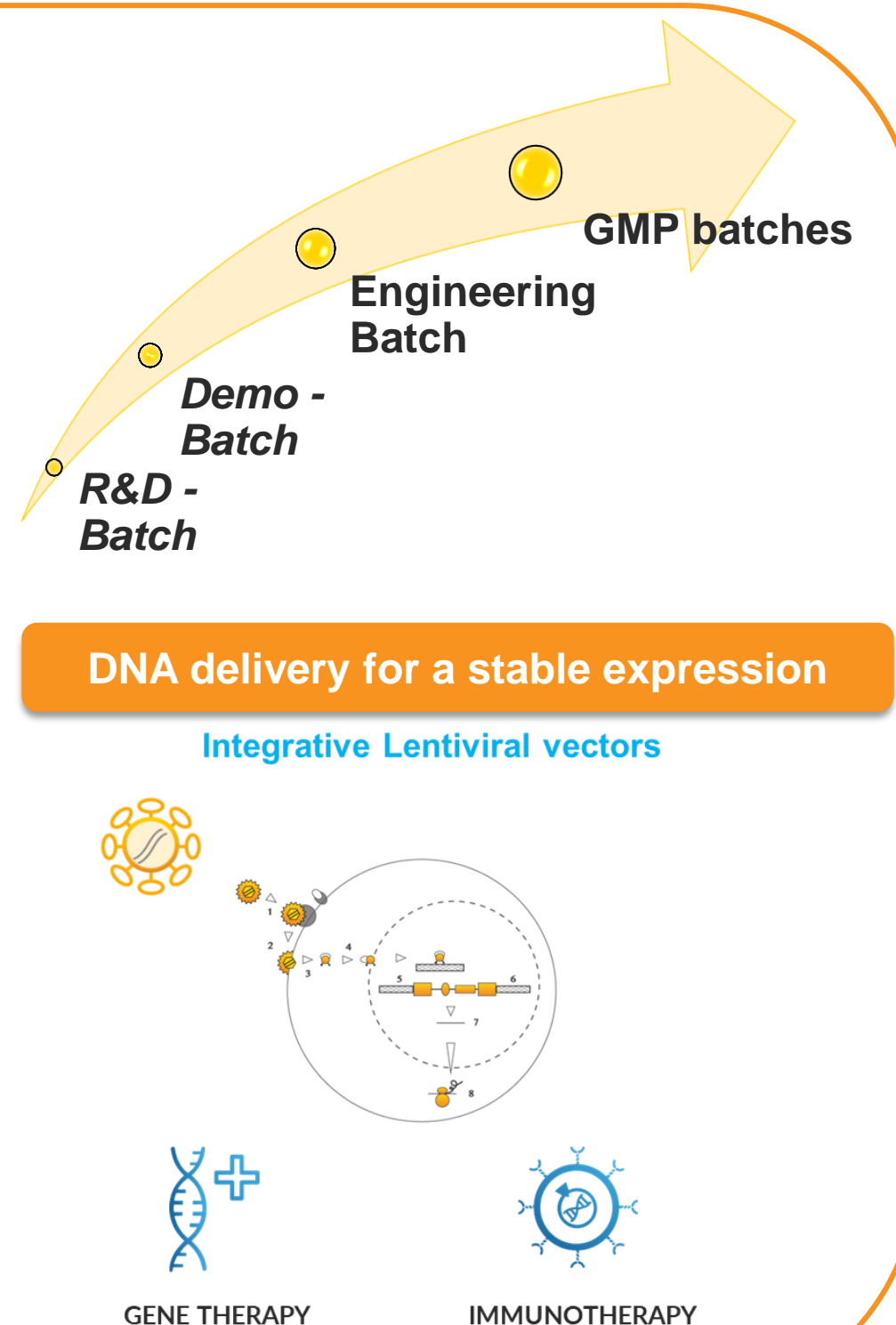


# LENTIVIRAL VECTOR MANUFACTURING: A SUCCESSFUL AND REPRODUCIBLE CONTINUUM PROCESS FROM RESEARCH BATCHES TO CLINICAL APPLICATION

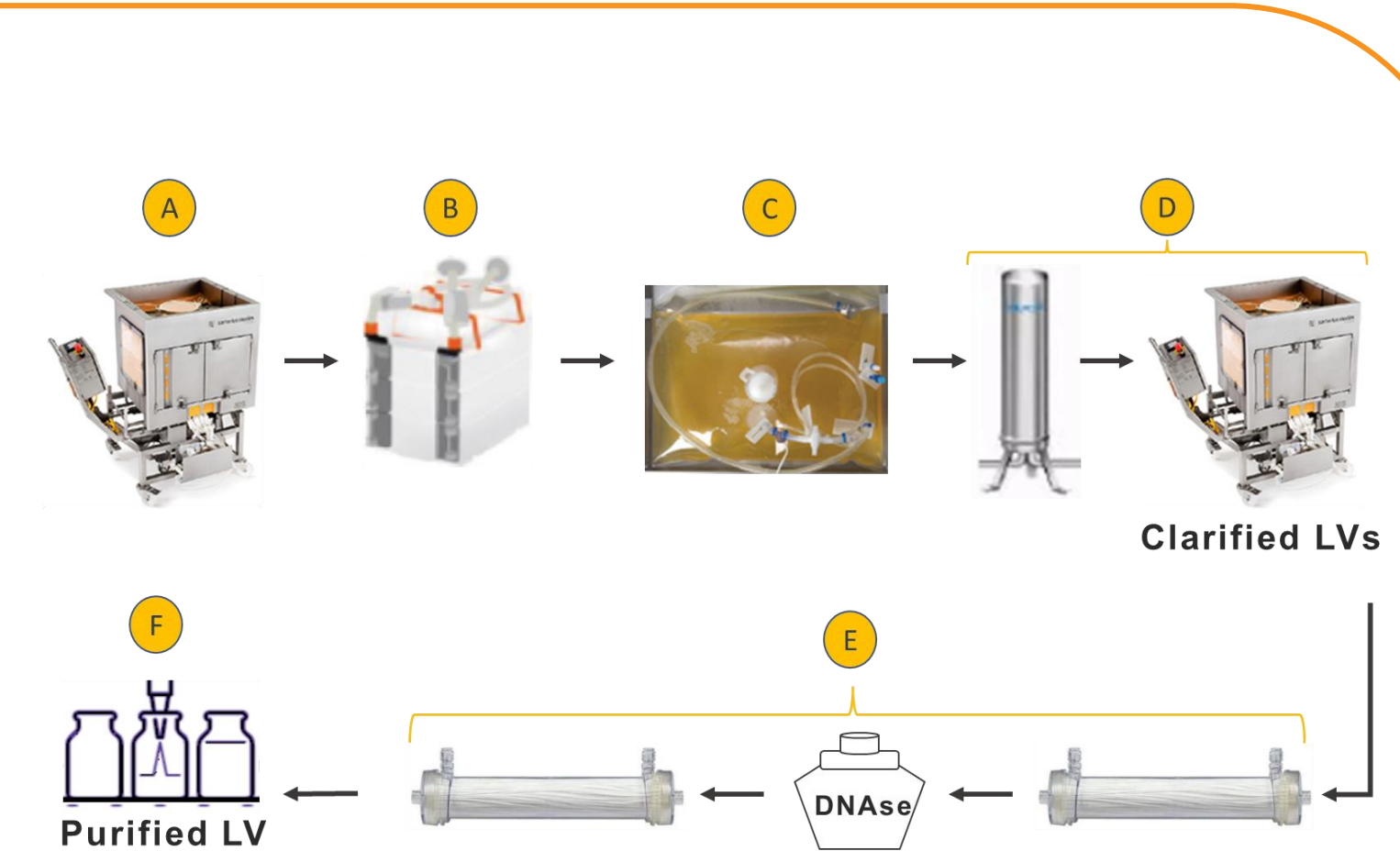
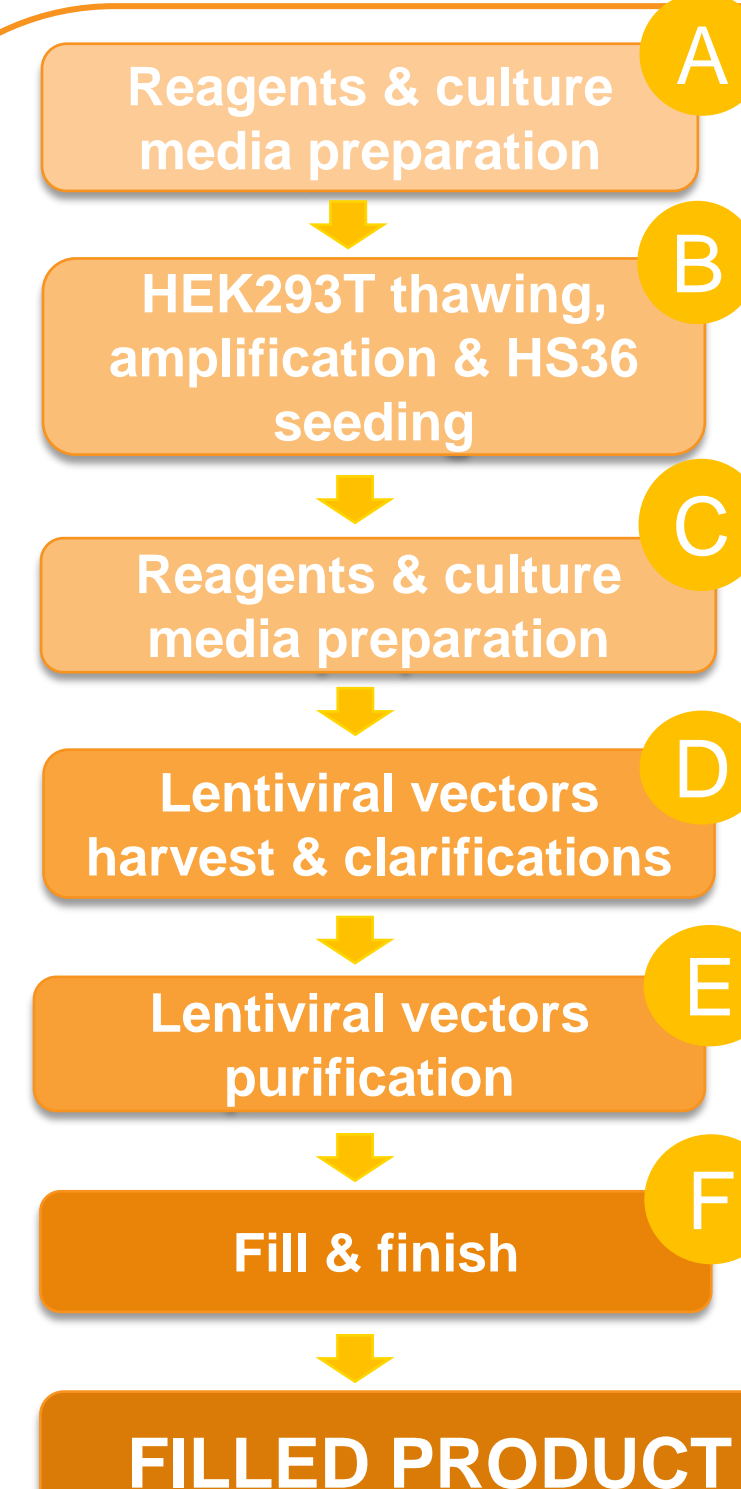
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## A. Challenges of lentiviral vector manufacturing scalability for ex vivo clinical applications

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.

