



Généthon's industrial approach for gene therapy products

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A UNIQUE organisation



- Created in **1991**
- Budget = **23 M€** (~ 90% for operational)
- 10 000 m²** for laboratories
- 220** collaborators
- 6 European projects
- ETGC**: pharmaceutical production sites

Within industrial area dedicated to therapeutic application from genomics knowledge

Actor of Meditech Santé

Spin off creation **GenoSafe**

Major actor of Génomôle Evry



A PIONEER IN GENETIC DISEASE

(Genome ⇒ Genes ⇒ Diagnosis ⇒ Therapies from gene knowledge)

Strategic mission: To cure by providing curative treatments for orphan genetic diseases

Exploratory candidate

Preclinical

DME

PhIa

PhIb

TUA

MA

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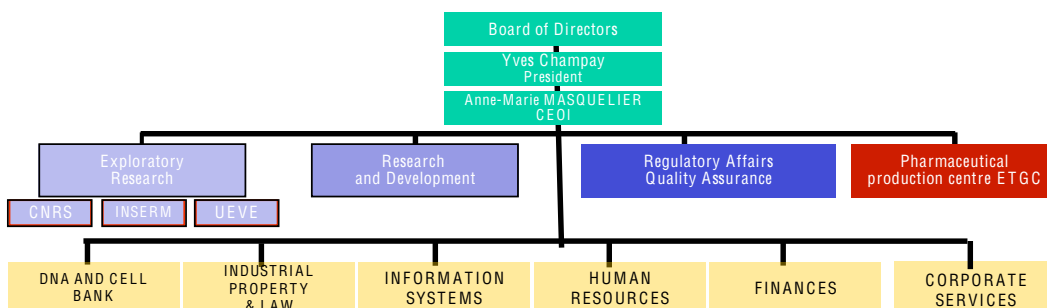
Généthon today

- 4 “orphan product” designations obtained
- Start of its first phase 1 clinical trial in 2006
- 3 clinical trials scheduled in the near future
- A rich portfolio
- Numerous academic, strategic, industrial, national and international collaborations

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A UNIQUE organisation

- Non-profit organisation for biotherapies,
 - Dedicated to development of innovative therapies derived from knowledge of gene
 - Rare and genetic diseases, particularly neuromuscular diseases
 - An integrated structure: Research, Development, Clinical production



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ETGC, Pharmaceutical Production Sites: Capacities

Establishment authorised by Afssaps: 2005 -2006
n° TG/06/O/007 and TG/06/O/016

- Within the Génopole ®
- 1900 m2
 - 1 L2 cell production line
 - 3 L2 & L3 vector production lines
 - 2 L2 & L3 aseptic filling suites
 - GMP preparation and storage zones
- Capacities
 - Up to 50 litres
 - 6 vector batches per year and per line



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ETGC, Production Centre: Competencies

Production technology transfer

GMP qualification of cell and vector production processes

Aseptic filling



AAV, HIV or MLV lots production for Phase 1 clinical trial

Facility control, raw material, finished products controls

Quality Assurance

Facility and equipments qualification
Audits, documents management

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Difficulties

Bioproduction in gene therapy:

For phase 1: Small batches

quantities needed for testing and sample collection
manual aseptic filling
fill and finish issues



European regulation regarding production

Gene therapy GMP not yet available

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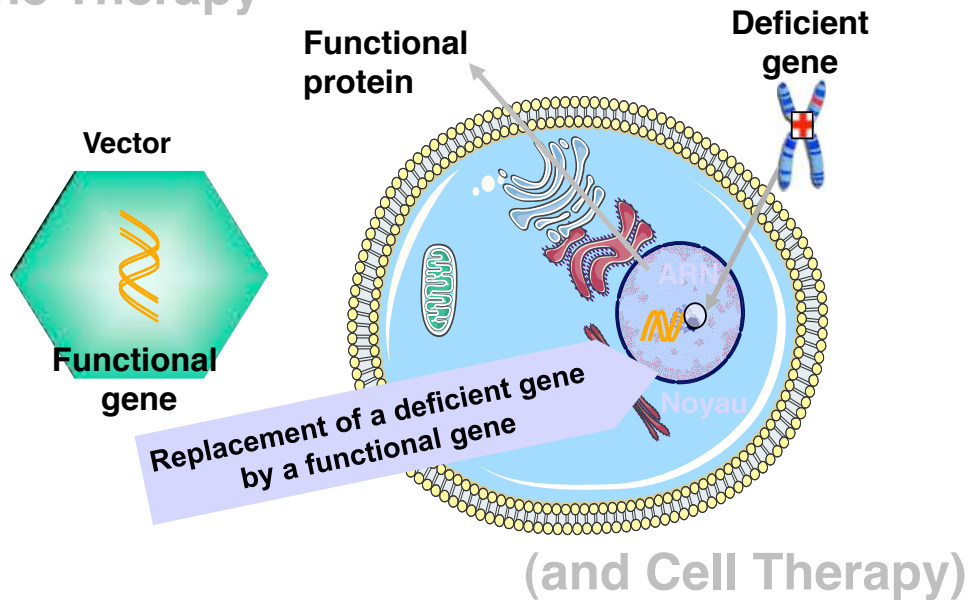
Challenges

