Manufacturing Challenges of AAV Gene Therapy Products

Rachel Legmann, PhD
Director, Gene Therapy & Viral Vectors Technology

11 May, 2020

CMC STRATEGY FORM EUROPE VIRTUAL

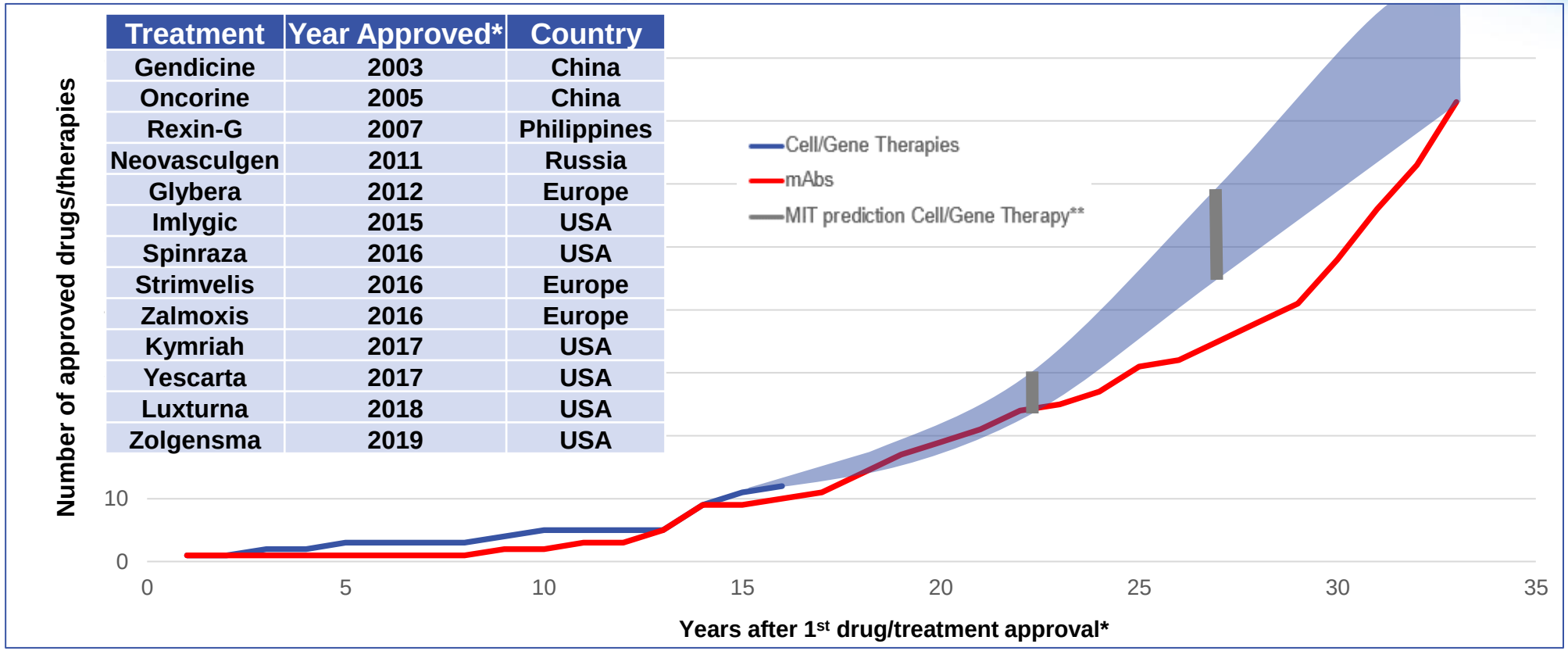
Your Journey. Your Way.

