825. LVV Production At-Scale: From Cell Factory™ Systems to iCELLis® 500 Bioreactor

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Evolving from the well-established GMP 48L Nunc™ Cell Factory™ (CF) process, AGC Biologics has developed a robust, scalable, high quality and quantity process for the industrial-scale production of lentiviral (LV) vectors for use in gene-modified cell therapy or *in vivo* gene therapy. This platform includes vector production in the iCELLis® bioreactor, downstream purification and concentration by chromatography and tangential flow filtration (TFF) steps and sterile filtration and filling in different formats. Upstream process was developed leveraging the scale down iCELLis® Nano system where key process parameters were screened in order to increase productivity, producing a bulk vector with consistently low contaminant profile. Data obtained on full-scale iCELLis® 500 system confirmed full scalability and equivalent performance of the process.

	Infectious viral titer (TU/mL)	Physical viral titer (ng p24/mL)	HCPs (ng/mL)	DNA (µg/mL)	
Bulk vector iCELLis® Nano	Average ± St. dev.	1.7E+07 ± 0.7E+07	330 ± 84	1073 ± 380	Negative
Bulk vector iCELLis® 500	Average ± St. dev.	2.5E+07 ± 0.8E+07	428 ± 221	1173 ±734	Negative

The downstream process was designed to remove main process related contaminants (Host Cell Proteins, Host Cell DNA, plasmid DNA and BSA), thus maintaining vector infectivity. The process consists of an anion exchange chromatography step that captures and concentrates the vector, enabling 80% recovery while removing HCPs, DNA and BSA. Eluted vector is then concentrated and diafiltered with TFF using hollow fibers. With this step, the vector is further concentrated with 80% recovery, and additional contaminant removal is achieved. The vector is then 0.2µ sterile filtrated and filled in vials. Total process recovery is approximately 30%.

	Infectious viral titer (TU/mL)	Physical viral titer (ng p24/mL)	HCPs (ng/mL)	DNA (µg/mL)	
Purified vector iCELLis® Nano	Average ± St. dev	3.3E+08 ± 1.4E+08	4820 ± 1274	negative	1.7 ± 0.8
Purified vector iCELLis® 500	Average ± St. dev	4.9E+08 ± 1.7E+08	9951 ± 3238	negative	1.5 ± 0.8

Final purified vector data shows a similar impurity profile as the established 48L CF's process. The system has already been successfully implemented for the manufacture for different vectors with different Gene of Interest (GoI). With the ability to produce a volume of 4x without a loss in quality, the iCELLis® 500 platform offers a scalable and cost-effective solution with regards to the number of patients treated versus the cost of production and quality control. Moreover, the possibility to leverage the analytical platform already in use for the GMP 48L CF's process accelerates transition from development to GMP clinical and commercial production with reduced comparability exercises.

826. Intensified Production of Vaccinia-Based Oncolytics in the High Density Cell Respirator (HDCR) Bioreactor Improves Vaccine Logistics and Economics

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Intensification of cell-based production processes is key to improving oncolytic vaccine logistics and economics by saving on GMP space, time, labor, and feedstock. Many promising vectors, including those based on vaccinia virus (e.g. CF33, JX-594/Pexa-Vec) are still produced using flask-based culture due to the cost, effort, and uncertainty involved in adapting to stirred-tank or perfusion processes. Based on prior success culturing adherent cells (e.g. HEK293, A549, CV-1) to high densities ($10^{7\text{-}8}$ cells/mL) using the scalable high-density cell respirator (HDCR) bioreactor, we hypothesized that the platform could support orders-of-magnitude-intensified production of replication competent viruses. Here we report on CF33 virus production as a proof-of-concept for oncolytic virotherapy. Bioreactor cartridges were produced based on a 4-stack of 600 cm^2 HDCR membranes, featuring a proprietary gas perfusable and permeable microarchitecture that optimally oxygenates cells even at high densities. Media perfusion was feedback-controlled based on glucose measurements in the waste stream. A549 cells were seeded into the bioreactor on microcarriers, expanded 10-fold to mid-10^7 cells/mL densities, infected with CF33 virus (MOI of 0.1), and harvested 48 hours post-infection. CF33 virus was gradient purified and titered for functional virus by plaque forming assay. Multiple production runs using different strains of CF33 virus validated the reproducibility of the process, as summarized in Table 1. Importantly, cell specific titers (PFU/cell) remained comparable to conventional flask-based production, leading to significant intensification due to the higher cell densities supported in the HDCR bioreactor. Volumetric productivity is on the order of 100× that of cell factories. The efficient usage of media due to the gas-media decoupled operation of the HDCR bioreactor enables gradient-purified virus costs of around $\$500/10^{10}$ PFU. This is significantly below the $\$10{,}000/10^{10}$ PFU charged by CMOs and supports our mission of democratizing access to life saving medicines. The straightforward adaptation of CF33 virus production from a flask-based to a high yield, intensified process highlights the logistical and economical advantage of the HDCR platform for oncolytics.