- Increase the production capacities in terms of
 - Particles concentration
 - Particles total quantities
- Implementation of a new facility to produce at industrial scale of gene therapy products in Evry
 - In 2010, 4000 m2 facility (up to 1000L)

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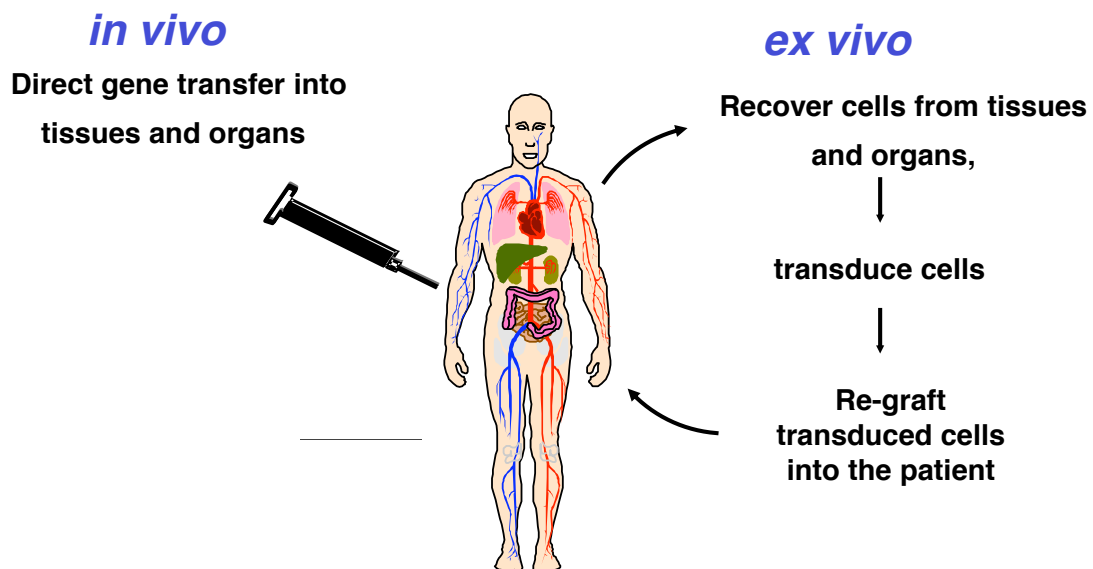
Innovative therapies

Gene Therapy



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Two gene therapy strategies: *in vivo* and *ex vivo*



e.g.: treatment of neuro-muscular diseases (Gene Therapy)

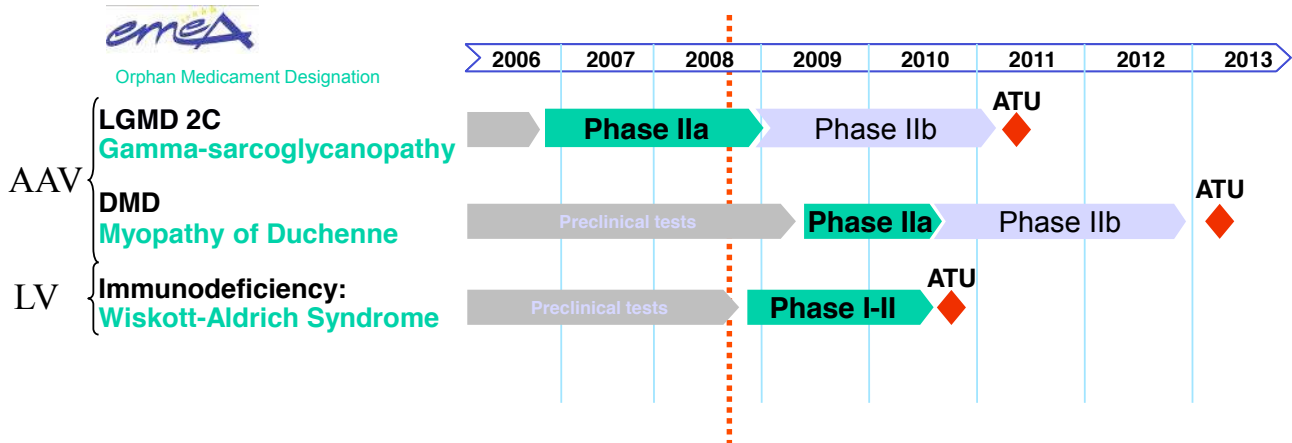
e.g.: treatment of severe combined immunodeficiencies (Combined Gene & Cell Therapy)

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Généthon's projects

September 2008: 3 clinical projects

3 pathologies: 3 novel therapeutic concepts



and more than 10 other projects in preparation

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Vector production – quantities needed for a phase I trial

AAV:

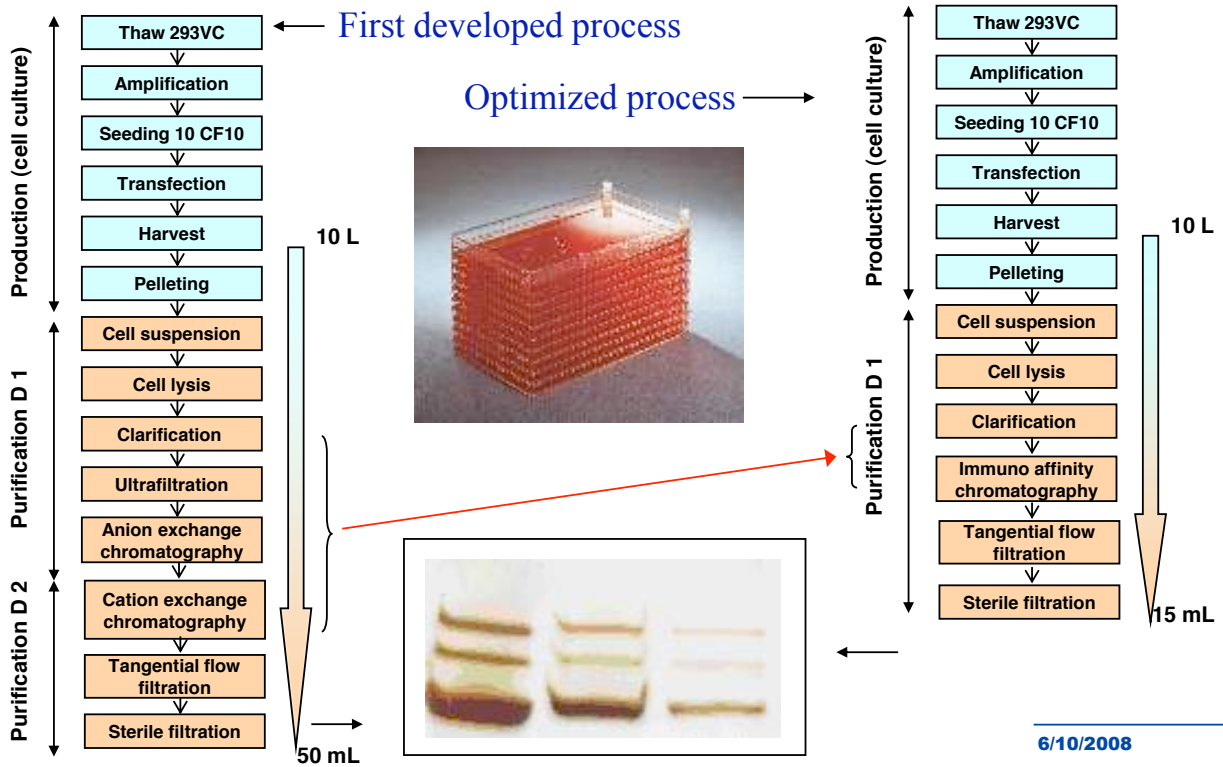
- Phase I, clinical trial, im (γ -sarcoglycanopathy):
- 10^{13} vg in total for administration of 3 different doses to 3 patients (Σ 9 patients)
- Production can be done by triple transfection of HEK 293 cells