Gene Therapy: The Golden Age

CAGR 2017-2024

Vaccines	4 %
Plasma	9 %
rProteins	8 %
mAbs and other Ab modalities	10 %
Gene and Cell Therapy	33 %

Treatment	Year Approved*	Country
Gendicine	2003	China
Oncorine	2005	China
Rexin-G	2007	Philippines
Neovasculgen	2011	Russia
Glybera	2012	Europe
Imlygic	2015	USA
Spinraza	2016	USA
Strimvelis	2016	Europe
Zalmoxis	2016	Europe
Kymriah	2017	USA
Yescarta	2017	USA
Luxturna	2018	USA
Zolgensma	2019	USA



Gene Therapy approvals on pace with mAbs

*1st mAb approved: 1986, Muromonab; 1st Cell/Gene therapy treatment approved: 2003, Gendicine

**https://newdigs.mit.edu/sites/default/files/FoCUS%20Research%20Brief%202018F210v027.pdf

Matching Manufacturing Scale to Therapy Demand

Indication (drug)	Dose/Patient (vg or TU)
RPE65 Inherited Retinal Disease (Luxturna)	1.5 x 10^{11}/eye
Hemophilia A and B	~1 x 10^{14}
Spinal Muscular Atrophy (Zolgensma)	~5 x 10^{14}
Duchenne Muscular Dystrophy	~1 x 10^{15}
CAR-T (MOI 4)	4 x 10^{9}

- Incidence/prevalence population
- Therapy access/reimbursement
- Market share

$$\text{Scale needed} = \frac{\#\ \text{patients/year} \times \text{patient dose}}{\#\ \text{manufacturing runs/year}}$$

Too few = operator error, inefficient use of capital

Too many = high risk in supply chain

AAV Process Scale Required to Meet Demand

Number of Manufacturing Rooms/Facilities @ 20 Batches/Year

Patients/Year	Total vg/Year	Flatware (2.5 m^2)*	iCELLis® Bioreactor (500 m^2)**	Allegro™ STR Bioreactor (2000 L)**
1000	1 x 10^{17}	1	1	1
10000	1 x 10^{18}	7	1	9
50000	5 x 10^{18}	35	2	42
100000	1 x 10^{19}	70	4	84

*Up to 10 assemblies of 4 x 2.5m^2 stacked flatware devices running in parallel per manufacturing room
**Up to 5 bioreactors running in parallel per manufacturing room

Assumptions

- Dose: 5 x 10^{14} vg/dose
- Virus titer:
  - Adherent: 5 x 10^{14} vg/L
  - Suspension: 1 x 10^{13} vg/L
- Overall process yield: 30%

Bioreactor Technology for the Production of Gene Therapy Viral Vectors

Suspension Cell Culture

Allegro™ STR
Single-Use Bioreactors

50 L 200 L 1000 L 2000 L

Single-use stirred tank reactors (STRs)

STR 500

Adherent Cell Culture

Up to 2.5 m^2 per unit

Flatware devices

iCELLis® Bioreactors

0.53 to 4 m2

66 to 500 m2

Fixed bed reactors

Upstream Platform (AAV): Scalability Challenges

Media and Buffer Preparation

Media/Buffer Mixing
Magnetic Mixer system

Media/Buffer Filtration
Viral clearance filter
Allegro™ MVP system

Media/Buffer Storage/Handling
Allegro plastic/stainless steel totes

Seed Bioreactor

Adherent Cell Seed Train
Xpansion multiplate bioreactor

N-1 Seed Train

MEDIA STRATEGY

INOCULATION

DNA:PEIpro Complex

TRANSFECTION

Transient Transfection

Production Bioreactor

Adherent Virus Production
iCELLis 500+ bioreactor

$>10^{16}$ Vector Genomes

Viral Vector Manufacturing Process Platform (AAV)

Process time ~5 weeks: 10^{16} vg* → **Process time ~1 day : >45 % product loss**

