Lentiviral vectors – considerations for their use in gene-modified cell therapy production

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James Miskin, Chief Technical Officer, Oxford BioMedica

CASSS Cell & Gene Therapy Products Symposium

Oxford BioMedica – an overview

OxfordBioMedica

>20 years as a specialist in lentiviral vectors

- ✓ **1st** to administer *in vivo* (both brain and eye)
- ✓ **1st** approved advanced therapy in the US using LentiVector Enabled technology, Novartis's Kymriah™
- ✓ **>200** patients treated by Oxford BioMedica or its partners
- ✓ **Four** Phase I/II studies completed with encouraging safety and efficacy

LentiVector Enabled gene delivery platform

- ✓ **IP** – extensive IP comprising both patents and know-how
- ✓ **Facilities** – state-of-the-art bioprocessing and laboratory facilities
- ✓ **Employees** – over 370 full time employees
- ✓ **Capabilities** – Manufacture, Analytics, Quality (lentiviral vectors)

Partners

NOVARTIS
Bioverativ
Orchard therapeutics
Green Cross LabCell
IMMUNE DESIGN

Product / IP licences

AXOVANT
SANOFI
Orchard therapeutics

Oxford BioMedica Facilities in the UK

OxfordBioMedica

Facilities less than 1 hour from London Heathrow Airport:

Windrush Court

- Corporate HQ & Laboratories
  71,955 sq.ft (6,684 sq.m)
- GMP Warehouse Hub
  2,691 sq.ft (250 sq.m).


Harrow House & Chancery Gate

19,375 sq.ft (1,800 sq.m)

- cGMP production facility
- Two clean room suites
- GMP QC microbiology laboratories
- Raw material testing
- GMP cold chain warehouse & office space


Yarnton

18,300 sq.ft (1,700 sq.m)

- cGMP production facility
- One clean room suite


FDA MHRA

APPROVED

Source: https://resources.oncourse.iu.edu/access/content/user/leema/profilepage/oxford.html

Cell and Gene Therapy

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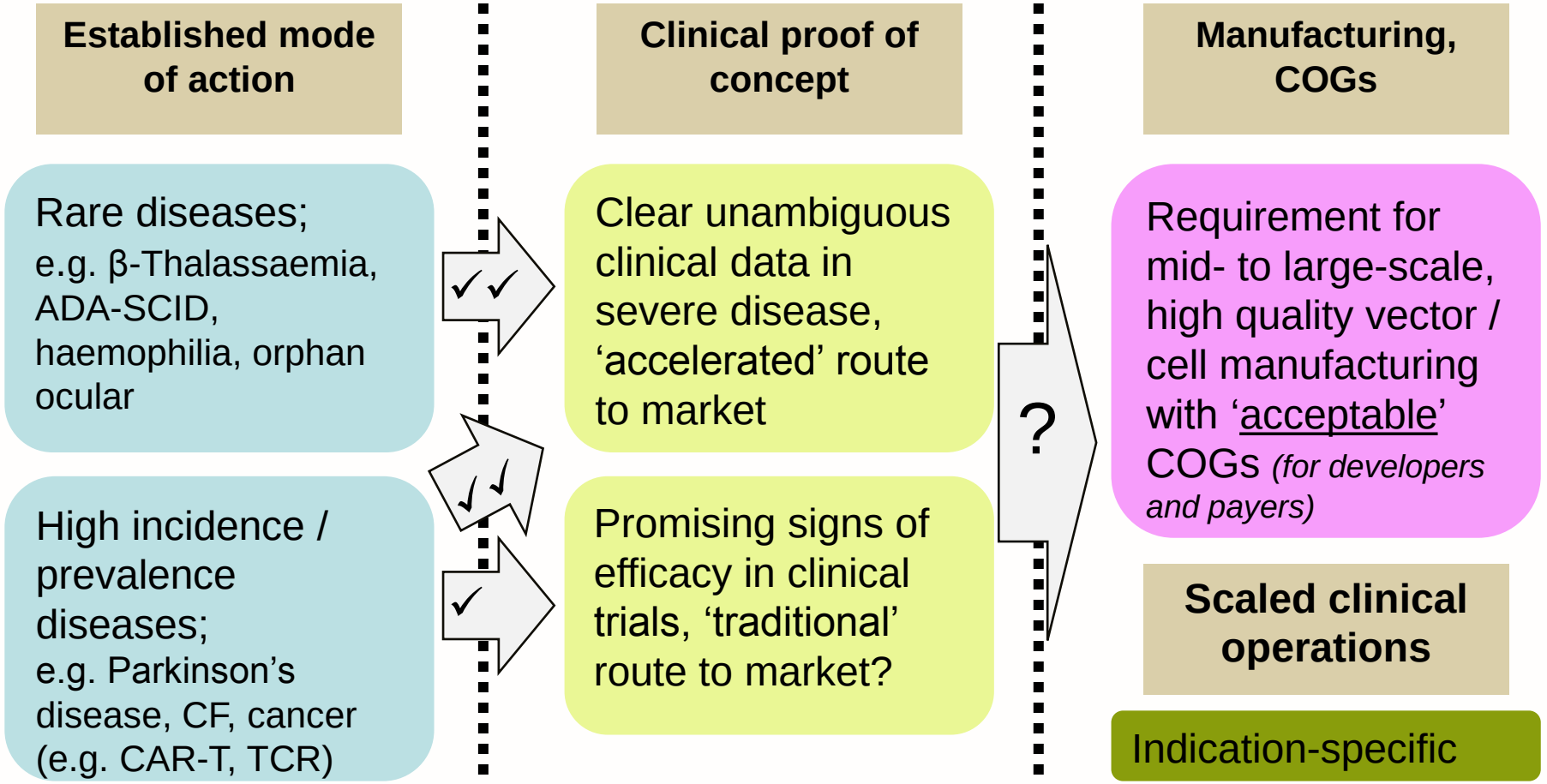
Towards successful commercialisation



