DEBOTTLENECK PROCESS DEVELOPMENT FOR TARGETED PRECLINICAL ENVELOPED VECTOR PRODUCTION

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ABSTRACT

This poster details a strategic approach to optimize the process development of targeted enveloped vectors for preclinical applications. A critical challenge encountered in our development process was the substantial product loss during the sterile filtration step after applying ultrafiltration and diafiltration (UF-DF) to the vector. After meticulous investigation, we ruled out factors such as filter chemistry and operation parameters as the cause of filtration loss. Nanoparticle tracking analysis (NTA) of the samples revealed that aggregation of vector occurred during the diafiltration step, regardless of frozen or fresh material. After identification of the critical step causing the product loss, diafiltration buffer screening experiments were performed to decrease vector aggregation. Three different diafiltration buffer pHs and four excipients were screened. By optimizing the ionic strength of the diafiltration buffer, a substantial reduction in vector aggregation and product loss was achieved. This finding underscores the significance of diafiltration buffer composition in preserving vector integrity and ultimately improving the overall efficiency of targeted enveloped vector production.

INTRODUCTION

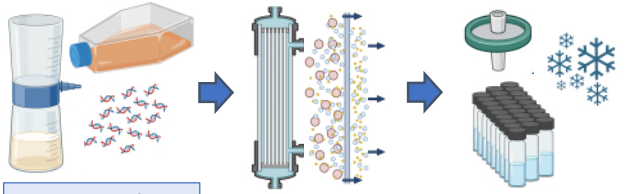
GenVivo has developed a platform to produce enveloped therapeutic gene transfer vectors with high transduction efficiency to treat solid tumors. GEN2 is a product currently in Phase I clinical trials which was generated from this platform (GenVivo IND#29718). Therapeutic genetic payloads in GEN2 consist of a cancer-killing suicide gene Herpes Simplex Virus enhanced Thymidine Kinase (HSV-eTK), and an immunocytokine gene for Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF).

GEN2 can be integrated by cells during cell division, making it particularly efficient for gene transfer in rapidly dividing cancer cells. GenVivo has successfully developed vector systems that target vector delivery to specific tumor cell types after intravenous administration. High specificity targeting of tumor cells can improve the therapeutic effect of the vector while reducing the consequences of potential off-target gene delivery.

While the targeting efficiency of these pseudotyped-envelope vectors is high, modifications of the vector envelope brought challenges for process development. To shorten the product preparation timeline for pre-clinical studies, the process development team developed a general process flow for new vectors generated by the R&D team. The process flow includes clarification through 0.45 µm filters after harvest, host cell DNA digestion by endonuclease, concentration of the vector through ultrafiltration, buffer exchange into formulation buffer via diafiltration, and finally sterilization through 0.22 µm filters before storage at -80°C for animal study use. After tuning the process parameters in individual unit operation steps, this general process flow is quite successful for new vectors. However, significant loss in the final product titer was seen for the targeting vectors during the final sterile filtration step.

To investigate this issue of targeting vector loss during the final filtration step, analytical techniques including nanoparticle tracking analysis (NTA) were applied. Vector aggregation during the diafiltration step was identified as the cause for the product loss during filtration. Several approaches were explored to mitigate aggregation and increase product yield, including pH adjustment, addition of excipients, and increasing the ionic strength of the diafiltration buffer. We found that manipulation of ionic strengths in the final formulation buffer could significantly reduce the level of vector aggregation, and we were able to improve final product yield by ~80%.

METHODOLOGY



Transient Transfection, Clarification, hcDNA Digestion	Ultrafiltration and Diafiltration (UF-DF)	Final Filtration, Sterile Vialing, Freezing
• Targeting vector produced by transient transfection. • Clarify to remove cell debris using 0.45 µm filter. • Digest host cell DNA using endonuclease.	• Ultrafiltration (UF) concentrates physical titer by reducing volume while removing small impurities. • Diafiltration (DF) buffer exchanges product into desired formulation buffer.	• After concentration and diafiltration, product is filtered through 0.22 µm or 0.45 µm filter. • Filtered product is aliquoted into sterile vials and frozen for future use.

Schematic 1. General process flow for the development of targeted retrovector.

Vector generation: Vectors were generated by transient triple transfection of 293T cells with *gagpol*, pseudotyped envelope, and payload plasmid. For the experiments shown here, the vector payload was green fluorescent protein (GFP).

Vector preparation: The vector was harvested, clarified through 0.45 µm filters, and incubated with endonuclease for 75 minutes for digestion of host cell DNA (hcDNA). The vector was concentrated via tangential flow filtration (TFF) using Repligen hollow fiber filters with a molecular weight cut off of 750 kDa. The concentration factor varied from 4X-5X. After the concentration, the vector was exchanged into final formulation buffer with 3 dialfiltration volumes. Finally, the vector went through a 0.22 or 0.45 µm sterile PVDF filter before it was aliquoted into small vials and stored frozen at -80°C.