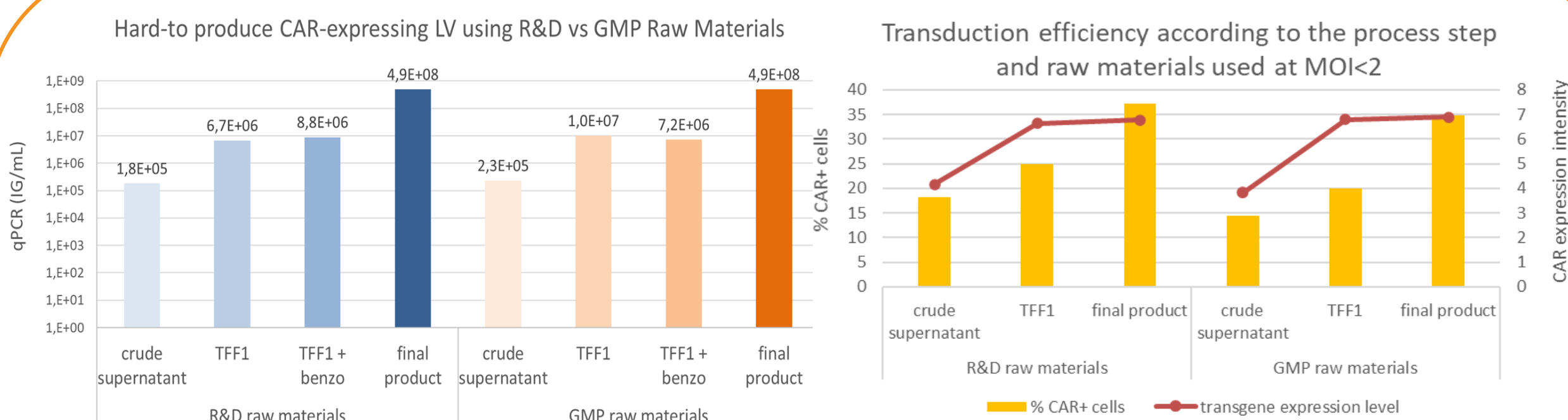


## B. LVs Manufacturing Process flowchart



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<sup>12</sup> infectious particles.

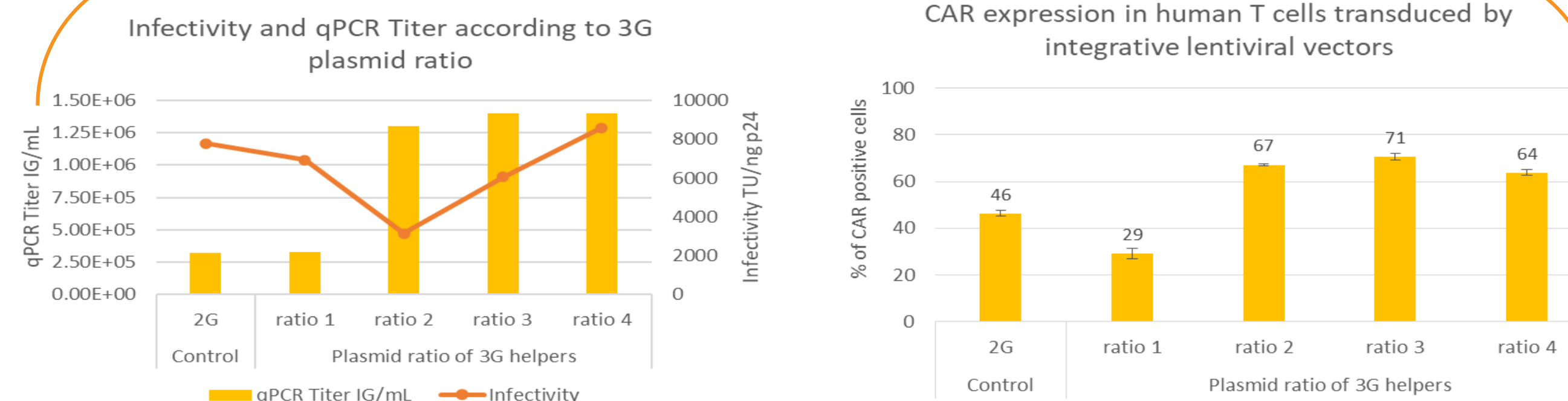
## C. Demonstrated raw material representativeness from R&D to GMP



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.