Requirements for vectors in *ex vivo* products

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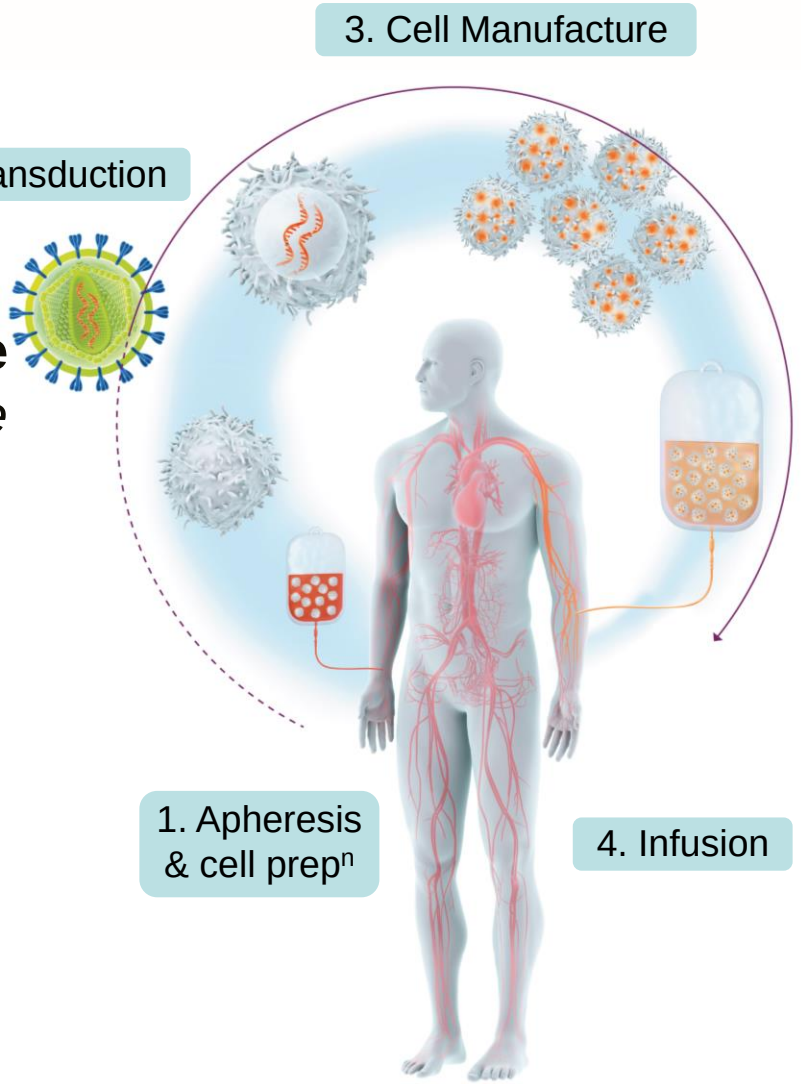
- Current focus on use in autologous products (i.e. individual patient batches)
- Vector is treated as a **drug substance** – *there is no opportunity to sterilise the cell product after vector addition*
- Regardless of terminology (Raw Material, Starting Material, etc.), need to apply similar CMC approach to parenteral (sterile) products
- Drastically increased demand (in-house and partners) – ***since 2012***
- Plus considerable demand for ***in vivo***



