Key engineering challenges in the biomanufacturing of lentiviral viral vectors

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Key engineering challenges in the biomanufacturing of lentiviral vectors

January 29, 2019

Peter Jones, Head of Operational Strategy, Oxford BioMedica
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**Problem statement**

The New York Times

- Gene and cell therapy revolution
  - £9 -14 bn by 2030
- Market expansion driven by recent approvals, driving significant growth:
  - Gene & gene-modified cell therapy sector raised $7.8 Bn YTD 2018 (34% increase YOY)
  - Surge in demand for lentiviral vectors
    - Greatly underpinned by the success of the T-cell therapies
- OXB estimates the lentiviral vector bioprocessing market
  - $200m in 2017 and will grow to $800m by 2026

Source: Q3 2018 Data Report, Alliance for Regenerative Medicine

**Gene Therapy Hits a Peculiar Roadblock: A Virus Shortage**

Source: Oxford BioMedica management estimates
Agenda

1. Company overview & facilities
2. LentiVector® gene delivery platform
3. Manufacturing strategy considerations
4. Scale-up & manufacturing challenges
5. Suspension process development – USP considerations
6. – DSP considerations
7. Development of advanced process analytical technologies
8. Concluding remarks
Oxford BioMedica – An overview

>20 years as a specialist in lentiviral vectors

- **Founded** in 1996, based in Oxford, UK, IPO on LSE April 2001 (OXB.L)
- **Mission** – Delivering life-changing gene therapies to patients
- **Core LentiVector® technology platform** based on lentiviral vector *in vivo* and *ex vivo* gene delivery system
  - 1st world-wide to administer lentiviral vector gene therapy *in vivo* (both brain and eye)
  - 1st approved advanced therapy in the US using LentiVector® enabled technology, [Novartis’s KYMRIAH® (tisagenlecleucel)]
  - 1st commercial supplier of lentiviral vectors, post CAR-T approval
- >400 patients treated by Oxford BioMedica or by its partners
- **Four** Phase I/II studies completed with encouraging patient 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** – >400 full time employees
- **Capabilities** – Development, Manufacture, Analytics, Quality (lentiviral vectors)
Oxford BioMedica facilities in the UK

**Windrush Court**
- Corporate HQ & Laboratories
  - 71,955 sq.ft (6,684 sq.m)
- GMP Warehouse Hub
  - 2,691 sq.ft (250 sq.m)
- GMP QC Release & stability testing facilities

**Harrow House & Chancery Gate**
- 19,375 sq.ft (1,800 sq.m)
  - GMP 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)
  - GMP production facility
  - Two clean room suites
  - GMP QC microbiology laboratory
  - 1Q2019 implementation of suspension platform

Source: https://resources.oncourse.iu.edu/access/content/user/leema/profilepage/oxford.html
Oxford BioMedica facilities in the UK

Innovation Centre
- 33,000 sq.ft (3,066 sq. m)
- Located adjacent to Windrush Court
- Offices & Laboratories
- Allows for further expansion of R&D and platform innovation activities on one site

The Future “OXBox”
- 84,000 sq.ft (7,800 sq.m).
- Phase I – 45,000 sq. ft (4,200 sq. m)
- GMP production facility
- Four clean room suites
- Two Fill & Finish suites
- Offices, warehousing and QC laboratories

(Under construction – operational early 2020)

Source: https://resources.oncourse.iu.edu/access/content/user/leema/profilepage/oxford.html
Strategy: Leveraging our LentiVector® Enabled delivery platform

LentiVector® Platform

IP – patents and know-how | Facilities | Expertise | Quality systems

Arising IP

R&D Investment
Technical Developments

Arising IP

Technical and scientific knowledge transfer

Investment into internal & external assets up to early clinical stage

Spin out/out-licence

Product development

Product development partner

Platform and process development

Partners’ Programmes

Multiple income streams
Process development fees
Process development incentives
Bioprocessing revenues
Royalties

OXB products

Upfront & milestones
Royalties
Development funding

Advancing Manufacture of Cell and Gene Therapies IV, ECI, Coronado, CA, USA, January 27-31 2019
Gene and cell therapy technologies