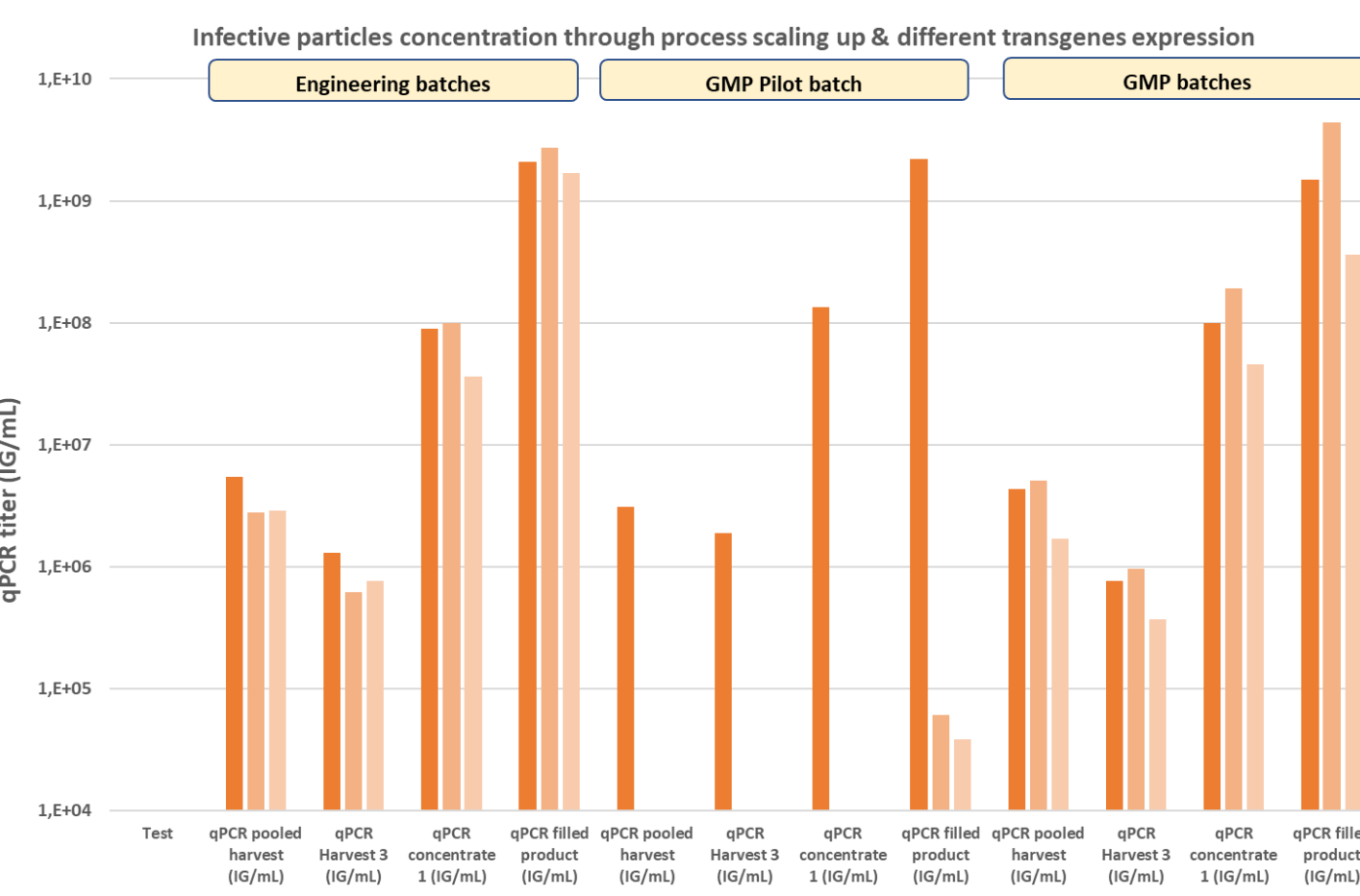
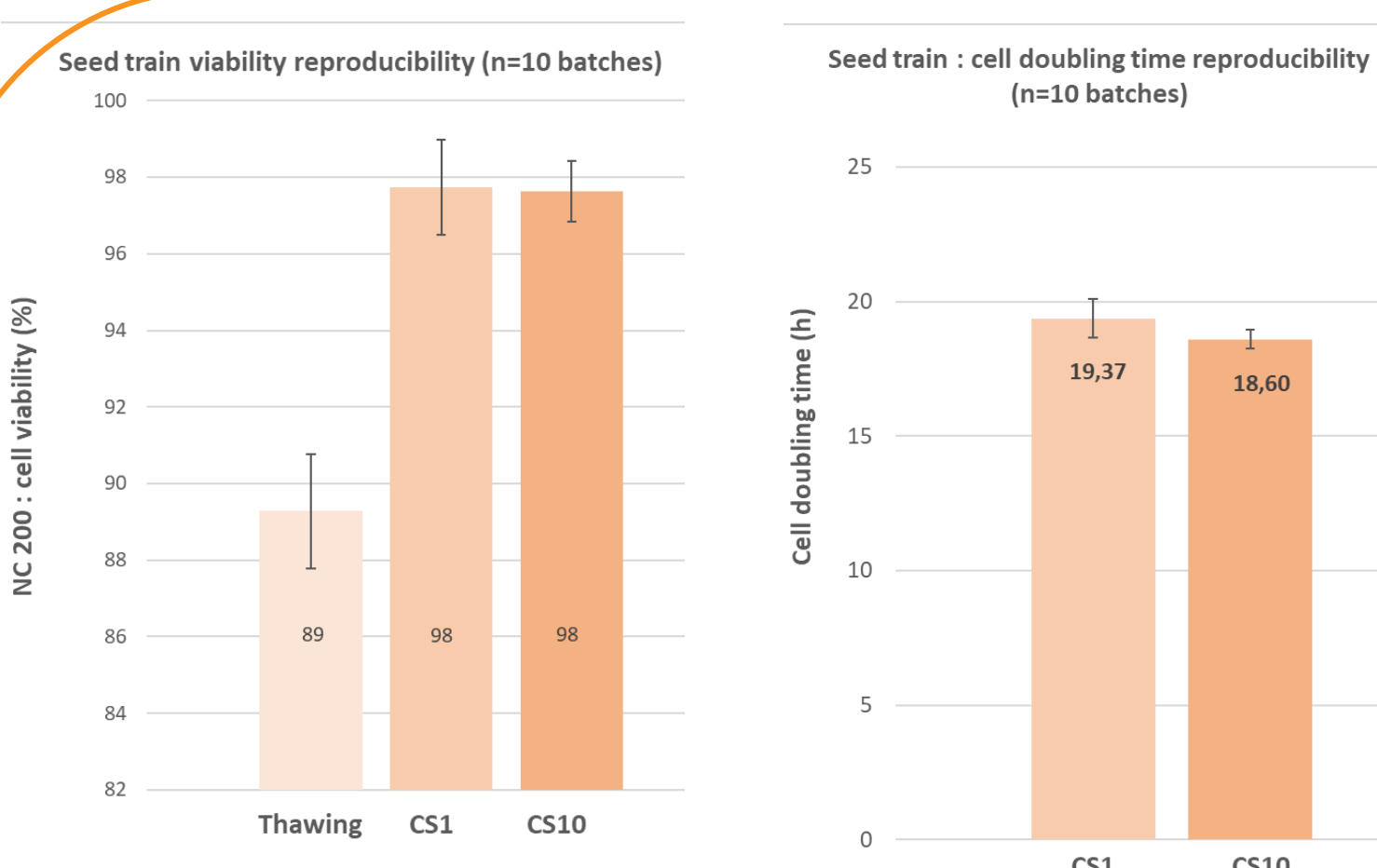
## D. Transgene specific plasmid ratio optimization prerequisite before scaling up



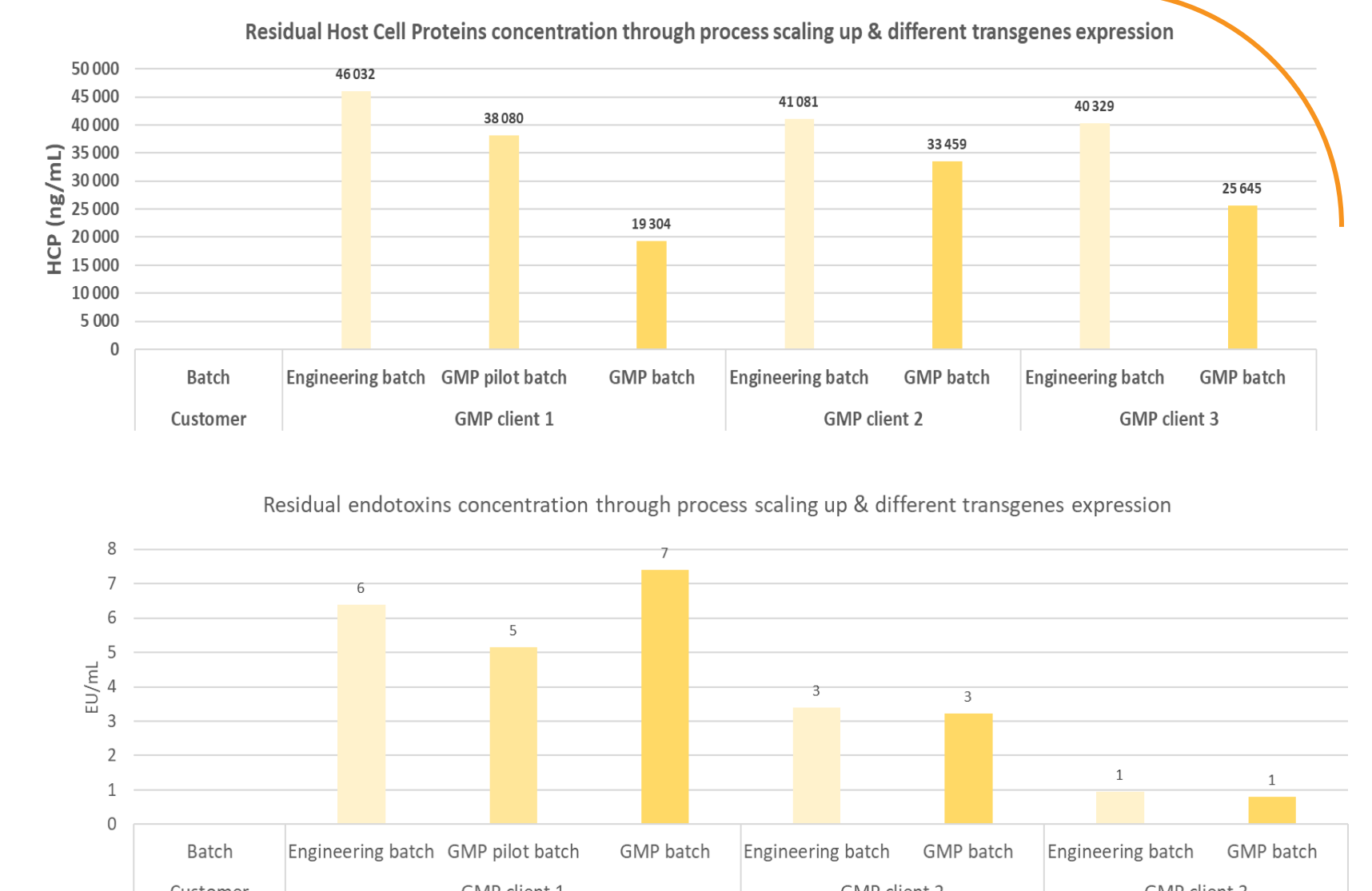
Crude batches produced in 3rd generation, compared to 2nd generation. Infectious titer (Integrated Genome/mL) is quantified by qPCR after transduction of HCT116 cells. p24 titer is quantified by ELISA assay. Titer and infectivity are plasmid ratio dependent.

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)



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.