Lentiviral vector system

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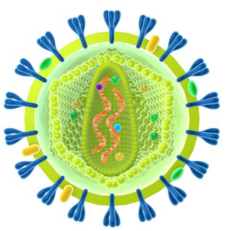
Benefit(s)	Feature
Safety—absence of replication-competent lentivirus (RCL) Yield—efficient vector production	Vector components segregated on 4 separate plasmids
	Open Reading Frames (ORFs) of nonessential accessory genes and Tat removed
	Codon-optimized Gag/Pol
	Self-inactivating long terminal repeat sequence (SIN LTR)
	Heterologous envelope (VSV-G)
High expression in target cells	Regulatory sequence for enhanced expression

Vector – overview of testing



- Broad testing panel to ensure vector quality, safety, and consistent manufacture - guided by process/vector structure and design
- **Platform approach** – potency and identity product specific
- **Validated** analytics to support commercial

Testing	**Comment**
Appearance and description	Appearance consistent with vector suspension
Identity	qRT-PCR (e.g. to CAR-specific sequence)
Safety	Assures absence of microbial contamination and RCL – **see next 3 slides**
Purity	Measures both vector and impurities
Quantity	p24 protein and vector RNA measured
Biological activity	Measurement of transduction of target cells

Vector – safety assessment

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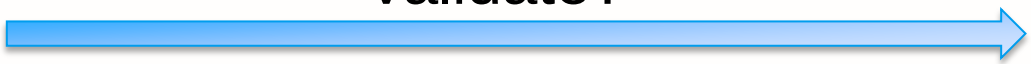

Mycoplasma

Bioburden

Residual production cell DNA

Plasmid DNA

In vivo inapparent viruses

residual antifoam

Control Cell testing

Endotoxin

Sterility

Replication competent virus RCR/RCL

Host Cell Protein

Integration site analysis

Vector particle concentration

Potency

In vitro virus detection

residual nuclease

residual induction reagent

End of production cells (latent viruses)

Bovine Serum Albumin

Actual testing performed may be dependent on production process, stage of clinical development

Vector – RCL testing

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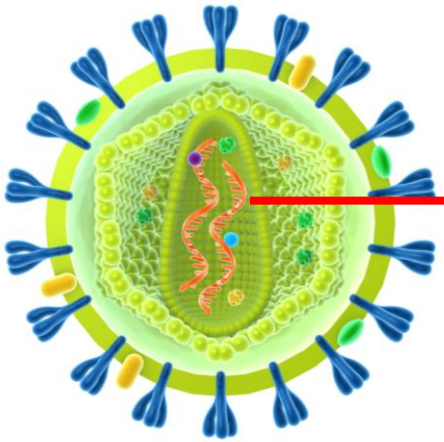
- Classic virus detection can't be used
  - 'Standard' infectivity assays cannot be used for lenti (e.g. focus formation)
  - PCR for partial recombinants presumes some prior knowledge of RCL
- Advantages of **P**roduct **E**nhanced **R**everse **T**ranscriptase (PERT) as endpoint detection as part of an infectivity assay:
  - Highly sensitive qPCR-based detection of RT activity
  - RT is common feature of all retroviruses
  - Method will detect any putative RCL without knowledge of structure



