Development of a Scalable, Suspension Cell Culture-Based Manufacturing Process for VivoVec, A Lentiviral Vector Platform for *In Vivo* CAR T-Cell Generation

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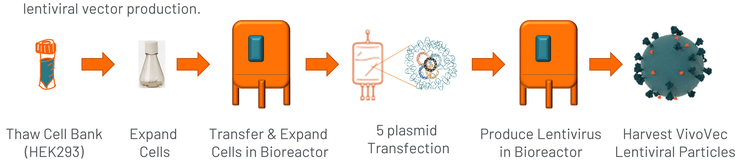
Introduction

Ex vivo CAR T-cell therapies have shown significant clinical success in treating hematological cancers. However, access to these lifesaving therapeutics has been severely limited due to the critical challenges in cost, supply chain, and manufacturing. To address these challenges, Umoja has developed an *in vivo* CAR T-cell platform based on an off-the-shelf, direct injection lentiviral vector platform (VivoVec). The VivoVec manufacturing process platform was developed using a scalable, suspension cell culture-based process. The functions of upstream and downstream process steps in producing and purifying VivoVec lentiviral vector particles for *in vivo* use are described. Data are presented showing consistent cell culture titers for bioreactor scale up from 3 L to 10 L to 40 L. Data are also presented demonstrating downstream purification process removal of HEK293 host cell DNA and protein, which are critical quality attributes that must be effectively controlled to ensure drug product safety. The knowledge gained from process development and scale-up lab studies will be used to enable successful process transfer to clinical and commercial scale manufacturing for supplying VivoVec to patients.

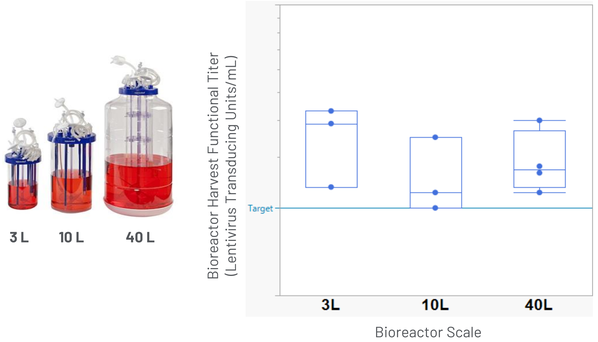
VivoVec Upstream Process Flow

The VivoVec upstream manufacturing process platform uses scalable suspension cell culture to produce engineered lentiviral particles. The platform uses an HEK293 cell line and 5 plasmid transfection to produce particles containing CAR and RACR genes. Development of this platform included selection of the cell line, cell culture growth medium, plasmid transfection ratios, and process parameters for consistent lentiviral vector production.



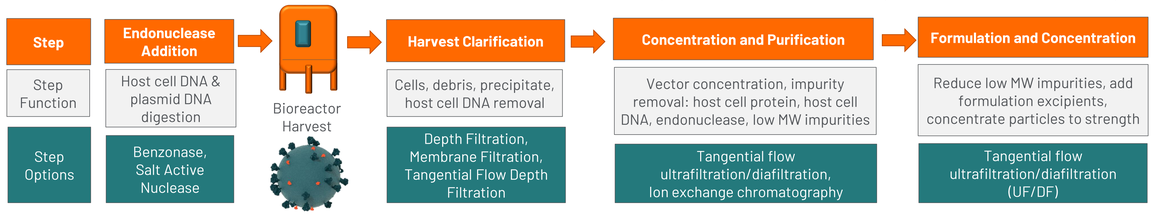
VivoVec Upstream Process Scale Up

The VivoVec suspension cell culture process development runs produced consistent product titers when scaling up the bioreactor from 3 L → 10 L → 40 L.



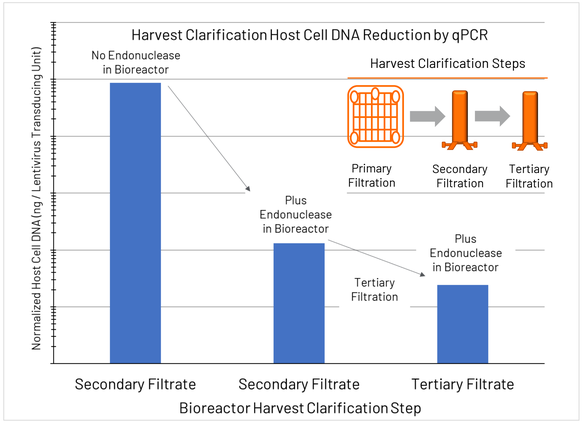
VivoVec Downstream Process Flow

The bioreactor at harvest contains VivoVec particles that must be purified, formulated, and concentrated to the product strength for *in vivo* use. The downstream purification process was developed to remove and/or control process-related impurities and product-related variants. Key functions of the downstream purification process include removal of residual HEK293 host cell DNA , host cell protein, and plasmid DNA, which are critical quality attributes that must be effectively controlled to ensure product safety.