LV:

- Phase I/II, clinical trial, ex vivo of CD34+ c. (WASP):
- $>5 \times 10^{10}$ ip in total for treating 5 patients (mono-dose)
- Production can be done by quadri transfection of HEK 293T cells

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Manufacturing process for production of AAV1



Performances of AAV1 manufacturing protocols

	Ion exchange chromatography (initial protocol)	Immunoaffinity chromatography (improved protocol)
	→ transferred to ETGC	→ to be transferred to ETGC
Bulk quantity of AAV (vg)	3.3×10^{14}	6.4×10^{14}
Yield (vg)	5-10%	36%
Final purified AAV (vg)	3.4×10^{13} 3.4×10^{13}	2.3×10^{14} 4.3×10^{13}
Final concentration (vg/ml), volume	$>10^{12}$ (50 ml)	$>10^{13}$ (15 ml)

(The bulk quantity of AAV1 is produced by triple transfection of HEK293 cells)

Limitations of the transfection process

- **Considerable limitations with respect to scale-up = limitations with respect to the quantity of AAV which can be produced**
 - Variations with respect to process performances
 - Elevated concentration of residual plasmid DNA
 - Possibility of recombination events of different AAV functions during transfection → generation of rcAAV
- **a more robust, safe and scalable manufacturing process is necessary**

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Production of larger quantities? → industrial phase

AAV – treatment of neuro-muscular diseases

- Phase I/II trial, whole body treatment via systemic administration.
- **Dose:** 5×10^{13} – 2×10^{14} vg/kg
- **Clinical lot:** 5×10^{16} vg of purified viral particles – treatment of 2 patients
- Needs for a large scale manufacturing process using bioreactors
- **Généthon's option:** use of the Sf9-baculovirus system

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AAV production: the insect cell/baculovirus technology – WHY?

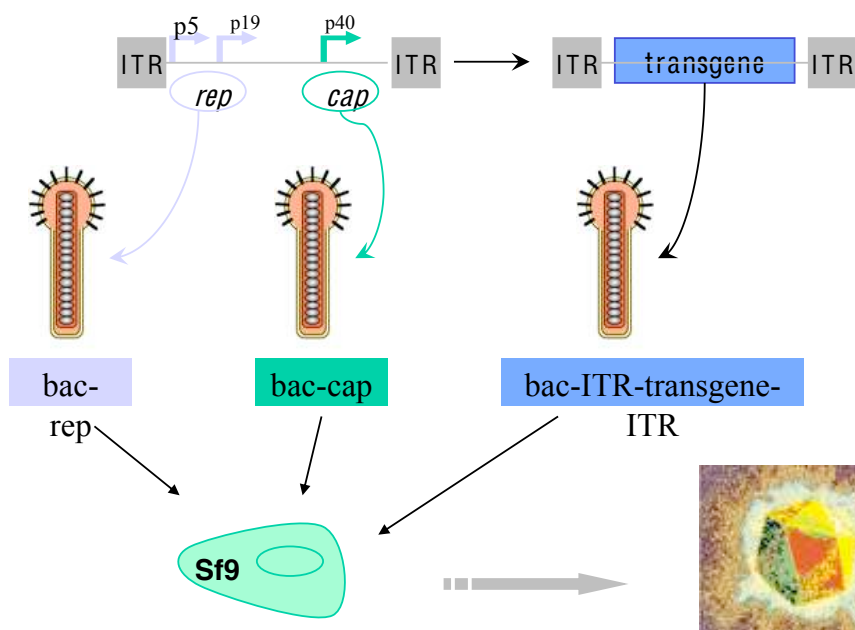
- **Insect cells (Sf9): culture ease**
 - Suspension culture – possible scalable reactor process
 - Compared to mammalian cells: higher cell density, increased tolerance to high osmolality and by-product concentration.
 - Serum-free media available.