- Consistent co-packaging of RT enzyme in vector particles
- Estimated 10-100 copies per virion
- Allows for semi-quantitative (relative) assessment of vector / virus concentration
- Detects all retroviral RT

Vector – RCL testing

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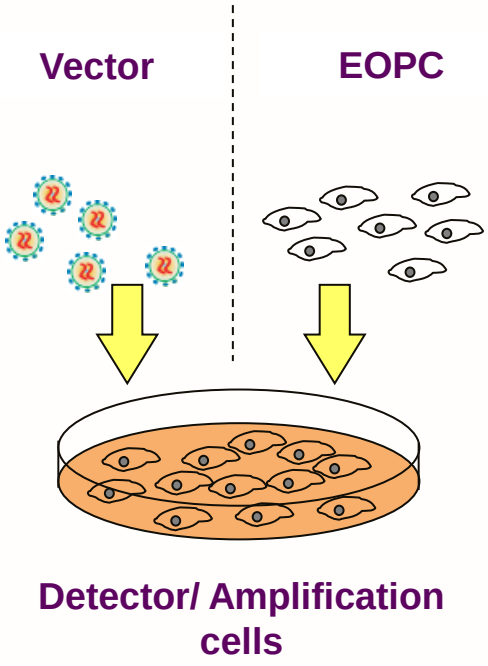
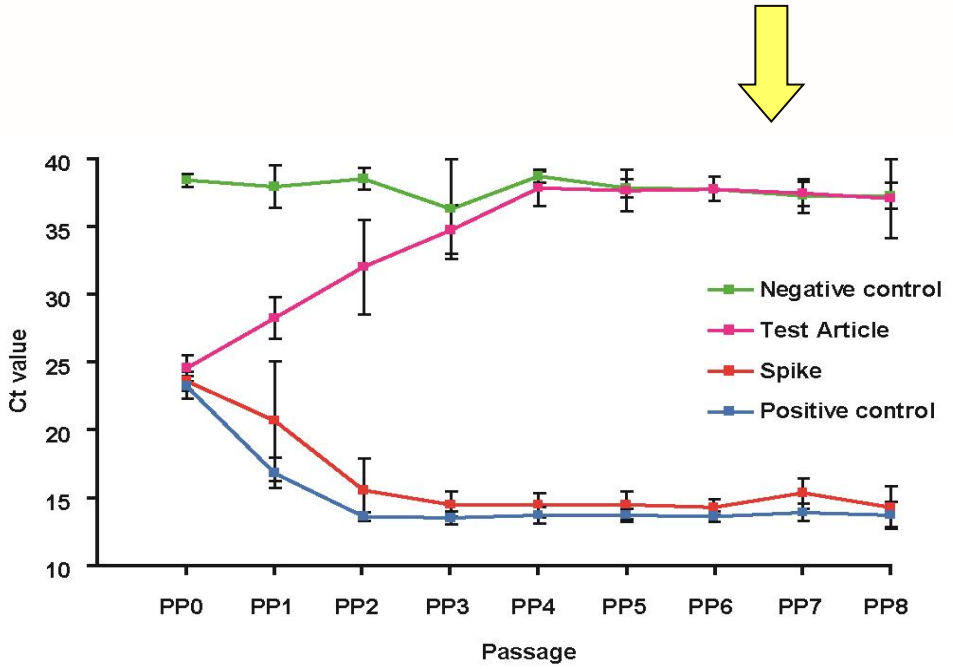
1. Inoculation → 2. Multiple Passages (~4-5 weeks) → 3. Collect cell supernatant → 4. PERT Real-time PCR (TaqMan)





Routine analysis requires testing of final passage supernatant only

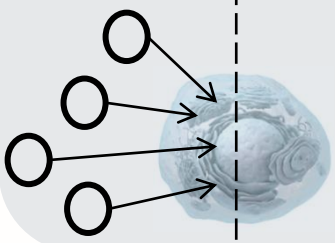
OXB vector process development strategies

- Holistic view of the development of ‘next generation’ lentiviral vector manufacturing, utilising closed systems wherever possible:

Production cells

Transient
Plasmids
Tfx reagent
Short USP phase

Stable
Packaging cell line
Producer cell line



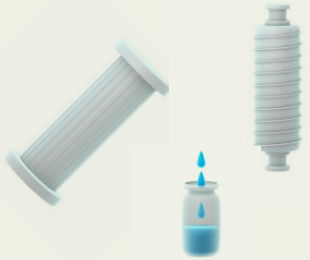
USP

Adherent
Up to 72L
Serum-containing
100% single use

Suspension
50L – 2000L
Serum-free
100% Single use

DSP

Flexible
Scalable
Gentle
100% single use
Rapid, optimised yield



Manufacturing strategy

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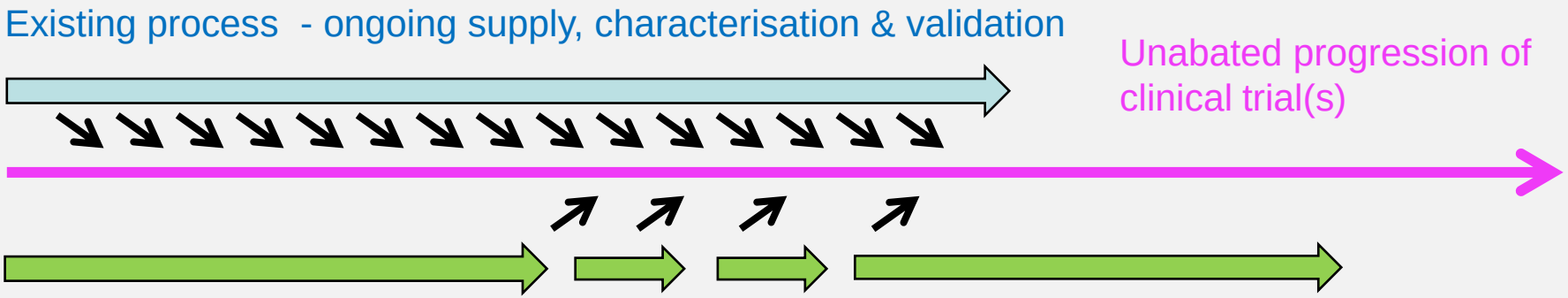
Satisfying current / future demand

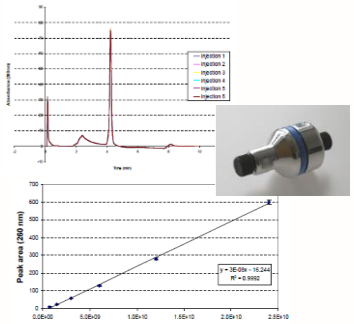
- Considerations for current and future processes (*strategy dependent on indication & phase of development*):
  - Process complexity (multiple vessels vs single)
  - Manual handling requirements vs single use closed systems (risk, COGs)
  - Reliance on raw material supply (e.g. FBS vs serum-free)
  - Output per clean room suite (COGs), number of independent suites
  - Need to support OXB and partner projects and programmes

Overall strategy:



