CASE STUDY: SINGLE-USE PLATFORM FOR COMPLETE PROCESS DEVELOPMENT AND SCALE-UP OF AN ADENOVIRUS

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Virus production for vaccines and gene therapy relies heavily on adherent cell culture based processes – typically trays, roller bottles or microcarriers. The introduction of the iCELLis® fixed-bed bioreactor and single-use purification operational units provides a full solution for rapid process development at small-scale in the iCELLis Nano bioreactor with direct, predictable process transfer to the large-scale iCELLis 500 bioreactors. The iCELLis 500 bioreactor provides a fixed-bed with 66 - 500 m² of surface area for cell growth. A 500 m² fixed-bed is equivalent to 5,882 roller bottles of 850 cm² or 278 HYPERStack 36. Coupled with other single-use upstream and downstream technologies, the iCELLis bioreactor and purification membranes provide a complete single-use platform for virus production. We have performed the complete process development and scale up of an adenovirus (Ad5) process using HEK-293 cells. The reference process using 2D flatware was transferred, optimized and scaled-up into the iCELLis Nano bioreactor (surface areas of 0.53 - 4.0 m²). Based on results in the iCELLis Nano bioreactor and virus requirements, the 66 m² large-scale iCELLis 500 fixed-bed bioreactor was selected. In order to simplify the seed train, the fully-closed, single-use Xpansion® multiplate bioreactor was used. The upstream yield from the iCELLis 500 66 m^2 bioreactor was 1.04 x 10^{16} of Infectious Units (IFU). In this study we optimized and developed adenovirus purification manufacture processes using Mustang® Q with bind/elute strategy managed to reduce significantly the impurities such as HCP and HC-residual DNA and enriching full vs. empty capsid (>90%). The eluted Ad5 from Mustang Q membrane is immediately processed through the UFDF for further concentration and buffer exchange to final virus formulation buffer. Final purified product was then sterile filtered and vialed for potency studies. Downstream processing utilized single-use systems with 62% recovery for a final purified yield of 6.42 x 10^{15} IFU. Analytical characterization of the virus met specifications and *in vivo* GLP toxicology testing results were comparable to material produced using the reference process. Scalable upstream and downstream strategies for production and purification of virus based product as such described here offers a fast-to-market, more cost effective alternative to traditional processes. We will review the iCELLis bioreactor platform and downstream purification platform and present as a case study the process development and scale up of the complete adenovirus (Ad5) process.

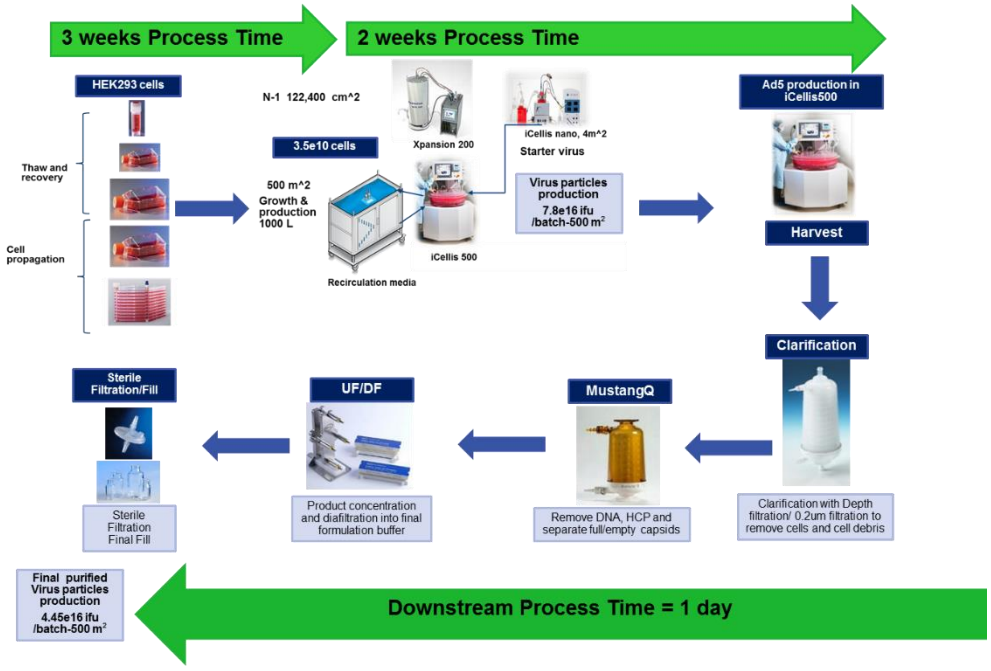

Figure 1: Virus-based product production and purification workflow process