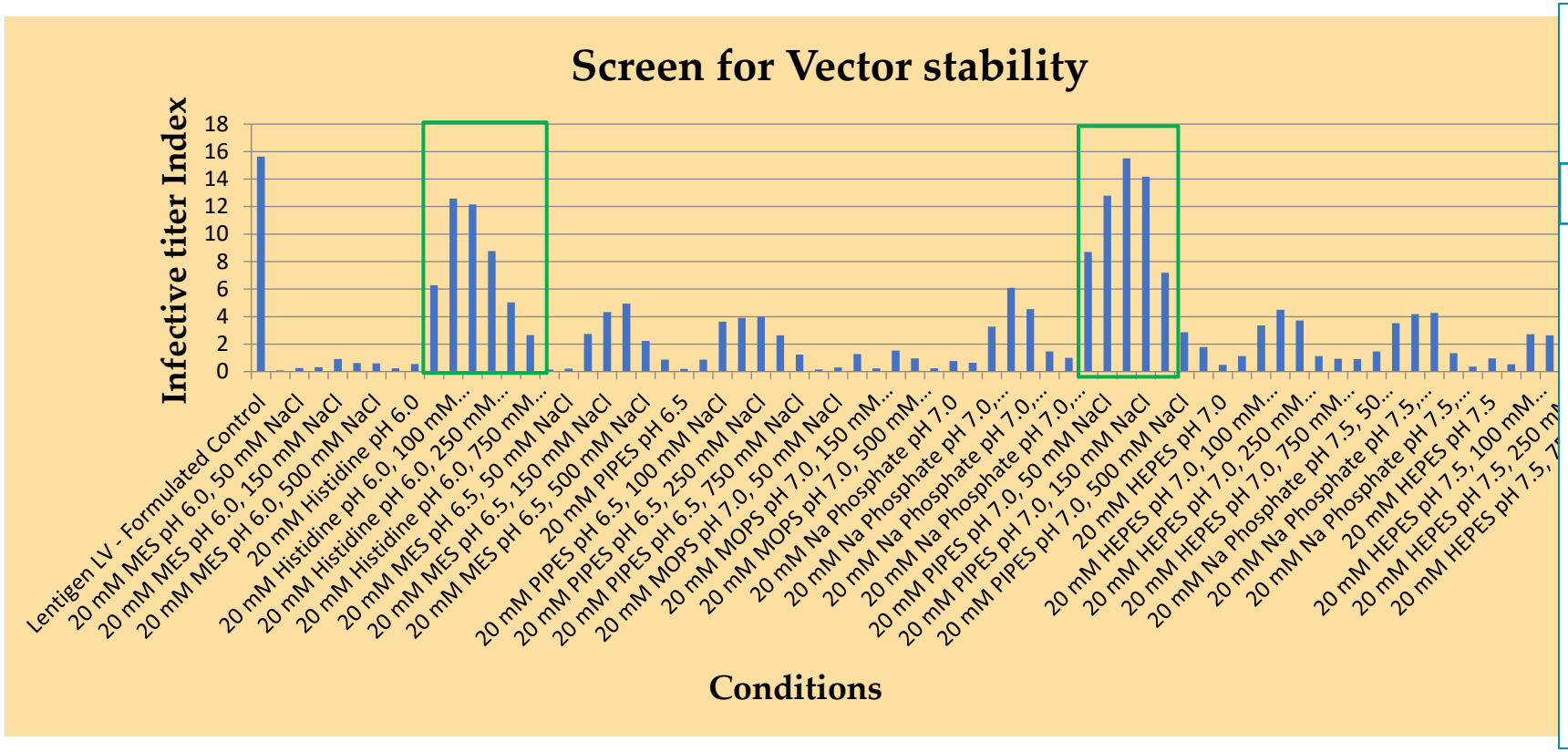
DLS used as the 'widely applicable analytical tool'