Scale up & manufacturing challenges

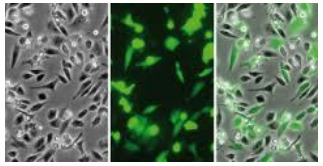
OXB process development roadmap



Development of rapid analytics for in-process and release testing

Automated, high through-put cell line generation

Cells

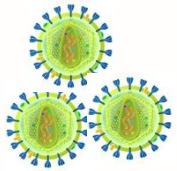


Stable, high producer packaging/producer cell lines

Analytics

Advanced Manufacturing Platform

Vector



Vector design & optimisation

Scale-up Studies >200L

Formulation, Fill & Finish

Process

Purification development programme

Media

Serum-free suspension culture in single-use bioreactor

Establish media & feed platform

Suspension process development

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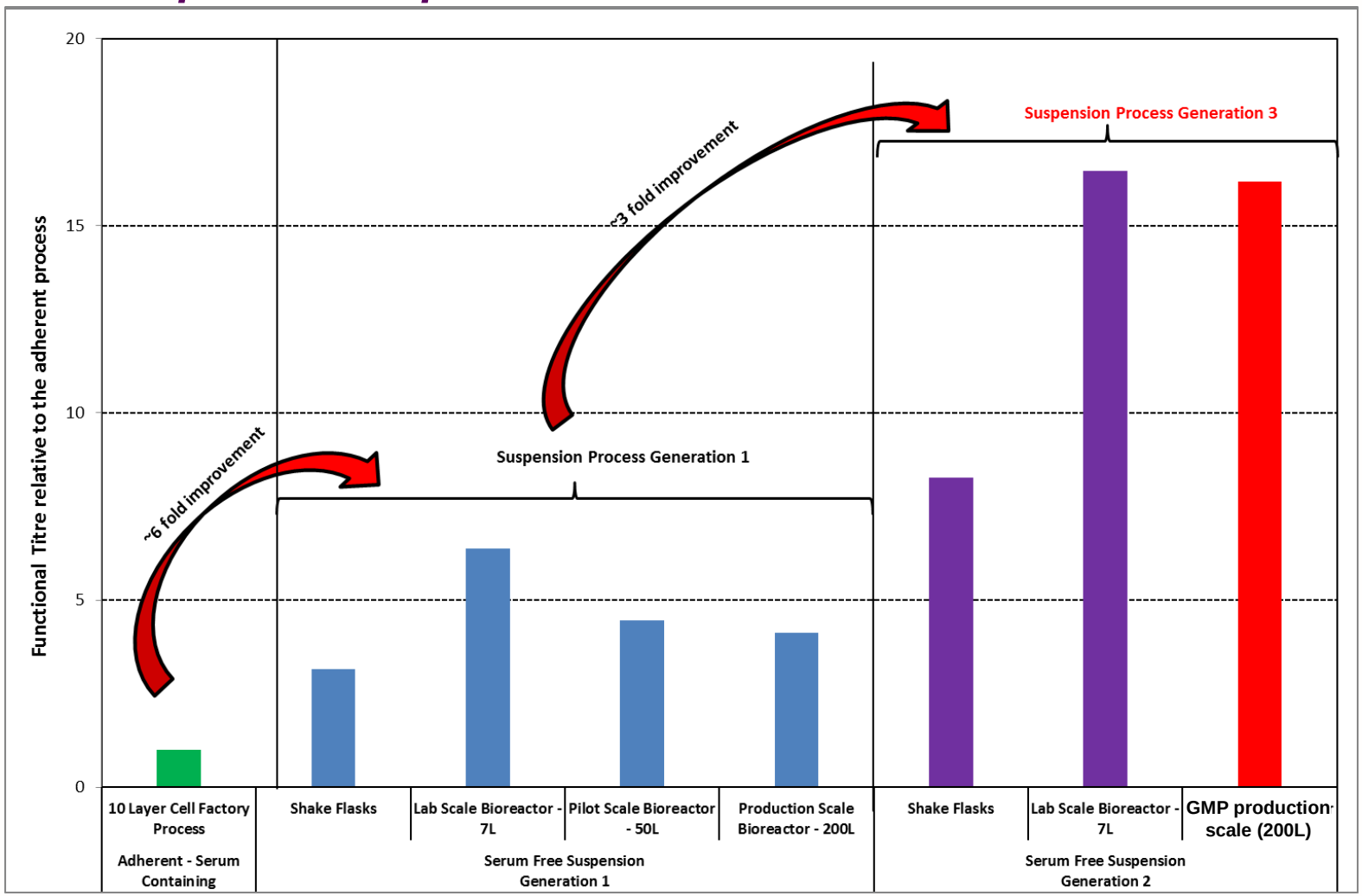
Continuous process improvement



GMP clean room facilities – operational

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Matching capacity to demand

Flexible independent clean room suites for GMP vector manufacture, with segregated air handling, material transfer and personnel routes.