Oxford BioMedica is involved at early stage of development of lentiviral based products either our own or with partners – strong IP position

Oxford BioMedica’s LentiVector® Platform

In vivo & Ex vivo development

- Direct administration in vivo of lentiviral vectors to target organs such as the eye, brain, liver and lung
- Administration ex vivo to target stem cells, T-cells and other cell types
- Permanent modification of dividing and non-dividing cells
- Broad tissue tropism
- Single administration with sustained or permanent efficacy
- No toxicity or adverse immune reaction

Lentiviral vectors vs AAV vectors

<table>
<thead>
<tr>
<th></th>
<th>Lentiviral Vectors</th>
<th>AAV Vectors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Efficient in vivo gene delivery</td>
<td>✓ ✓ ✓</td>
<td>✓ ✓ ✓</td>
</tr>
<tr>
<td>Safe and well tolerated</td>
<td>✓ ✓ ✓</td>
<td>✓ ✓ ✓</td>
</tr>
<tr>
<td>Large therapeutic payload</td>
<td>✓ ✓ ✓</td>
<td>✗</td>
</tr>
<tr>
<td>No pre-existing immunity</td>
<td>✓ ✓ ✓</td>
<td>✗</td>
</tr>
<tr>
<td>Permanent modification of dividing cells</td>
<td>✓ ✓ ✓</td>
<td>✗</td>
</tr>
<tr>
<td>IP protection</td>
<td>✓ ✓</td>
<td>✓</td>
</tr>
<tr>
<td>Ease of manufacture</td>
<td>✓</td>
<td>✓ ✓ ✓</td>
</tr>
</tbody>
</table>
OXB’s LentiVector® gene delivery platform

- Codon-optimised gag-pol
- Mutated WPRE
- SIN-LTR
- Next generation vector engineering
- TRiP Technology
- Producer and packaging lines
- Transient vector production: Adherent/serum Suspension/serum free 200L bioreactors
- State of the art DSP & 2000x concn

- Clinical/commercial ready process development and characterisation
- OXB vectors familiar to regulators

- TRiP System

Advancing Manufacture of Cell and Gene Therapies IV, ECI, Coronado, CA, USA, January 27-31 2019
Cohort 2
Dose
Administration
3 months (UPDRS)
6 months (UPDRS)
1 year (UPDRS)
2 years (UPDRS)

1, n=3
Original
Mean 27%
Max. up to 30%
Mean 30%
Max. up to 50%
Mean 29%
Max. up to 44%

2, n=3
Original
Mean 28%
Max. up to 53%
Mean 34%
Max. up to 53%
Mean 29%
Max. up to 56%

3, n=3
Enhanced
Mean 26%

OXB LentiVector® platform – safety features

Minimal LentiVector system
- Third generation
- Separated on 4 plasmids with minimal sequence homology

Genome cassette
- Minimal viral nucleic acid (RNA) remaining
- No viral open reading frames or lentiviral enhancers
- Self inactivating LTR
- Mutated WPRE

Gag-pol
- Codon optimised gag-pol
- No sequence homology with genome cassette

Contains only ~ 10% (861 bp) of wild type genome
• During manufacture, transgene is normally expressed
• Can reduce vector yield activity, and impact product purity and yield
• Ideally transgene expression should be repressed to allow consistent vector production and purification, irrespective of transgene identity
• **Transgene Repression in vector Production [TRiP] cell system** is used to recover vector titres compromised by transgene expression
• Bacterial protein TRAP and RNA binding sequence inserted within transgene leader sequence

**TRiPLenti**

- Also available: TRiP Retro, TRiP Adeno, TRiPAAV

Source: Published PCT number WO 2015/092440
Maunder HE et al., Nat Coms 2017

Potent repression of GFP transgene in cells transfected with TRiP system components
**Transgene Repression In vector Production**

**HIV-1**

Improved therapeutic lentiviral vector crude titers using the TRiP system in HEK293T cells

![Graph showing improved titers](image)

**EIAV**

Improved therapeutic lentiviral vector crude titers using HEK293T-TRiP cells

![Graph showing improved titers](image)

**Adeno**

![Graph showing improved titers](image)

**TRiP System™**

**TRiPLenti**

Also available: TRiP Retro, TRiP Adeno, TRiP AAV

System expected to support any viral vector system

Source: Published PCT number WO 2015/092440
Maunder HE et al., Nat Coms 2017

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Producer cell line development

**Transient System**
- ✓ Does not require selectable markers
- ✓ Immediate production
- ✓ Fast development timelines
- ✗ Expensive at large scale
- ✗ Resource intensive
- ✗ Generates cells with varying gene expression levels

**Stable Cell Lines**
- ✓ Cell ‘combinations’ can be engineered to suit purpose
- ✓ Large scale vector production over extended periods
- ✓ Reduces batch-to-batch variation
- ✓ Reduces costs
- ✓ Generates clones with high expression levels
- ? Requires lengthy cell-line stability studies
- ✗ Development time and costs can be high

Advancing Manufacture of Cell and Gene Therapies IV, ECI, Coronado, CA, USA, January 27-31 2019
Proprietary technologies: packaging/producer cell lines

- HIV packaging & producer cell lines have been developed using the ACSS
- LV production yields are equivalent or better that standard transient process
- New platform is available to partners
## Manufacturing strategy considerations

**Real world thinking – hypothetical example**

### Potential impact of indication and clinical development phase on production capacity requirements:

<table>
<thead>
<tr>
<th>Phase of clinical development</th>
<th>‘Low demand’ indication</th>
<th>‘High demand’ indication</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of subjects</td>
<td>Volume (L)</td>
</tr>
<tr>
<td>Preclinical</td>
<td>12 primates</td>
<td>10-20L</td>
</tr>
<tr>
<td></td>
<td>30-60 rodents</td>
<td></td>
</tr>
<tr>
<td>Phase I/II</td>
<td>10 -15 patients</td>
<td>100L</td>
</tr>
<tr>
<td>Phase II</td>
<td>20</td>
<td>200L</td>
</tr>
<tr>
<td>Phase III</td>
<td>50</td>
<td>500L</td>
</tr>
<tr>
<td>BLA/MAA Commercial</td>
<td>100’s</td>
<td>≥1000L</td>
</tr>
</tbody>
</table>

- Manufacturing strategies are influenced by indication and development phase
- Major driver for process design/development/improvements in upstream volumetric productivity, recovery/downstream purification, sterile manufacturing, % step recoveries etc.

### Assumptions:
- 1L gives approximately one dose to account for process losses, testing etc.
- USP yield improvements targeted to realise benefits in COGS and access to high demand indications

[Diagram with RESEARCH and DEVELOPMENT stages]
### Late clinical & commercial supply options

#### Process evolution of the LentiVector® manufacturing platform

<table>
<thead>
<tr>
<th>Planar technologies</th>
<th>Fixed-bed systems</th>
<th>Suspension platform</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scale-out based on surface area</td>
<td>Scale-up based on fixed bed compaction</td>
<td>Easy to scale-up Up to 2m³ in SUB format</td>
</tr>
<tr>
<td>Serum-containing</td>
<td>Serum containing / serum-free</td>
<td>Serum-free</td>
</tr>
<tr>
<td>More laborious, time consuming, limited monitoring of mono-layer, multiple manipulations, higher risk profile</td>
<td>Process simplification, on-line monitoring, reduced manipulations, lower risk profile</td>
<td>Batch/fed-batch or perfusion Process simplification, on-line monitoring, reduced manipulations, lower risk profile</td>
</tr>
</tbody>
</table>

#### Production cells
- Transient, packaging & producer cell line development
- Current adherent process with serum
- 20µm

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**OXB vector process development strategies**

Holistic view of the development of ‘next generation’ lentiviral vector manufacturing

**Production Cells**
- **Transient**
  - Plasmids
  - Tfx reagent
  - Short USP phase
- **Stable**
  - Packaging cell line
  - Producer cell line

**Upstream Processing**
- **Suspension culture**
  - Animal
  - Component free
  - Media and feed
  - 100% single use
- **Process Control**
  - pH
  - Temperature
  - Dissolved oxygen
  - Identification of critical process parameters
- **Scale up**
  - P/V ratio
  - KLa
  - Tip speed
  - 100% Single use

