A Chimeric Approach to Purifying Lentiviral Vectors for CAR-T

[1,3]Adam T.P. McLeod, [1,3]Stephie Leung, [1,3]Joannes P. Yuh, [2,3]Atefeh Ghorbani, [1,3]Rajan Ramanuj, and [2,3]Spencer Hoover

[1]GE Healthcare Cell & Gene Therapy, Toronto, ON, Canada, [2]CCRM, Toronto, ON, Canada, [3]Centre for Advanced Therapeutic Cell Technologies (CATCT), Toronto, ON, Canada

Introduction

Lentiviral vectors (LVV) are the most common delivery method for transducing T cells for CAR-T therapies, and their production and purification are major cost-drivers in manufacturing; an industry "gold standard" of 10-20% recovery results in oversized and expensive production batches. Effective and consistent purification strategies for LVV are a serious challenge due to 1) the labile nature of the virus, 2) physically segregating LVV from the cells from which they bud, 3) removing host cell DNA and protein, and 4) 0.2 µm sterile filtration for 0.08-0.12 µm particles at high concentrations. Although unit operations from mAB and vaccine bioprocessing are readily available, they have yet to be successfully commercialized for LVV (e.g. affinity chromatography) or are detrimental to infectivity (e.g. anion exchange). Thus, a host of wildly divergent, open, and non-scalable schemes are being developed across industry and academia, resulting in poor recoveries, inconsistency of product, and risk of contamination.

Objectives

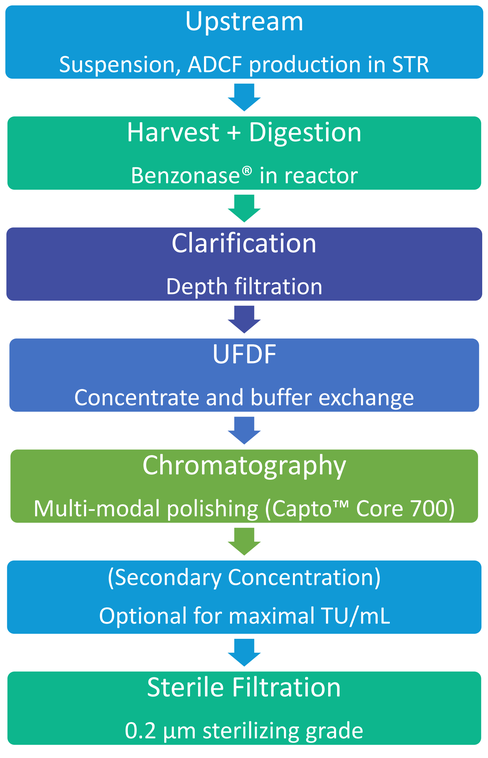
To develop a closed, single-use, ambient temperature, single-day downstream lentiviral vector purification process workflow to:

- Robustly and consistently purify and concentrate LVV to minimums of 1E8 TU/mL and 25% recovery with 2-3 log reduction of host cell protein and DNA
- Replace non-scalable ultracentrifugation with depth filtration
- Replace non-existent affinity chromatography with TFF
- Apply a novel multimodal chromatography for purification
- Develop effective methods for sterile filtration

The combination of single-use consumables, reduced processing time, and increased recovery will greatly reduce COGs for the entire CAR-T manufacturing process and thus increase access to these powerful new treatments.

Methods

LVV Production and Purification Process



Analytics

- Infectious titre (IT) assay (HEK293T adherent cells are infected with LV particles for 48 hr) was used to determine LVV titre by measuring GFP transgene expression using flow cytometry.
- Physical titre (PT) was measured using the HIV-1 p24 ELISA Kit from XpressBio.
- Host cell protein (HCP) was quantified using the HEK 293 HCP ELISA Kit from Cygnus Technologies.
- Host cell DNA (HCD) was quantified using the Quant-iT™ PicoGreen™ dsDNA Assay Kit from Invitrogen™.

Results

Upstream

LVV were produced using a stable inducible LV producer cell line (NRC[1]) in 1-3 L STRs. This presents a closed, scalable, ADCF, suspension-based alternative to adherent HEK293 LVV production requiring serum supplementation.

