LENTIVIRAL VECTOR MANUFACTURING: A SUCCESSFUL AND REPRODUCIBLE CONTINUUM PROCESS FROM RESEARCH BATCHES TO CLINICAL APPLICATION

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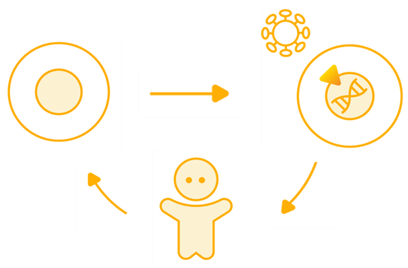
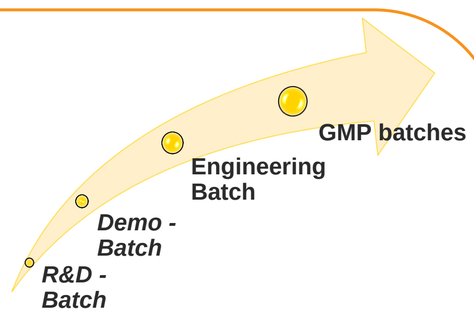
A. Challenges of lentiviral vector manufacturing scalability for ex vivo clinical applications

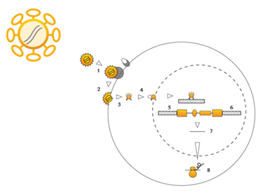
Advanced Therapy Medicinal Products (ATMP) needs for CAR-T/CAR-NK cells applications have intensified and require highly purified **lentiviral vectors** (LV) as **starting material for *ex vivo* clinical trials**. Ensuring **a continuum from discovery to clinical applications requires** to successfully shift between production scales while **maintaining process performance attributes** and **critical quality attributes**.





