A Robust and Scalable Platform Process for GMP Manufacturing of Lentiviral Vectors

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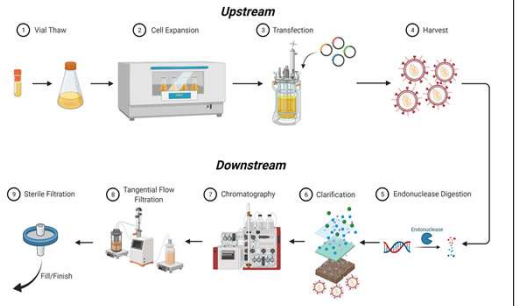
INTRODUCTION

Lentiviral vectors (LV) are a potent tool in the growing field of cell and gene therapy as they enable efficient delivery of genetic material into cells for therapies such as CAR-T and HSC-based gene therapies. With the increasing number of clinical applications and interest in the field, robust, scalable, and cost-effective platforms for GMP manufacturing of high-quality lentiviral vectors are urgently needed.

We describe here a well-established platform process for LV production based on transient transfection of serum-free cells grown in suspension. Cell growth and transfection production parameters were determined using DoE studies to achieve optimal vector yields. The crude LV harvest from upstream operations shows a high infectious titer (> 1E7 TU/ml) and a low particle-to-infectious titer ratio (< 1000 particles/TU). More importantly, the robustness and scalability of upstream process has been demonstrated in different reactor configurations (e.g., Ambr® 250, bench-top and pilot scale bioreactors). The downstream unit operations have been established and optimized for both small and large-scale production requirements. The purified and highly concentrated LV final product shows efficient and potent transduction of T cells and remarkable reduction in the host cell protein (HCP), host cell DNA and plasmid DNA impurity content.

Aiming to provide a robust, scalable and GMP compatible process, this platform also focused on timeline acceleration and seamless transition from PD to GMP manufacturing in the following aspects: ability to produce LVs coding for different genes of interest (GOI) including CAR/TCR constructs, employment of the single-use technologies, employment of non-animal-derived raw materials and alignment of equipment, protocols, and data collection tools between PD labs and GMP manufacturing suites.

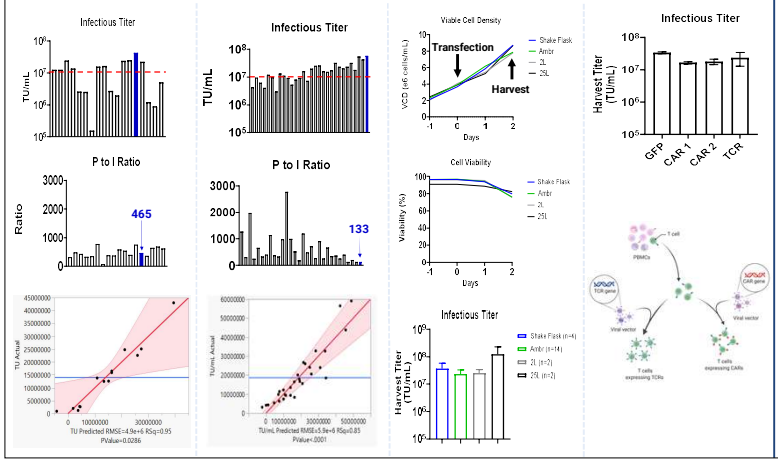
Lentiviral Vector Upstream and Downstream Production Workflow



RESULTS

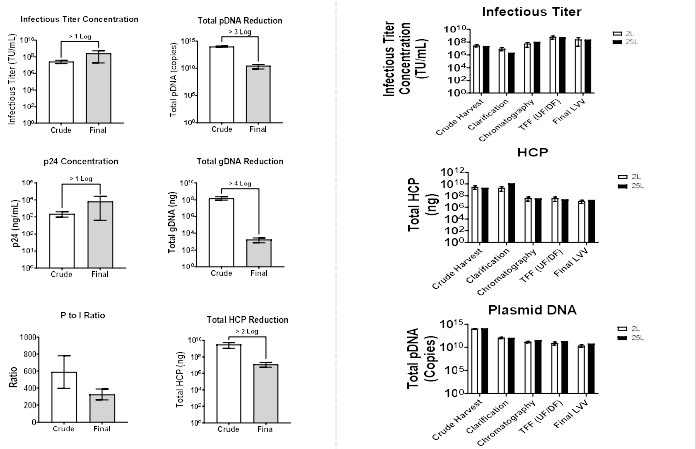
UPSTREAM DEVELOPMENT

DoE 1: Improving Titer | DoE 2: Improving Quality | Good Scalability | Robust Platform Process



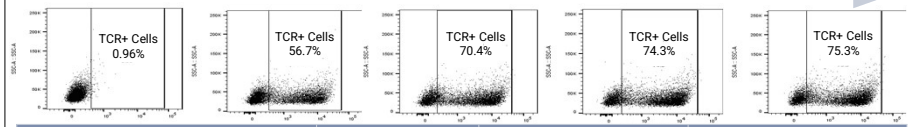
DOWNSTREAM DEVELOPMENT

Improved Quality from Crude Harvest to Final LVV | Robust Scale-up from Lab to Production Scale



POTENCY ASSESSMENT

CONTROL | INCREASING TRANSDUCTION VOLUME



Percent Transduction Volume	Calculated MOI	TCR+ Expression	Vector Copy Number
2.5%	5.9	56.7%	1.59
5.0%	11.8	70.4%	2.58
10.0%	23.7	74.3%	3.18
20.0%	47.4	75.3%	3.85

CONCLUSION

Our data demonstrates the robustness, scalability and GMP compatibility of LV productions using a platform process. The major advantages include:

- High titer, low impurities and high potency in T cells
- Platform process for multiple GOIs to accelerate the development and tech transfer timeline to GMP
- Robust and consistent LVV production for both up- and downstream processes
- Scalable process from shake flask to production reactors with linear scaling downstream applications

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