Single use systems (SUS) used throughout

- **GMP1** Grade C/D (ISO 7/8) – 4,198 sq. ft (390 sq. m) clean room suite
  - OXB's original clean room facility, acquired in 2011
  - Planar 2D technologies e.g. CF-10, areas for cell expansion and downstream processing
  - **Operational and MHRA licenced (since 2012)**

- **GMP2** Grade C/D (ISO7/8) - 2,691 sq.ft (250 sq. m) clean room suite
  - Suspension platform 2 x 50/200L Duo SUB
  - Planar 2D technologies e.g. CF-10
  - Operational readiness **Operational and MHRA licenced (since May 2016)**

- **GMP4** Grade C/D (ISO7/8) - 6,028 sq. ft (560 sq. m) new off-site API Facility
  - Suspension platform up to 2 x 50/200L Duo SUB
  - Planar 2D technologies e.g. CF-10
  - Areas for cell expansion, media make-up, buffer preparation and downstream processing
  - **Operational and MHRA licenced (since Jan 2016)**

2018 – OXB announced next phase of capacity expansion

Considerations for commercial vector supply

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Production and testing capability

Adequate production scale

Materials

Dual supply, Single use

Ensure process robustness through development & optimisation

Scientifically sound analytics and initial specifications

Process Characterisation
Process Validation

Analytical validation
Final specifications

Process Control strategy (NOR, PAR)

Key supply agreements (materials, components, suppliers, contractors)

Robust QMS

Regulatory support

PLI & audit readiness

Batch manufacture and release process

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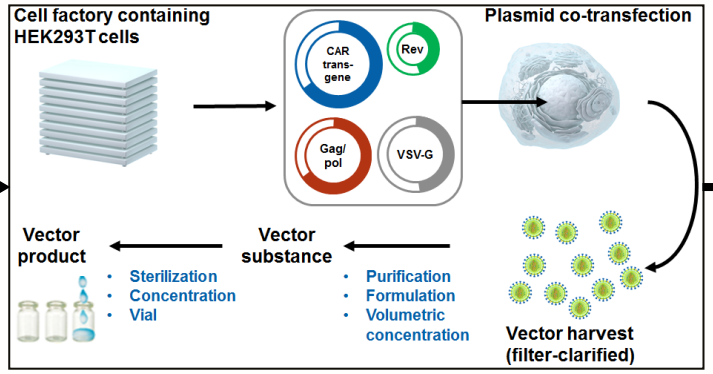
Manufacture | Testing | Release

Ordering, S&OP, Raw Materials



Sampling, shipping

Complex methods, manual

Certification, shipping

✓

Ongoing & future improvements

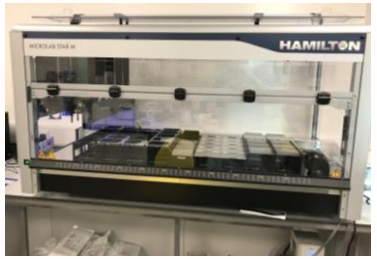
ERP system ✓

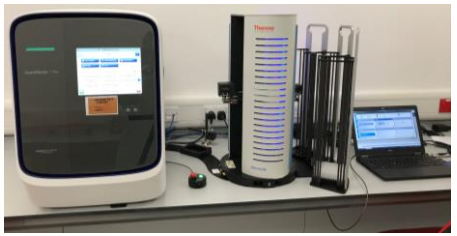
Doc Mgmt ✓

Automation
Ongoing

eQMS
Ongoing

LIMS
Ongoing





Concluding remarks

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- Gene and cell therapy has reached the stage where several very promising therapies have reached the commercial phase

  Strimvelis® – retroviral vector-based cell therapy product for ADA-SCID, launched summer 2016

  CTL019 - worldwide first commercial product based on lentiviral vector technology (Kymriah™ FDA approved Aug 2017, CHMP positive opinion Jun 2018)

  Kite/Gilead – approval of Yescarta (FDA approved Oct 2017, positive opinion Jun 2018)

  Spark Therapeutics Voretigene Neparvovec for RPE65-mediated IRD (Luxturna™ FDA approved Dec 2017)

- ***Conclusion*** *– need to continue to develop and evolve technologies and CMC strategies to support anticipated level of patient demand*

Contact us

James Miskin
Chief Technical Officer

+44 (0) 1865 783 000
j.miskin@oxfordbiomedica.co.uk

Further information:
http://www.oxfordbiomedica.co.uk/