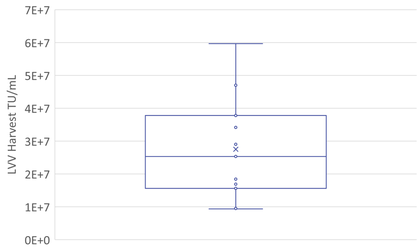


Figure 1: Harvest Concentration of LVV Produced in an STR. Small molecule inducers were added to the bioreactor to produce LVV from an ADCF suspension cell line (n=11).

Clarification

Depth filtration was chosen as a scalable, closed alternative to centrifugation to remove producer cells, debris, and large aggregates. Single-use filters, bags, and pressure sensors with sterile connectors allow for a closed system.

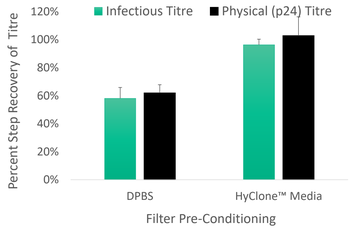


Figure 2: Pre-Conditioning of Filters Effects Performance. Filters pre-treated with HyClone™ culture media showed an ~ 40 % increase in both physical and infectious titre.

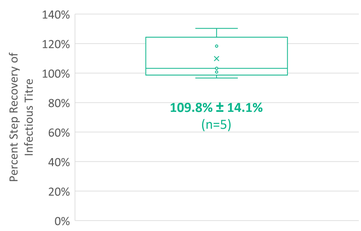


Figure 3: Clarification Recovery. IT recovery in clarification unit op using primary and secondary depth filters in line with a tertiary microfilter.

Ultrafiltration/Diafiltration

In the absence of a commercially available affinity column for LVV (the primary capture step in mAB purification), UF/DF was selected to concentrate and buffer exchange while simultaneously acting as the primary contaminant (host cell protein/DNA) clearance step.

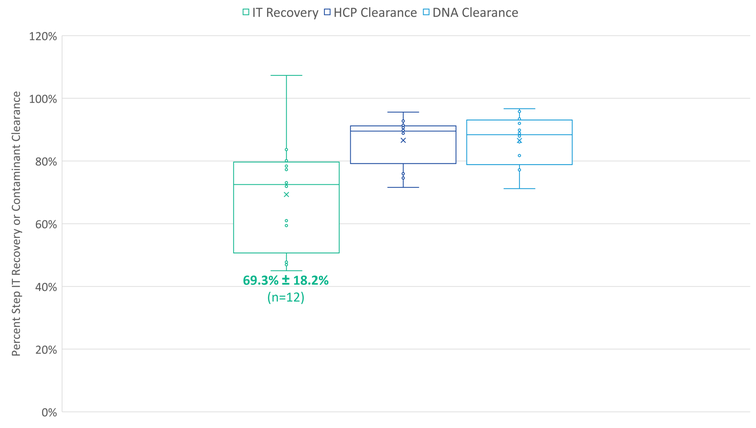


Figure 4: Performance of UFDF Step. An average step IT recovery of 69.3% ± 18.2% was achieved with an average step clearance of 86.6% ± 7.9% of host cell protein and 86.6% ± 8.0% of host cell DNA (n=12).

Chromatography

Anion exchange, a common purification step for mABs and recombinant proteins, led to massive loss of IT and PT and did not result in volume concentration as the high salt elution required immediate dilution to retain LVV infectivity. Instead, a novel multi-modal flow through chromatography resin (Capto™ Core 700) was selected for gentle processing of the LVV as well as a simpler workflow.

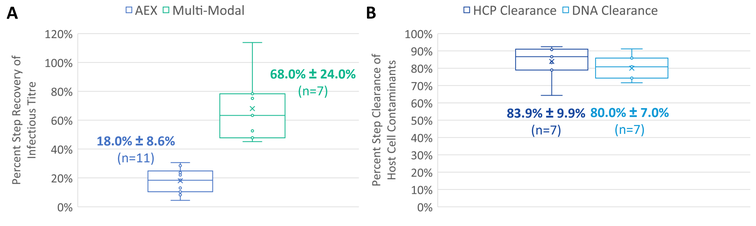


Figure 5. A) Comparison of Step Infectious Titre Recovery From Anion Exchange (left) and Multi-Modal (right) Chromatography. B) Host Cell Contaminants Clearance in Multi-Modal Chromatography. Flow-through chromatography leads to greater IT recovery, perhaps partly due to the gentler conditions and low salt buffer required. This leads to contaminant clearance of ~ 80% for both HCP and HCD, although optimization may lead to better results.