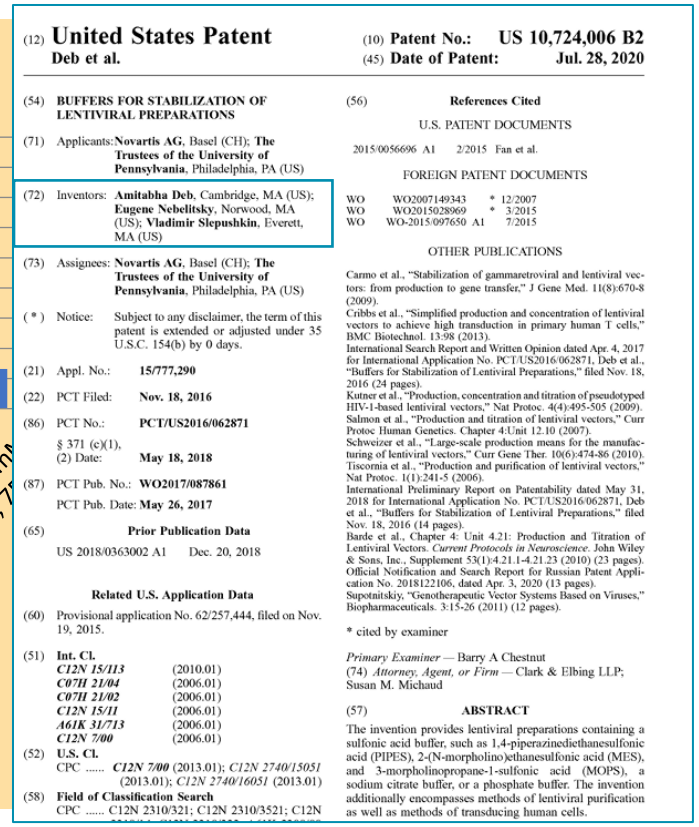


- LVV is a critical raw material or the final product
  - Quality expectations from Health authorities on LVVs as the final product
  - DLS is used as an analytical tool for aggregation measurements; similar strategy can be used for a functional readout/potency assay

High-throughput Screen to identify LV stabilizing buffers

Amitabha Deb, Eugene Nebelitsky et. al; Case Study #6a





(12) **United States Patent**
Deb et al.

(10) **Patent No.: US 10,724,006 B2**
(45) **Date of Patent: Jul. 28, 2020**

(54) **BUFFERS FOR STABILIZATION OF LENTIVIRAL PREPARATIONS**

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(72) Inventors: **Amitabha Deb**, Cambridge, MA (US); **Eugene Nebelitsky**, Norwood, MA (US); **Vladimir Slepushkin**, Everett, MA (US)

(73) Assignees: **Novartis AG**, Basel (CH); **The Trustees of the University of Pennsylvania**, Philadelphia, PA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

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§ 371 (c)(1),
(2) Date: **May 18, 2018**

(87) PCT Pub. No.: **WO2017/087861**
PCT Pub. Date: **May 26, 2017**

(65) **Prior Publication Data**
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Related U.S. Application Data
(60) Provisional application No. 62/257,444, filed on Nov. 19, 2015.

(51) **Int. Cl.**
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C07H 21/04 (2006.01)
C07H 21/02 (2006.01)
C12N 15/11 (2006.01)
A61K 31/713 (2006.01)
C12N 7/00 (2006.01)

(52) **U.S. Cl.**
CPC ***C12N 7/00*** (2013.01); *C12N 2740/15051* (2013.01); *C12N 2740/16051* (2013.01)

(58) **Field of Classification Search**
CPC C12N 2310/321; C12N 2310/3521; C12N

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Primary Examiner — Barry A Chestnut
(74) *Attorney, Agent, or Firm* — Clark & Elbing LLP; Susan M. Michaud

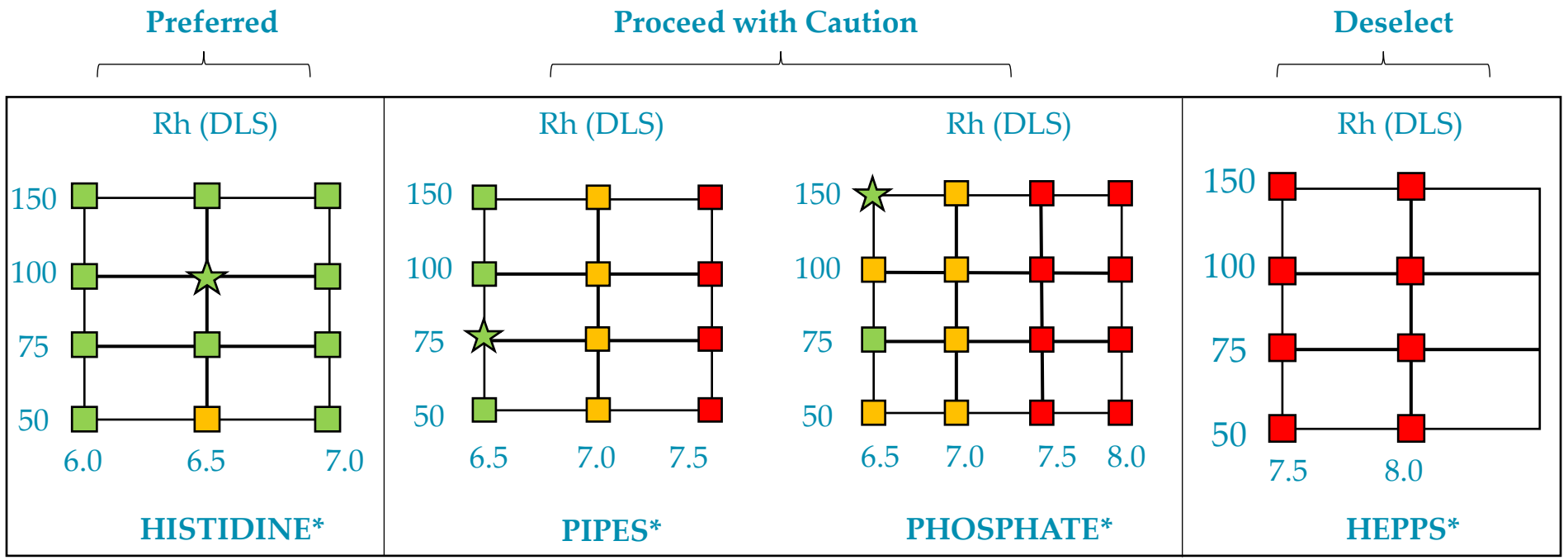
(57) **ABSTRACT**

The invention provides lentiviral preparations containing a sulfonic acid buffer, such as 1,4-piperazinediethanesulfonic acid (PIPES), 2-(N-morpholino)ethanesulfonic acid (MES), and 3-morpholinopropane-1-sulfonic acid (MOPS), a sodium citrate buffer, or a phosphate buffer. The invention additionally encompasses methods of lentiviral purification as well as methods of transducing human cells.

