Viral vector production for cell and gene therapy

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**About NRC**

- 19 Research Facilities
- Several Industrial Research Assistance Program (IRAP)
- **3,700** employees and 650 volunteer and independent visitors
- Wide variety of disciplines and broad array of services
  - support industry,
  - advance knowledge and technology,
  - fulfill government mandates
Viral Vectors

- Viral vectors are derived from viruses that have been modified by deleting genes necessary for replication or having toxic effects.
- Used to deliver genetic material into the body to treat a disease (In vivo gene therapy).
- Used to modify cells needed for cell therapy (Ex vivo gene therapy).
Ex vivo gene therapy

- Cell harvested from patients
- Modified with viral vectors (most often retrovirus or lentivirus)
- After modification cells are re-injected into patients
  - The origin of the cells can be from the same individual (autologous source) or from another individual (allogeneic source).
Vectors derived from adeno-associated virus (AAV) and lentivirus are important for gene therapy

- **In vivo gene therapy (current preclinical and clinical studies)**
  - Rare non-oncologic diseases (85% AAV)
  - Cancer (50% adenovirus, 25% Vesicular stomatitis virus, 5% AAV)
  - Sensory diseases (80% AAV)
  - Neurological diseases (80% AAV)
  - Blood and clotting diseases (90% AAV)

- **Ex vivo gene therapy (current preclinical and clinical studies)**
  - Cancer (45% lentivirus, 25% retrovirus, 10% adenovirus)
  - Rare non-oncologic diseases (50% lentivirus, 40% undisclosed virus)
  - Blood and clotting diseases (35% lentivirus, 20% retrovirus, 20% adenovirus)
Recent successes for in vivo gene therapy

Approved In vivo Gene therapy treatments

• **Glybera**, first gene therapy treatment approved in 2012 (Europe)
  • Lipoprotein lipase deficiency
  • Intramuscular injection of \( \text{AAV1-lipoprotein lipase} \)
  • Manufacturer: UniQure

• **Imlygic**, approved in 2015
  • Treatment for Melanoma
  • Intratumoral injection of oncolytic virus (herpes virus)
  • Manufacturer: Amgen

• **Luxturma**, approved Dec 2017
  • Treatment of Retinal dystrophy (biallelic RPE65 mutation)
  • Intraocular injection of \( \text{AAV2-RPE65} \)
  • Manufacturer: Spark Therapeutics, Inc
Approved *ex vivo* gene therapy treatments

- **Strimvelis**, approved June 2016
  - Treatment of Severe Combined Immunodeficiency due to Adenosine Deaminase (ADA) deficiency
  - Autologous transplantation of Stem cells (CD34+) modified with a retrovirus encoding ADA
  - Manufacturer: GSK

- **Kymriah™**, approved in August 2017 - Manufacturer: Novartis
  - Acute lymphoblastic leukaemia (ALL)

- **Yeskarta** approved October 2017 - Manufacturer: Kite Pharma
  - B-cell lymphoma
  - Both Treatments: autologous transplantation of T-Cells modified with **Lentivirus Vector** expressing CAR against CD19
Production of vectors derived from AAV and lentivirus

- Both vectors are produced using mammalian cells
- Human Embryonic Kidney cells (HEK293 cells) are most often used to produce AAV and Lentivirus
  - Note: AAV can be produced also in insect cells through infection with baculovirus
- There is a growing demand for AAV and lentiviral vector (academia and industries)
  - We need efficient and scalable processes of production
HEK293 expression platform at NRC

A clone of HEK293 cells was isolated by NRC in 1995: **293SF-3F6**

- Grow in suspension (Scale-up)
- Serum-free medium (regulatory compliance)
- Patented in 1998

- cGMP cell bank: 2003; 2015
  - Can be used to manufacture biologics for human application

- Cell storage
  - ATCC (Manassas, VA)
  - Core Cryolab (Toronto)
Process development to scale-up 293SF-3F6 cells in suspension culture

Shake flasks: 125 ml to 2 Liter

Bioreactor 3 Liter

Wave bioreactor 5 to 25 liter
State of the Art Animal Cell Culture Facility

Animal cell pilot scale production facility equipped with bioreactors up to 500L working volume

60 L Bioreactor

200 L Bioreactor

293SF-3F6 scaled up to 450 L working volume for viral vector production
Production of vectors derived from Adeno-Associated virus (AAV)
Adeno-Associated virus (AAV)