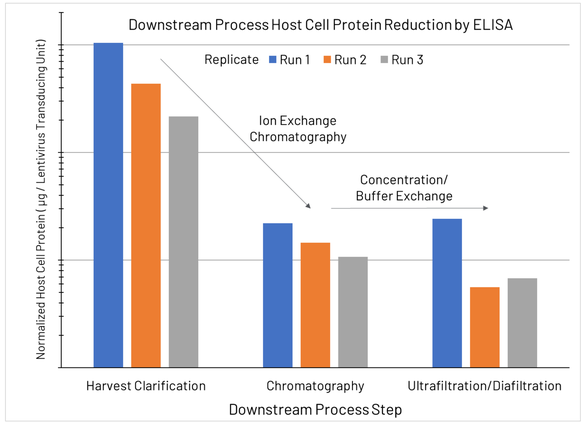
Host Cell DNA Reduction

Endonuclease digestion in the bioreactor reduced host cell DNA impurities measured by qPCR by ~3 log. Harvest clarification tertiary filtration (polishing) further reduced host cell DNA prior to downstream vector purification. DNA electrophoresis data indicated remaining host cell DNA fragment size were less than 200 base pairs, demonstrating effective endonuclease DNA digestion.

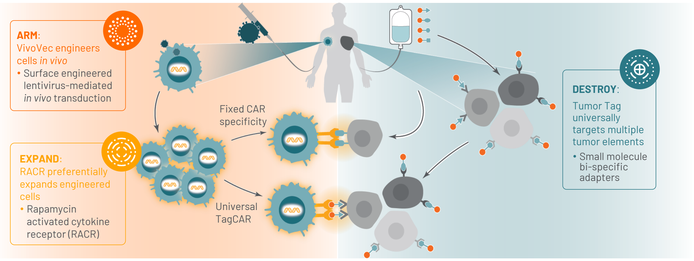


Host Cell Protein Reduction

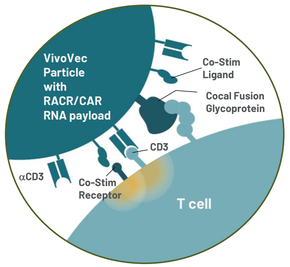
Ion-exchange chromatography reduced host cell protein impurities measured by ELISA in clarified harvest by 1-2 logs. There was less reduction during the subsequent ultrafiltration/ diafiltration step, indicating that some host cell proteins were retained by the ultrafiltration membrane and therefore may be associated with the lentivirus. Mass spectroscopy analysis is on-going to identify remaining host cell protein species.



Umoja's integrated immunotherapy platform provides solutions to the challenges in both blood and solid tumor CAR-T therapies.



VivoVec Lentiviral Vector Delivery Platform: *In Vivo* Gene Delivery



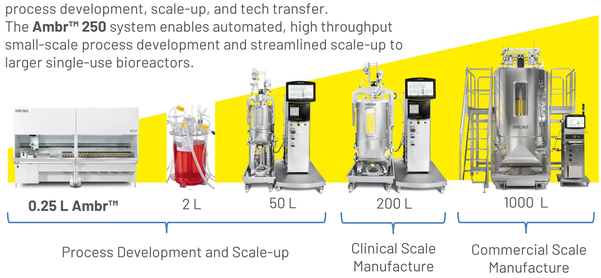
Differentiated RNA/nanoparticle-based delivery vehicle

- Based on 3rd generation lentiviral vector technology
  - Two-decade safety record in clinical gene transfer
- Enzyme-catalyzed membrane fusion for efficient cytoplasmic access
- Capsid encased RNA payload blocks cytoplasmic RNA restriction
- Genomic integration for persistent gene expression
- Established manufacturing processes

Proprietary surface engineering designed to enable *in vivo* transduction

- Resists serum inactivation
- Transduction targets T cells as primary pharmacology
- Maintains low ratio of physical to transducing particles vs. alternative approaches

The VivoVec cell culture process is using the Sartorius suspension bioreactor platform going forward for further process development, scale-up, and tech transfer.
The **Ambr™ 250** system enables automated, high throughput small-scale process development and streamlined scale-up to larger single-use bioreactors.



Conclusions

- To meet anticipated VivoVec particle production requirements for *in vivo* CAR T-cell therapy, Umoja has developed a scalable manufacturing process capable of producing lentiviral vector product with the quality characteristics necessary for patient direct injection.
- VivoVec upstream suspension cell culture process development studies demonstrated consistent lentiviral vector titers when scaling up from a 3 L to 40 L bioreactor
- The VivoVec downstream purification process was developed to remove and/or control process-related impurities, including host cell DNA and host cell protein, and was successfully scaled up from 3 L to 40 L to produce VivoVec product for pre-clinical testing.
- Process knowledge gained from upstream and downstream lab scale process development will be used to further scale-up and transfer the VivoVec process to the Umoja GMP manufacturing facility under construction in Louisville, Colorado to deliver innovative VivoVec pipeline therapeutics to patients.

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