DNA delivery for a stable expression

Integrative Lentiviral vectors

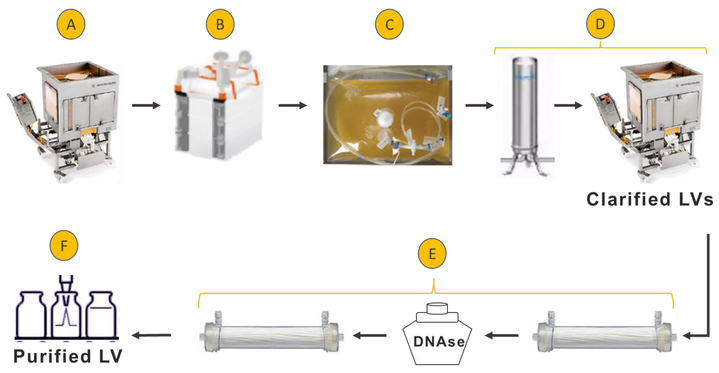


GENE THERAPY

IMMUNOTHERAPY

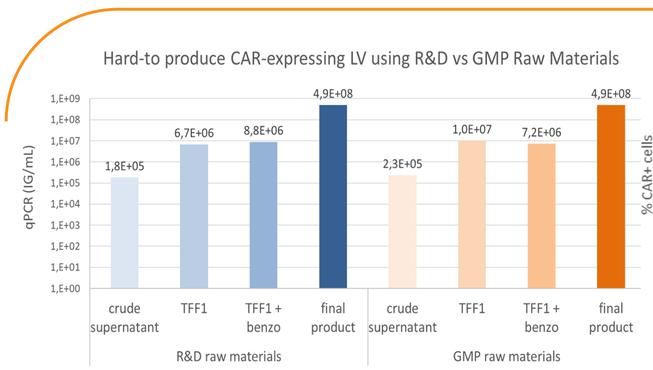
B. LVs Manufacturing Process flowchart

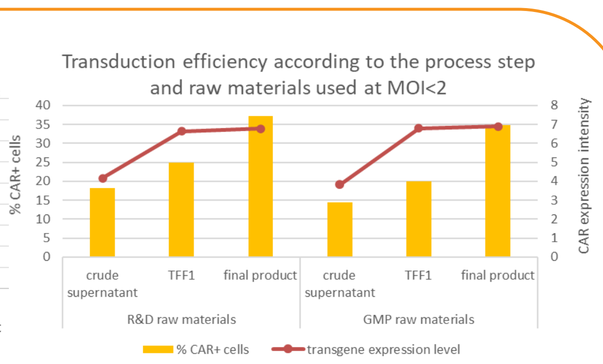
- A — Reagents & culture media preparation
- B — HEK293T thawing, amplification & HS36 seeding
- C — Reagents & culture media preparation
- D — Lentiviral vectors harvest & clarifications
- E — Lentiviral vectors purification
- F — Fill & finish
- **FILLED PRODUCT**



A GMP manufacturing platform using Hyperstack® (HS36) technology was implemented for both integrative & non integrative lentiviral vectors production (RNA delivery, LentiFlash®), able to produce **up to 10^{12} infectious particles**.

C. Demonstrated raw material representativeness from R&D to GMP

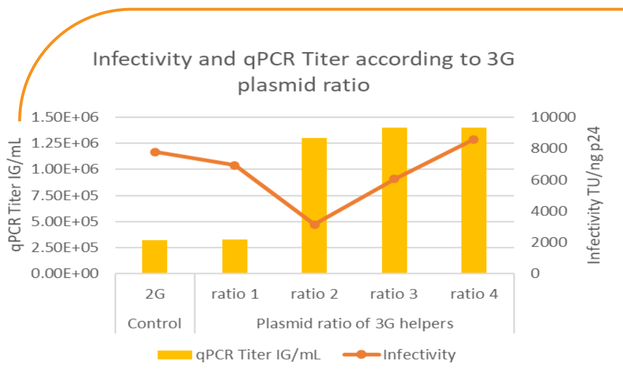


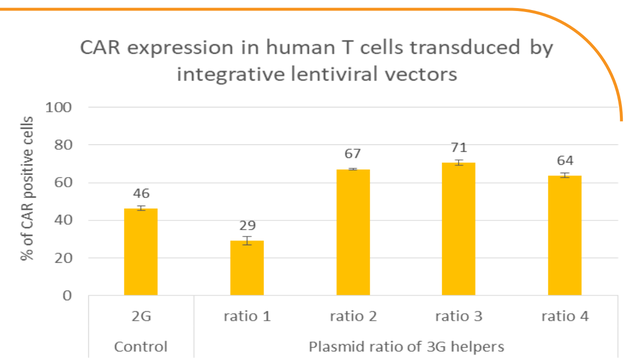


Infectious titers (Integrated Genomes/mL) were compared for Hyperstack process with either R&D or GMP grade raw materials. **Similar process yields** were obtained **at all different process steps**.

Product quality with both raw material grades was further assessed *via* T-cell **transduction efficiency** and **transgene expression level at low MOI**, showing **no raw materials effect** and **increased quality through purification**.

D. Transgene specific plasmid ratio optimization prerequisite before scaling up



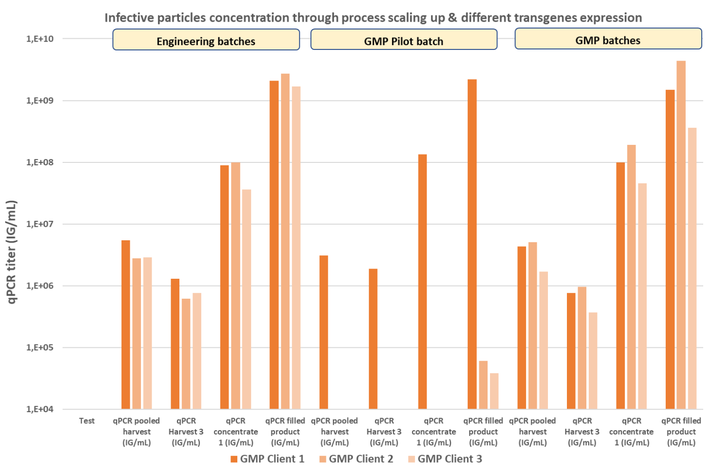


Crude batches produced in 3rd generation, compared to 2nd generation. Infectious titer (Integrated Genome/mL) is quantified by qPCR after transduction of HCT116 cells. p24 titer is quantified by ELISA assay.
Titer and infectivity are plasmid ratio dependent.