- **AcMNPV:**
 - No size limitation for foreign genes
 - Strong promoters available
 - Non pathogenic virus for vertebrate
 - Unable to replicate in mammalian cells

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AAV production using the insect cell/baculovirus technology

- In 2002, Urabe et al. described a new approach



(C. Gény-Fiamma, O.-W. Merten)

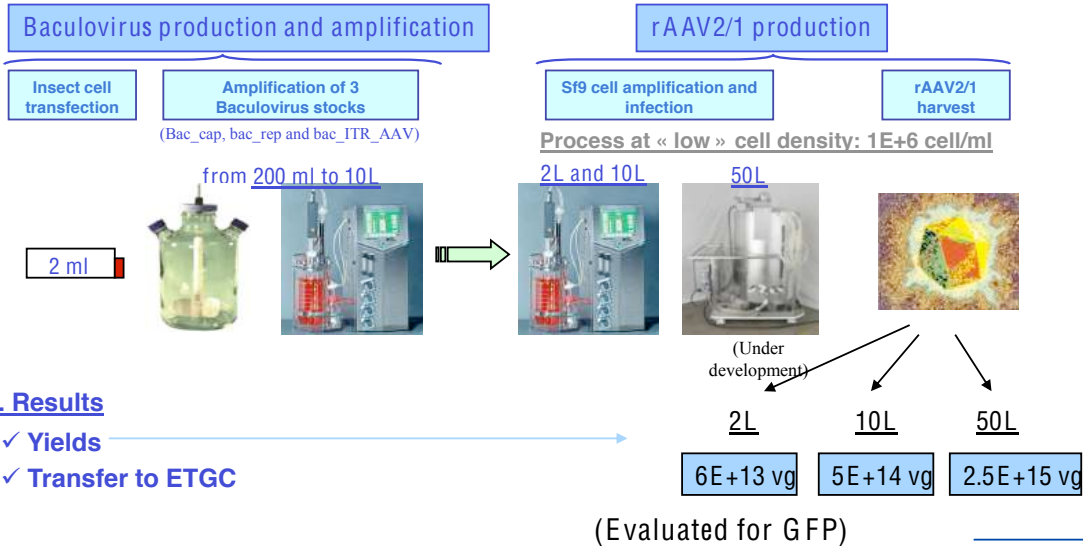
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Production of rAAV2/1 using insect cells/baculovirus technology – achievements

- I. Goal:** 1. Development of performing and robust process at large scale (2L and 10L) to produce rAAV2/1
2. Technology transfer to GM P production unit of Généthon (ETGC)

II. Objective: Process to produce up to 50 litres (>2.5E¹⁵ total vg)

III. Process overview and results



IV. Results

- ✓ Yields
- ✓ Transfer to ETGC

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Future developments in AAV manufacturing



- Scale-up to 50 litres – use of a disposable reactor system
- Transfer to the GMP production unit of Généthon (ETGC)
- Process intensification – high cell density process (>5E+6 cell/ml)
- Evaluation of the production process for the production of other AAV serotypes
- Scale-up to 200 – 300 litres → Transfer to ETGC

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LV – treatment of SCIDs and ‘less’ orphan diseases

- Increased number of patients to be treated (e.g. FANC A)
- Phase I/II trial, ex vivo treatment of patient cells – in vivo treatment of patients’ bone marrow
- Dose: GALV: MOI 1-10 – bone marrow cells → 5×10^8 – 5×10^9 particules/kg → 10^{10} – 10^{11} particles/patient (child – 20 kg)
- Clinical lot: 10^{11} – 10^{12} (5 patients/QC-needs)
- Needs for a large scale manufacturing process using bioreactors