Sterile Filtration

Final sterile filtration presents a huge challenge as LVV (~ 0.08 – 0.12 µm) are very similar in size to the 0.2 µm pores. Filter material, layout (single vs. multiple layer), surface area, flow rate, and pre-conditioning must all be optimized for successful recovery.

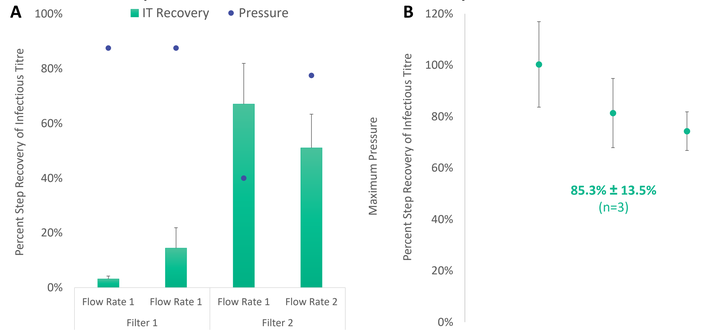


Figure 6: Effects of Filter Type and Flow Rate on IT Recovery and Pressure. A) Filters from two manufacturers were tested with two different flow rates (Filter 1 tested at only one due to very high pressure). B) The filter 2/flow rate 1 condition was carried forward to three full process purifications from 1 L STRs for an average step IT recovery of 85.3% ± 13.5% (n=3).

Recovery vs. Concentration

We have observed that in most unit ops there is a trade-off between total LVV recovery (total TU) and concentration (TU/mL). By modifying, or removing, product recovery flush steps we can drive unit op performance towards either end of the spectrum.

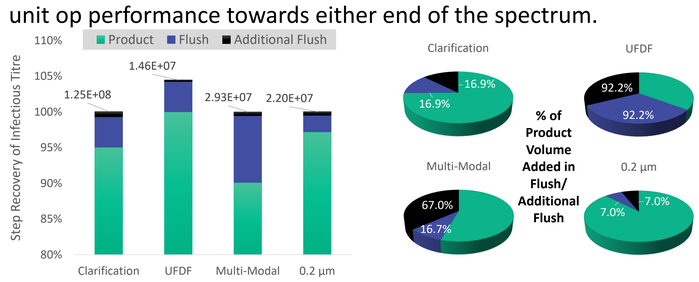


Figure 7: Driving Recovery or Concentration Based on Flush Amount. For each unit op, the percentage of LVV in flush and additional flush is shown, as is the total amount of TU recoverable with additional flush. Pie charts show how much flush volume (as a percentage of product volume) must be added to achieve maximum recovery.

Conclusion

Although much work needs to be done to fully optimize each unit operation, we present here our results to date in designing a fully scalable, single-use consumables, closed, lentiviral vector purification process. By producing LVV in a suspension cell based, ADCF system we allow for full scalability, process closure, and eliminate the regulatory burden of FBS. Depth filtration allows for effective clearance of LVV producer cells as well as debris and aggregates ~ 0.45 µm and larger without any loss of infectious titre. Until an effective commercially available LVV affinity resin is developed, we have shown that UFDF can be used to effectively concentrate LVV and exchange into a formulation buffer. Capto™ Core 700 presents a gentle method to remove ~ 80% of residual host cell contaminants, while greatly increasing IT recovery compared to AEX. Much needs to be done for both UFDF and multi-modal chromatography to fully understand and optimize process parameters to obtain a robust and reproducible result. Finally, we have demonstrated that sterile filtration step IT recovery of > 70% is possible at 1 L scale.

Future Work

Although great success has been achieved in all unit ops in isolation, challenges remain in maintaining recoveries when completing a full downstream purification. Optimization must be completed with both the previous and subsequent unit ops in mind. Once this is completed at 1 L scale, we will attempt to show scalability and robustness with 5 and 25 L productions/purifications.

References

1. Manceur, A. et. al. Scalable Lentiviral Vector Production using Stable HEK293SF Producer Cell Lines. Human Gene Therapy Methods. 2017. 28:6.

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Inquiries to: adam.mcleod@ge.com