Adherent seed train:
Xpansion® multiplate bioreactor

Adherent bioreactor:
iCELLis 500+ bioreactor

Clarification:
Stax™ depth filtration systems

Suspension seed train:
Allegro STR 50 L bioreactor

Suspension bioreactors:
Allegro STR 200, 500, 1000, 2000 L bioreactors

Concentration:
Cadence® TFF modules

Sterile filtration:
Kleenpak™ capsules

Affinity Chromatography

Purification:
Mustang® Q capsules

Concentration:
Cadence TFF modules

Sterile filtration:
Kleenpak capsules

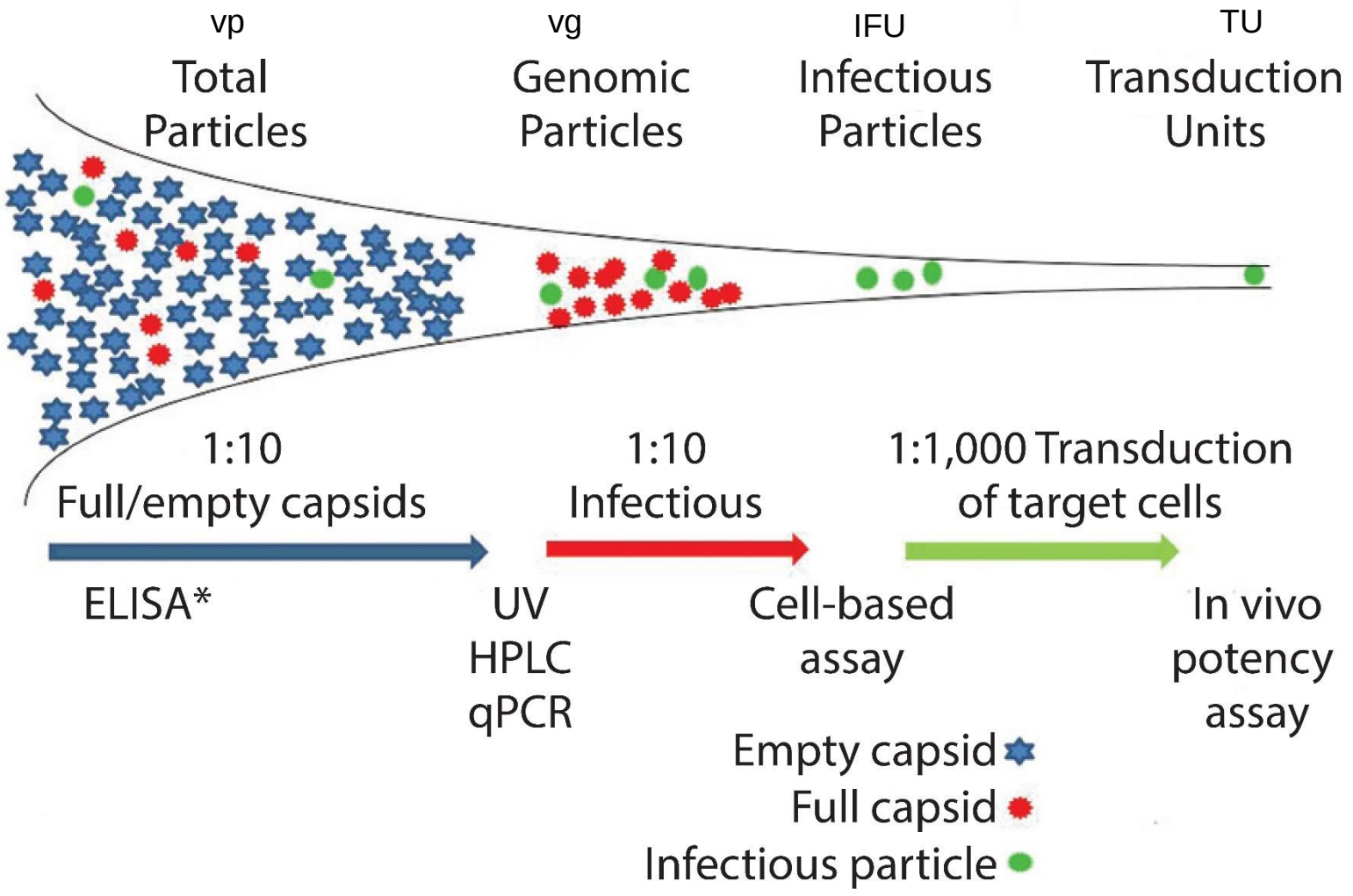
Media/buffer prep:
Allegro mixers

Media/buffer storage/handling:
Allegro plastic/stainless steel totes

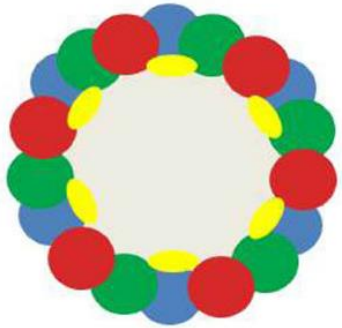
Your Journey. Your Way.