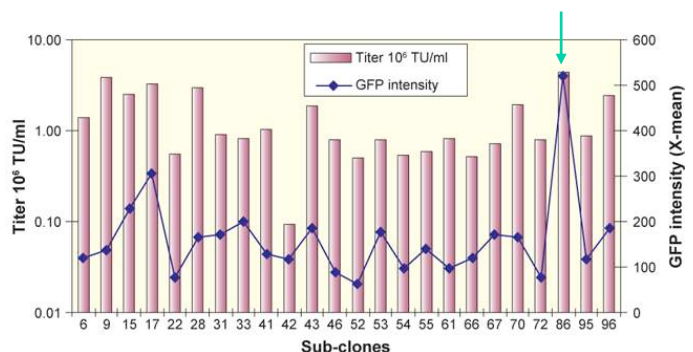
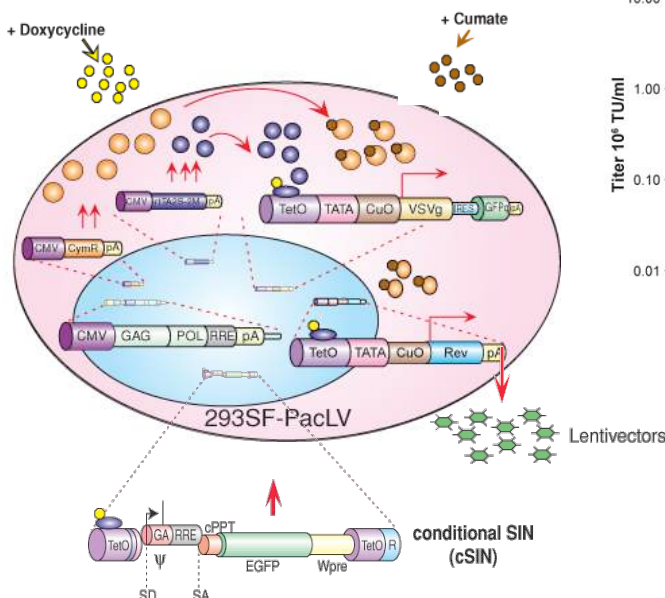
→ Option of Généthon: development of LV vector producer cell lines in a collaborative effort (with B. Massie from IBR-CNRC in Montréal) – development of a reactor based process

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Principle:

Generation of cSIN LV stable producer clones

- 1- Transient transfection of cSIN transfer vector
- 2- Infection of 293SF-PacLV at high MOI with cSIN LV
- 3- Limiting dilution of producer pool

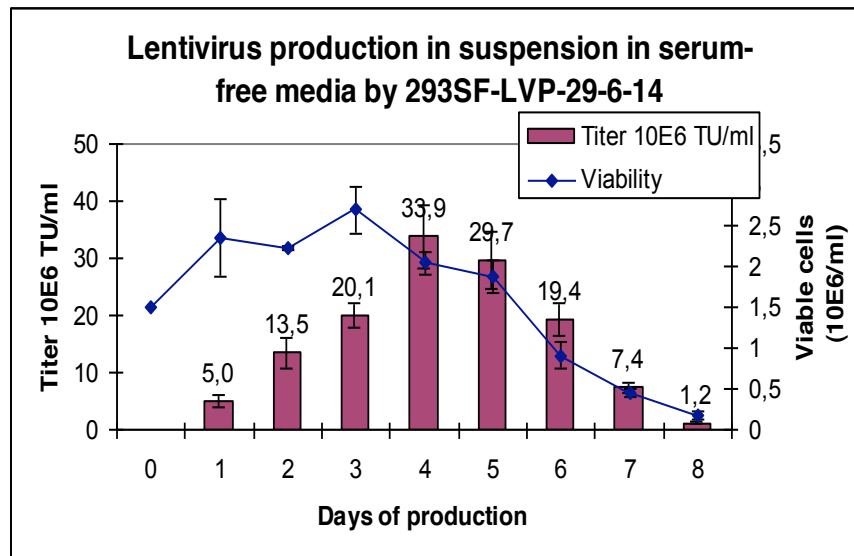


•Titers > 10E6 for majority of clones

•GFP level (from cSIN vector) is only indicative of best clone

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Production of LV in suspension serum-free culture



From a stable producer clone derived from 293-SF-PacL-29-6 clone, on average 1.8×10^7 TU/mL of LV-TR-GFP can be collected from day 1 to 7 for a total of 3.2×10^9 TU in a 25 mL culture

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Summarizing:

Future production scales available in Généthon:

- AAV (Sf9/baculovirus system):
 - 2L/10L (existing)
 - 50L use of disposable (under evaluation)
 - 200L/300 L – 1000 L use of disposable (to be evaluated in the near future)
- LV (producer clones):
 - 2L/10L perfusion process (to be developed)
 - 50L perfusion process, use of disposable (to be developed)



Thank you for your attention

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