- Selected buffers provide high stability to Lentiviral vectors
  - 1st report of leveraging DLS/light scattering tool for preformulation development

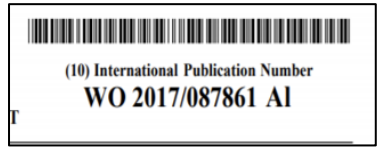
Stability of LVV: Aggregation vs. pH/NaCl

Case Study #6b demonstrating robustness of different buffer systems



Amitabha Deb, et.al.
Kymriah-related patent,
Novartis Pharma

Amitabha Deb, et. al



Small Rh: Low Aggregation
Medium Rh: Moderate Agg.
Large Rh: High Aggregation

* Buffer systems; X-axis: pH, Y-axis: mM Salt/NaCl
Rh: Hydrodynamic radius

Freeze-thaw study to test formulations

Case Study #6c

Conditions	Regularization Analysis		Analytics	
LV hCAR19*	Rh	% PD	Integration Titer (TU/mL)	% CV
Freeze/Thaw Study: Control **	135	61	7.4E08	4.8
1 Freeze / Thaw	170	64	7.2E08	8.0
2 Freeze / Thaw	162	61	7.6E08	5.9
3 Freeze / Thaw	197	103	8.1E08	2.1
4 Freeze / Thaw	187	82	7.4E08	6.8
5 Freeze / Thaw	213	118	7.9E08	6.4

Normal Operating range

'Knowledge' Space

*LV-CD19CAR: 100x Concentrated in Formulation Buffer with GRAS excipients

** Additional 1x F/T to account for thaw during Analytics

- Highly polydisperse population of the concentrated LVV, as expected.
- Significant insights can be gained from accelerated stability studies

Summary

- Develop integration strategy for process and analytics early during preclinical development: Focus on Manufacturability

- Rely on high throughput screening and analytics: Explore analytical methods for trending and characterization: Directly linked to cost-efficient manufacturing
  - Use of predictive and real-time analytics and process modelling can be beneficial
  - ML and AI are increasingly being employed to identify patterns in process data

- Identify GOI specific characteristics of viral vectors and their relation to biomanufacturing failures: Make highly potent molecules

  - Scientific innovation alone isn't enough to ensure the success of Cell and gene therapies
  - The CGT field must now address building potent therapies and business models that are truly scalable, accessible, and sustainable.

Acknowledgements

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The Future of The CGT Regulatory Paradigm

"Much of the clinical uncertainty today ties back to **uncertainties within the manufacturing process**, particularly as it relates to **achieving overall manufacturing consistency** and/or **fully understanding and characterizing the principles of your cells**".

(Scott Gottlieb, Feb 2022)

> *"In contrast to the traditional drug review, where 80% of the review is focused on the clinical portion of that process, and maybe 20% is focused on the product issues, I'd say that this general principal is almost completely inverted when it comes to cell and gene therapy…. The more challenging questions relate to product manufacturing and quality."*

1. Engineered cell line
2. Improved product stability
3. Intelligent Manufacturing

CellGene Consulting founded by Amitabha Deb, Ph.D.

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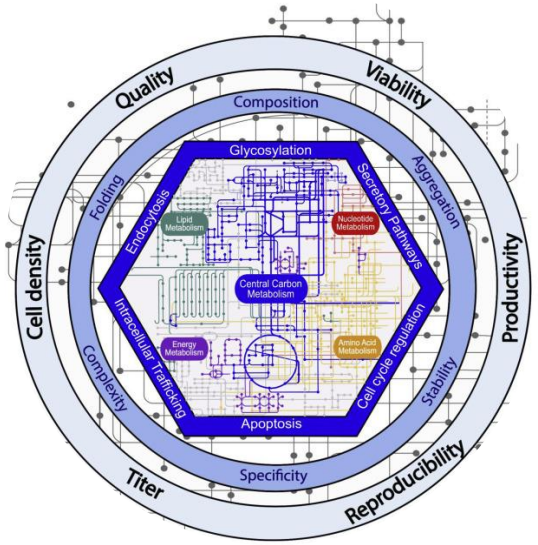

ENGINEERED CELL LINE,

AGNOSTIC TO VIRAL VECTOR TYPES & PRODUCTION PLATFORMS

INTELLIGENT MANUFACTURE

HIGHER YIELD & LOWER COST OF GOODS

REGULATORY COMPLIANCE

LOW COMPARABILITY BURDEN