PALL Biotech

Measuring Virus



Full capsid

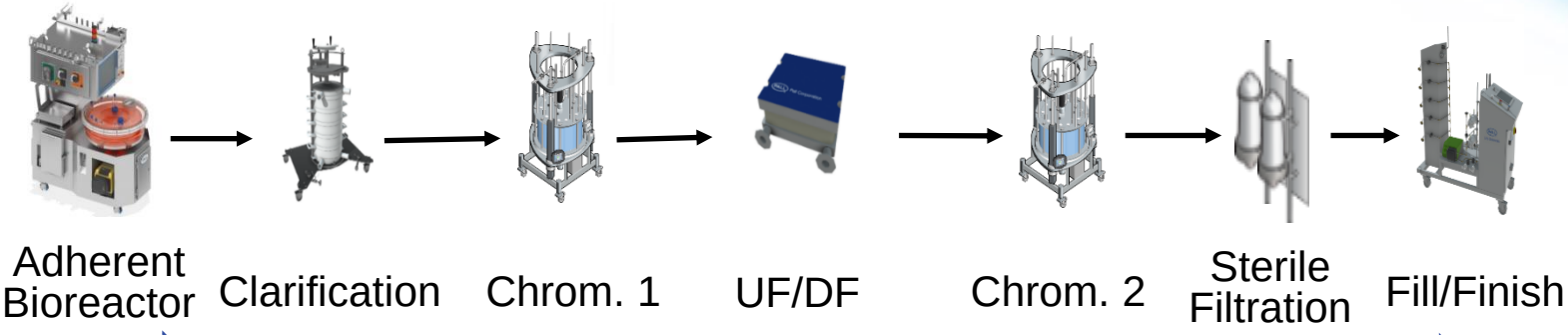


Empty capsid

vg = viral genome vp = viral particle TU = transduction units

Intensifying the viral vector manufacturing process: Continuous Viral Vector Manufacturing

Viral Vector Gene Therapy Process

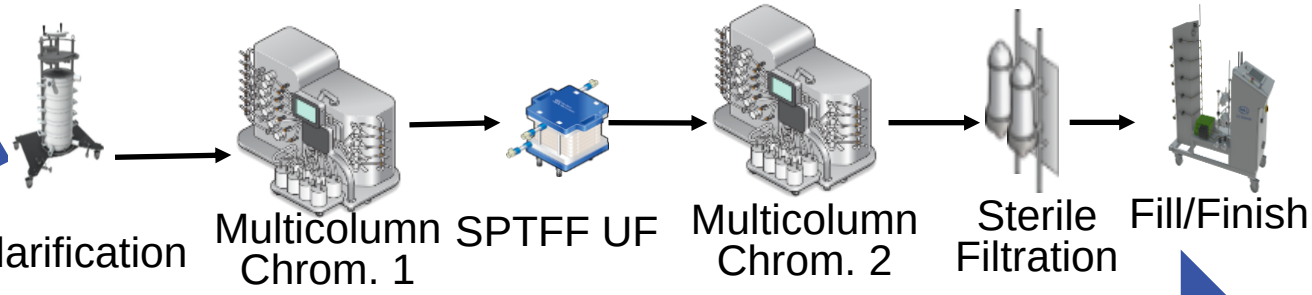


Upstream ~4 weeks: 10^{16} vg

Downstream ~ 3 day: 70-90% product loss

Implementation of Continuous Bioprocessing

- Improved quality & productivity
- Smaller foot-print
- Reduced cost
- Reduced time to market



Continuous Downstream ~ 1 day: >>50% product recovery

Summary

- Gene therapy development following the same path than that of mAbs 30 years ago
- Advanced upstream technologies already available to enable high titers and process scale-up, but improvements required to serve bigger populations and/or high dosage indications
- Significant technology development required on the downstream side to improve the yield of recovery
- Implementation of continuous bioprocessing to viral vector could solve remaining manufacturing issues to secure a broad adoption of gene therapy

THANK YOU!

QUESTIONS?

Your Journey. Your Way.