- Family of parvovirus
- Small virus (≈18-25 nm)
- Do not cause diseases
- Do not have an envelope
- Single stranded DNA as genome (5-kb)
Vectors derived from AAV (rAAV)

- Do not contain viral genes
- Do not integrate its genome inside the cell chromosome
- Weakly immunogenic
- Transduce dividing and non-dividing cells
- Long term transgene expression (in non-dividing cells)
- Small transport capacity (4.7-kb)
- Virus particle very stable
rAAV production in HEK293 cells

- HEK293SF cells
  - Suspension culture
  - Serum-free medium
  - 1 million cells/ml
- Optimize transfection procedure
  - Three plasmids + Polyethylenimine (PEI)
- Scale-up process in bioreactor
Production of AAV2 in 3L Bioreactor

Harvest Window
Reproducibility of AAV manufacturing

![Graph showing total genome containing particles (Vg) for different process steps and runs.]

- Lysed Cell Culture
- Filtered Cell Lysate
- UF/DF
- Affinity Column Eluate

Run labels: 45L-RUN 01, 45L-RUN 02, 45L-RUN 03.
Production of AAV: 293SF-3F6 cells by transient transfection using PEI

- Shake Flasks: 20 ml to 500 ml
- Wave bioreactors: 5 to 20 L (Manceur et al., Poster #213)
- Stirred Tank Bioreactors: 2.5 L, 65 L, 500 L
- Titers obtained: $0.5 - 2.0 \times 10^{13}$ vg/L; 5 000 - 20,000 vg/cell (after purification)
  - Note: titer varies with construct; it decreases with larger construct
Production of vectors derived from lentivirus (Lentiviral Vectors)
**Lentivirus**

- **Lentivirus are complex retrovirus**
  - Best example: Human immunodeficiency virus (HIV) causative agent of AIDS
- **It is an enveloped virus**
  - Relatively fragile
- **Genome consists of two fragments of RNA**
- **Viral cycle involved integration**
  - Integration can occur in non-dividing cells
Production of lentiviral vectors

Transient transfection with 4 plasmids and PEI

Plasmids used

- pMDLg/pRRE
- pRSV-REV
- pCMV-VSVg
- Transfer Vector

Envelope protein

Titer: $10^6$ to $10^7$ TU/ml

Lentivectors
Scale-up of LV production: LV-CAR

Process: robust, reproducible and can be scaled-up
Development of packaging cells for lentiviral vectors

Packaging cells
• Cells that contain all the genes necessary to produce lentiviral vectors
  • VSV-G (envelope protein)
  • Gag/Pol
  • Rev

Production of LV using packaging cells
• Transient transfection with transfer vector
• Generation of stable clones that have integrated several copies of transfer vector (producer)

• VSV-G and Gag/Pol are toxic to cells
  • Therefore transcription must be regulated.
    • During cell amplification: Genes are OFF
    • During LV production: Transcription is turned ON by addition of inducers
1. Titer = or > than transient transfection with four plasmids
2. Cells grow in suspension (scale-up)
3. Cells grow in serum-free culture medium (regulatory compliance)
4. Addition of inducers to turn on the production of lentiviral vectors
   - Dox + Cumate

Properties packaging cells developed at NRC
Stable producers: produce LV without transfection

**Generation of stable producer clones:**
- Packaging cells transfected with transfer vector (LV-CMV-GFP)
- Clones are isolated by limiting dilution
- LV production occurs after induction
  - Cumate + Doxycycline

Producers are stable for 4 months
Production modes in bioreactor

› **Batch**: No additional supplements or feeds are added in culture

› **Fed-Batch**: Feeds are added to increase cell growth

› **Perfusion**: Continuous process, fresh medium is added and spent medium is remove; Cells remain in the bioreactors
LV Production using stable producers in 3-L bioreactor

Comparison between batch, Fed-batch and perfusion

Growth kinetics with stable producer cell line
Improved LV Production: Fed-Batch & Perfusion Mode (3 L scale)

- Increased upstream yields: batch<<fed-batch<perfusion
Summary and conclusion

- The two most popular vectors for gene therapy
  - AAV: for in vivo gene therapy
  - Lentivirus: for ex vivo gene therapy

- Efficient processes to produce AAV and LV has been developed at NRC
  - Processes can be scaled-up
  - Reproducible
  - Amenable to cGMP manufacturing

- Current research
  - Construct and test better packaging cells (derived from 293SF-3F6 cells) for both AAV and LV
  - Develop a robust purification process for LV
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