Titer and Infectivity analysis: Titers were measured by RT-qPCR after isolation of the vector RNA. Human melanoma A375 cells were transduced with vector, and payload GFP protein expression was examined by FACS.

RESULTS & DISCUSSION

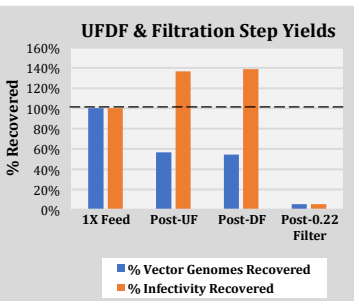
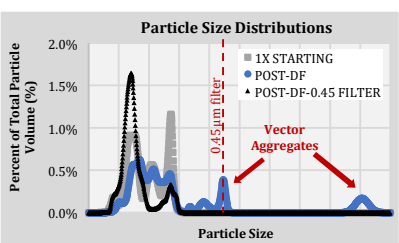


Figure 1: Vector recovery during ultrafiltration (UF), diafiltration (DF), and sterile filtration (0.22 µm pore size) process steps. Recovery of both physical vector (quantified in vector genomes by RT-qPCR) and vector infectivity (quantified by FACS for GFP expression) are shown, using 1X Feed as the 100% reference. Roughly half of the total vector genomes are recovered from the UF-DF process, while infectivity is improved during UF-DF. During the final sterile filtration step using 0.22 µm PVDF membrane, 90% of the remaining physical vector is depleted and infectivity is decreased by 96%.

Figure 2: Nanoparticle tracking analysis (NTA) confirms vector aggregation after diafiltration. Particle size distribution shows larger particle populations post-DF that would be lost through a 0.45 µm filter. After filtration, the particle size distribution shifts left to favor smaller particles, indicating that the larger vector aggregates were caught in the membrane.



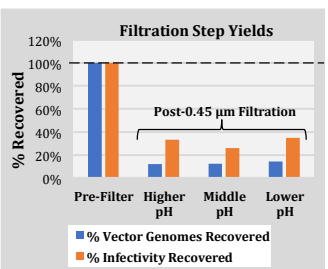


Figure 3: Vector recovery during final filtration step after vector underwent UF-DF process with diafiltration buffers at three different pHs (range pH 6.0 to 7.5). Vector was filtered through a 0.45 µm PVDF membrane after UF-DF process. Physical vector genome recovery pre- vs. post-filtration is shown, along with the change in vector infectivity (as measured by FACS for GFP expression) pre- vs. post-filtration. Both physical vector and infectivity recovery are similarly low regardless of DF buffer pH, indicating that the targeting vector aggregation is not pH-dependent.

RESULTS & DISCUSSION (cont.)

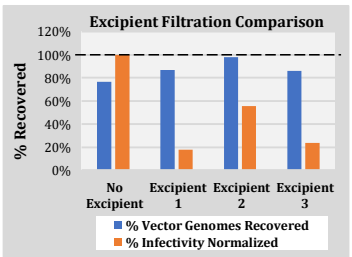


Figure 4: Screening comparison of 3 different excipients on filtration yields to determine if vector aggregation could be reduced by the addition of excipient. 3 different excipients were included in the buffer compositions, mixed 1:1 with 4X UF-concentrated vector product, then filtered through 0.45 µm PVDF membrane. Diafiltration using hollow fiber filter was not performed, as this was a screening study. Recovery of physical vector pre- vs. post-filter was similar for all excipient conditions. Infectivity (as measured by FACS for GFP expression) is normalized to the control "No Excipient" condition to compare the excipients to the control formulation. Compared to the same buffer with no added excipients, the addition of excipients harmed the targeting vector's infectivity.

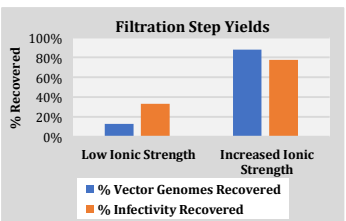


Figure 5: Vector recovery during final filtration step after vector underwent UF-DF process with diafiltration buffers with two different ionic strengths. Both physical vector (quantified in vector genomes by RT-qPCR) and vector infectivity (quantified by FACS for GFP expression) pre- vs. post-0.45 µm PVDF filtration are shown. Increasing the ionic strength of the diafiltration buffer improved filtration recovery of both physical vector and vector infectivity.

CONCLUSIONS

Composition of final formulation buffer can impact vector integrity. The diafiltration process caused the pseudotyped envelope vector to aggregate in a low-salt buffer. Increasing the ionic strength of the formulation buffer reduced vector aggregation, thus allowing the vector product to pass through a 0.45 µm membrane and improving product recovery.

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4. Schematic 1 made using Biorender.com

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GenVivo Inc. is a clinical-stage gene therapy company headquartered in San Marino, CA, currently advancing a cancer immunotherapy enveloped vector product candidate.