**Downstream Processing**
- Flexible
- Scalable
- Gentle
- 100% single use
- Rapid, optimised yield
- Throughput
- Vmax / Pmax / Tmax sizing
- Membrane chemistry compatibility
- Pore size selection
- Dynamic binding capacity
- Buffer selection

**Advancing Manufacture of Cell and Gene Therapies IV, ECI, Coronado, CA, USA, January 27-31 2019**
Scale up & manufacturing challenges

OXB process development roadmap

- Development of rapid analytics for in-process & release testing
- Automated, high through-put cell line generation
- Stable, high producer packaging/producer cell lines
- Vector design & optimisation
- Establish media & feed platform
- Serum-free suspension culture in single-use bioreactor
- Purification development programme
- Formulation, Fill & Finish
- Scale-up Studies >200L
- Advanced Manufacturing Platform

Cohort 2
Dose Administration
3 months (UPDRS)
6 months (UPDRS)
1 year (UPDRS)
2 years (UPDRS)

1, n=3
Original Mean 27%
Max. up to 30%
Mean 30%
Max. up to 50%
Mean 29%
Max. up to 44%
Mean 20%
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2, n=3
Original Mean 28%
Max. up to 53%
Mean 34%
Max. up to 53%
Mean 29%
Max. up to 56%

3, n=3
Enhanced Mean 26%
-
Upstream considerations

Design and scale-up challenges for lentiviral vector production

**Culture system**
- 15 mL ambr™ bioreactor mimic and Shake flasks
- 0.5 L Bioreactors
- 7 L Bioreactors
- 50 L Single Use Bioreactors
- 200 L Single Use Bioreactors

**USP Process Design Fundamentals**

**Vector**
- Type (HIV or EIAV-based)
- Envelope pseudotype
- Vector titre
- Volumetric productivity
- Stability
- Non-infective to infective particles (P:L ratio)
- Aggregation
- Shear effects

**Cells**
- Cell line
- Concentration
- Biomass
- Cell viability
- Cell debris
- Cell aggregation
- Shear effects

**Medium**
- Medium components
- Serum or serum-free
- Anti foam aspects
- Transfection reagent
- Induction reagent

**Operation**
- Adherent or suspension
- Transient transfection
- Packaging/producer
- Number of harvests
- Volume processed
- Batch
- Fed batch
- Perfusion
- Concentrated fed batch

**Contaminants**
- Nucleic acids
- Process component toxicity
- Process related impurities
- Host cell protein
- Endotoxins
- Total protein

Characteristics of feed stream impacts design of purification strategy

Advancing Manufacture of Cell and Gene Therapies IV, ECI, Coronado, CA, USA, January 27-31 2019
Access to stable, high producing packaging/producer cell lines

- Justification for investment in stable cell line development has to be evaluated case by case based on predicted patient population size
- Automation of clone selection process (ACSS)

Scale-up of transient transfection processes

- Development of a stable producer cell line removes this constraint, enabling process refinements such as higher cell density, fed-batch and perfusion
- Optimisation of transfection step using DoE studies
  - Minimises costs (DNA, transfection reagents) on scale up
- Automation reduces operator-dependence and minimises variability (suspension process)

Envelope proteins (such as VSV-G) are often cytotoxic

- DoE studies to determine optimum plasmid amounts for transfection

Other LV packaging components (Rev, Gag/Pol) induce extra metabolic burden

- Regulation of expression of LV packaging components (Rev, Gag/Pol)

Process component toxicity

- Transfection & induction reagents
Suspension process development
Continuous process improvement
Suspension Process Development – DSP considerations

Functional lentiviral vector Particles

By-product toxicity

Vector stability

Process related impurities

VSV-G vesicles / TBS

Vector heterogeneity

Significant concentration of gene therapy to attain clinical efficacy, especially for in vivo applications

Protein aggregates

Inactive / empty particles

Significant concentration of gene therapy to attain clinical efficacy, especially for in vivo applications