Table 1: Production metrics for CF33 virus produced in HDCR bioreactor.				
Virus Strain	**Crude Yield (PFU/Run)**	**Specific Yield (PFU/Cell)**	**Media Usage (Liters)**	**Cost Per 10^{10} Purified PFU**
CF33-hNIS-αPDL1	6E10	28	10.5	402
CF33-mCD19t	2.6E11	130	10.9	275
CF33-tk	2.8E10	18	10.6	540

827. Comparative Analysis of Endotoxin Removal Protocols for AAV Purification

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Endotoxin, otherwise known as lipopolysaccharide (LPS), is the major component of the outer membrane of Gram-negative bacteria. Endotoxin can be released by bacteria and co-purify with AAV therapeutics. *In vivo*, endotoxin can cause sepsis that leads to excessive inflammation, leading to multiple organ failure and potentially death. As such, endotoxin must be monitored as an adventitious agent that can adversely affect the safety and efficacy of AAV drug products. Preclinical research animal model endotoxin limits based on the threshold of 5 EU/kg for human doses are calculated to be approximately 1.5 EU/mL. We have investigated three endotoxin removal strategies to clear AAV vectors of endotoxin for potential use in mouse and primate studies. AAV viral preps with titers of 1 e13 GC/mL and 6 e12 GC/mL were spiked up to 980 EU/mL with LPS. A commercial endotoxin removal kit was compared to published endotoxin removal protocols based on detergents Triton X-100 and Triton X-114. The commercial product reduced endotoxin content by 30-fold but sacrificed 30 % of the titer. Triton X-100 reduced endotoxin by 3,500-fold but sacrificed 60% of the titer. Triton X-114 was the most efficient at removing LPS, up to 60,000-fold for final levels less than 0.100 EU/mL, while maintaining the highest titer recovery of 75%. Endotoxin removal using Triton X-114 is recommended for quick processing of LPS-contaminated AAV therapeutics.

828. Development and Optimization of Transient Transfection Process for Adeno-Associated Viral Vector Production

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Transient transfection is presently the primary approach widely used for recombinant adeno-associated virus (rAAV) production. In this study, we have explored the critical process parameters for rAAV production. We have evaluated multiple polyethylenimine (PEI)-based transfection reagents and identified PEIpro® as the lead candidate. We have also compared various cell culture media formulations and observed high compatibility of PEIpro® with different media types. Key transfection parameters, including transfection cell densities, plasmid DNA amounts, transfection complex volume, transfection/ harvest timings as well as bioreactor settings were also screened through DoE studies to optimize the manufacturing process. The optimized production process shows high robustness and improved batch-to-batch consistency and reproducibility.

829. Stealthed, Retargeted HIV-1 Vectors Incorporating Darpin-Displaying Canine Distemper Virus Envelope Glycoproteins without Cytoplasmic Tail Truncations

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Receptor-targeted lentivirus vectors (LV) are emerging as a key technology for selective gene delivery to key target cell populations both during *ex vivo* and *in vivo* transduction protocols. Currently, the best developed approach is to pseudotype the LV with paramyxovirus Fusion (F) and Hemagglutinin (H) glycoproteins whose natural tropisms are ablated and whose attachment specificity is redirected via cell specific polypeptide binding domains (eg. scFv) displayed on the C-terminus of the H protein. LV pseudotyped with engineered measles F and H glycoproteins can be efficiently retargeted but pre-existing anti-measles antibodies render them ineffective for *in vivo* gene delivery. An addittional limitation of current pseudotying strategies with receptor-targeted *paramyxovirus* glycoproteins is the requirement for extensive engineering of their cytoplasmic tail regions to allow for incorporation into lentiviral particles. Here we show that fusion-competent, full-length canine distemper virus (CDV) glycoproteins (ie without engineered cytoplasmic tails) can be efficiently pseudotyped onto LVs. Cytoplasmic-tail truncations of the CDV envelope glycoproteins did not improve efficiency of pseudotyping, while swapping the cytoplasmic tail of CDV-H with the heterologous tail from the measles virus H protein completely abolished it. To generate an EpCAM-targeted lentiviral vector, we engineered CDV glycoproteins to ablate their tropisms for cognate receptors, canine SLAMF1 and human/canine nectin4, and fused a designed ankyring repeat proteins (DARPin) specific for EpCAM to the C-terminus of the CDV-H protein. The receptor-targeted CDV-LVs showed improved EpCAM-specific transduction efficiency when compared with their MeV-based counterparts. Our current effort focus on the generalizability to other receptors of choice. Lentiviral vectors pseudotyped with full-length retargeted CDV glycoproteins might provide a valuable addition to the family of targeted LVs for gene therapy purposes.

830. Comparative Analysis of Anion Exchange Chromatography for the Enrichment of Adeno-Associated Virus Serotype 9 Full Particles Using a Conductivity Step Gradient

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Adeno-Associated Viruses (AAVs) are a well-established vector for gene therapy applications due to their ease of use and low pathogenicity. However, their production yields empty particles, that lack the genetic material required for the therapy, at ratios up