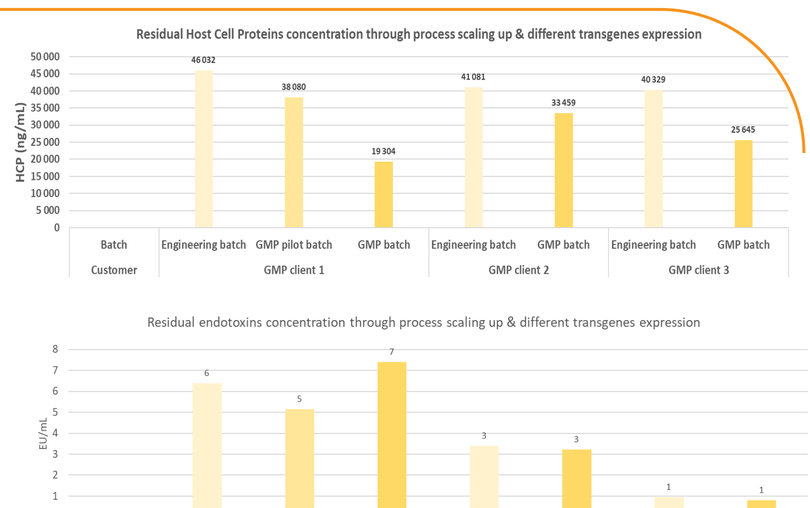
Human primary T cells are activated by CD3/CD28 beads prior being transduced by the same volume of crude supernatant. % of CAR positive cells is measured by flow cytometry after immunostaining of the CAR.
Transgene expression is ratio dependent.

E. Process scalability from engineering to GMP batches for 3 GMP projects in portfolio through Process Performance Attributes (PPA) & Critical Quality Attributes (CQA)

GMP manufacturing seed train consists in HEK293T cells thawing in Cellstack 1 (CS1) followed by amplification in Cellstack 10 (CS10) before Hyperstacks 36 seeding.
Batch-to-batch consistency is observed for both **cell viability** (left) at each step, with **highly reproducible cell growth for each step** (doubling time, right). Nucleocounter NC200® is used for both measurements.
Harvested cell suspension is then used for seeding either 1 line of 4 Hyperstack® (engineering or GMP pilot batch) or 3 lines of 4 Hyperstack® (GMP batch)



A detailed analytical characterization of the product was established with internal method (Integrated genome (IG/mL), and led to **equivalent process performance yields**, throughout the different process steps **for both engineering & GMP batches**, with equivalent ex vivo transduction efficiency at low MOI (data not shown).



Process related-impurities such as **HCP**, quantified using GMP QCs, was in a **3-fold magnitude order** between scales, **with low endotoxins content.**
Part of these data is associated with the **IRIS project** funded by the French National Research Agency (ANR) Investments for the Future program (PIA) under grant agreement No. ANR-18-RHUS-0003.

F. Conclusion

Here, we describe a successful and reproducible continuum process for LV manufacturing**, from Discovery to Clinical phases**, leading to highly reliable process yield & quality attributes to generate starting material LV batches for ***ex vivo* clinical applications.**

Ensuring a continuum from discovery to clinical applications requires to successfully shift between LV production scales while:
- Providing **batch-to-batch seed train consistency**
- Maintaining **LV productivity & high infectivity ratio**
- Ensuring **consistent process-related impurities level**

between scales, compliant with ex vivo use

These solid results provide relevant elements for the scalability of our continuum for GMP batches, **with manufacturing capabilities up to 180L of crude supernatant and between 100 and 300 mL of filled product**. It strengthens the claim of an easy and prompt transition from R&D activities to phases 1/2 clinical studies. Manufacturing capabilities.

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