Ineffective / empty particles

Aggregates

Suspension Process Development – DSP considerations

Cohort 2

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Mean 34%

Max. up to 53%

Mean 29%

Max. up to 56%

3, n=3

Original

Mean 26%

- -

Mean 29%

- -

Mean 20%

Max. up to 30%
Lentiviral vector product properties

Process development and manufacturing challenges

- Family: Retroviridae
- Genus: Lentivirus
- Enveloped
- Size: ~ 80 - 120 nm in diameter
- Genome: Two copies of positive-sense ssRNA inside a conical capsid
- Strong net negative charge
- Shear sensitivity
- Temperature sensitivity (inactivation) – product stability
- Freeze-thaw sensitivity
- Salt sensitivity (v. narrow range)
## Lentiviral vector product properties

**Process development and manufacturing challenges cont’d**

- Vector stability
  - Need to develop « soft » production methods
    - Minimise risk of high shear velocities, eddies, gas-liquid interfaces etc.
    - Reduce residence time in chromatography columns by using membrane chromatography or alternatives

- Sensitivity to temperature, pH and high salt, gas-liquid interfaces, shear effects
  - Avoid unwarranted intermediate storage steps
  - Minimise overall processing time
  - Add excipients or sugars into final formulation for cryopreservation
  - Develop formulation to stabilise vector at ambient temperature and upon freeze-thawing
Lentiviral vector product properties

Process development and manufacturing challenges cont’d

- Sensitivity to high salt concentration
  - Dilute vector after high salt contact as soon as possible
  - Use weak anion-exchangers (DEAE) and milder elution conditions
  - Use flow-through purification methods

- Sterility assurance issues
  - Terminal filtration using one or more 0.2 µm sterilising-grade filters can be technically challenging
  - Vector particles are relatively large (e.g. an average diameter of approx. 120 nm) compared to the maximal pore size of sterilising grade filters (i.e. 200 nm)
  - Aggregation – formulation buffer considerations important
  - Can lead to unacceptable product loss if the product is sterile-filtered at high concentration
Schematic of Serum-free, Suspension Process (200L Scale)

GMP manufacturing process for clinical supply

[Diagram showing the GMP manufacturing process]
**Sterile Manufacturing Process (Aseptic Fill & Finish)**

GMP manufacturing process for clinical supply

1. Vial to FDP: ~2000-fold volume concentration factor
2. Final volume determined by number of Ultra-diafiltered Drug Substance (UDFDS) lots and test data
Industrial Viral Vector Manufacturing using Advanced Process Analytical Technologies

- 2-year £2M collaboration project co-funded by Innovate UK
- Consortium led by OXB includes the CGTC and Synthace Ltd
- Apply novel advanced technologies to further evolve OXB’s proprietary suspension LentiVector® platform
- Support intensification of manufacture and drive higher viral titre yields to enable maximal productivity whilst limiting the need for increased process scale and higher capital costs.
  - Real time in-process monitoring
  - Improved product knowledge
  - Better process understanding and control
  - Improved consistency of manufacture
  - Optimised media and feeding strategies
  - Reduced product cycle times
  - Higher volumetric productivity and yields
  - Reduced COGS
Industrial Viral Vector Manufacturing using Advanced Process Analytical Technologies

- Temp
- pH
- DO
- Raman spectroscopy
- RI spectroscopy

Multivariate analysis

Big data analytics
In silico modelling
Real-time in-process monitoring
Adaptive control

Omics analysis

Metabolomics
Transcriptomics

Chemometrics
Process fingerprint development
Concluding remarks

• Gene and cell therapy has reached the stage where several very promising therapies have reached the commercial/market supply phase. However many challenges remain.

• OXB’s know-how and technologies in lentiviral vector development, production and analytics in addition to its ability to manufacture commercial quantities of viral vectors, provides significant competitive advantage.

• OXB is a technology innovator with ongoing investment to gain increased understanding enabling further improvements to the LentiVector® gene delivery platform.

• OXB recently completed a net £19.3m capital raise to fund the construction of additional manufacturing capacity (OXBox facility).

• This investment with Innovate UK viral vector grant support for OXBox will enable OXB to maintain its global leading position, and to be well-positioned to meet future demand.
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