20 YEARS OF EXCELLENCE IN INTERNATIONAL MEETINGS
alliedacademies.com
Joint Event on

2nd International Conference on
CELL AND GENE THERAPY
&
2nd World Congress on
PUBLIC HEALTH, EPIDEMIOLOGY AND NUTRITION

April 15-16, 2019 | Milan, Italy

Arch Gen Intern Med 2019, Volume 3 | DOI: 10.4066/2591-7951-C2-027

TOWARDS A 3rd GENERATION AAV MANUFACTURING PLATFORM AND IN-PROCESS CONTROLS

Ales Strancar
BIA Separations, Slovenia

Adeno associated virus (AAV) is the leading vector in the field of gene therapy because of its low toxicity, good overall safety profile and ability to maintain stable expression for long periods of time. It is therefore crucial to develop a robust and high efficiency platform for its manufacturing. One of the key challenges in manufacturing viral vectors is managing the interface between upstream and downstream processing. Experimental observations indicate that, in addition to losses due to shear, processing methods such as precipitation, freeze/thaw and Tangential Flow Filtration (TFF) promote formation of stable associations between virus, DNA and proteins. These complexes lower virus recovery and process capacity, depress robustness, and inflate contamination at each processing step. They accordingly have strong potential to influence long term clinical safety, especially with respect to residual DNA. This paper will present a 2nd generation AAV manufacturing platform engineered specifically to address these issues. After removal of cell debris by filtration, AAV is captured and fractionated by hydrophobic interaction chromatography (HIC). The AAV fraction from HIC is diluted and loaded onto a cation exchanger under conditions to dissociate AAV from virus-protein-DNA complexes and strongly bind remaining protein-DNA complexes. The cation exchange fraction is then applied to an anion exchanger as a final DNA dissociation-polishing step and to separate empty and full capsids. This orthogonal process achieves very high recoveries (>70%) with AAVs that are secreted from the host cells. It is fully scalable, very robust, and has proven effective for all AAV serotypes evaluated to date. In addition, the 3-step chromatographic process without preliminary TFF is very efficient and improves overall reduction of all contaminating viruses. A 3rd generation process is under development to fully extend these benefits to AAVs processed by cell lysis and to reach extra low DNA impurity profile. Rapid sensitive analytics using multiple in-line HPLC detectors to provide insightful process development and manufacturing documentation will also be presented.

Note: