LEVERAGING VECTORED VACCINE CANDIDATES MANUFACTURING TO GMP COMPATIBLE BIOPROCESSES

Peixoto C, IBET, Instituto de Biologia Experimental e Tecnológica, Portugal
peixoto@ibet.pt
Tomás HA, IBET, Instituto de Biologia Experimental e Tecnológica; Universidade Nova de Lisboa, Portugal
Faria T, IBET, Instituto de Biologia Experimental e Tecnológica, Portugal
Rodrigues AF, IBET, Instituto de Biologia Experimental e Tecnológica; Universidade Nova de Lisboa, Portugal
Moleirinho M, IBET, Instituto de Biologia Experimental e Tecnológica; Universidade Nova de Lisboa, Portugal
Coroadinha AS, IBET, Instituto de Biologia Experimental e Tecnológica; Universidade Nova de Lisboa, Portugal
Carrondo MJT, IBET, Instituto de Biologia Experimental e Tecnológica, Portugal
Alves PM, IBET, Instituto de Biologia Experimental e Tecnológica; Universidade Nova de Lisboa, Portugal

Background

Vectored vaccines are very efficient in the *in vivo* delivery of antigens either in the form of antigen protein and peptides or genetic material. The bioprocess of vectored vaccines poses however several challenge since the viral particles to be effective must maintain their infectivity. Lentiviral and adenoviral vectors are among the particles more used in the treatment of cancer diseases modulating the immune system. Both viral vectors are currently produced in transient upstream process. While the adenoviral vectors are produced at high titers the lentiviral vector upstream process still requires further improvement. The non-lytic nature of lentivirus enables the design of stable cell lines which may improve its yields through perfusion and longer term productions, reducing costs. The application of novel methods for the downstream processing such as continuous purification will contribute to increase the yield and lower the overall cost of the manufacturing processes.

Experimental approach

At the upstream process, many of the challenges lentiviral bioproducts present in its manufacturing are related to the apoptosis-leading cytotoxicity of some of the vector components. Supported on our long track experience and enabling tools developed for gammaretrovirus manufacturing, we undertook the challenge of establishing a constitutive stable lentiviral producer cell line. To address this challenge we proposed to eliminate or reduce the cytotoxicity of the lentiviral vector expression components. At the downstream process lentiviral vectors face the challenges common to retroviridae family of vectors namely short half-lives at room temperature, sensitivity to pH variations and salt concentrations, and shear stress. The purification strategy developed was designed to be based on disposable and easily scalable technologies. A final concentration achieving 10^8 TU mL^{-1} was targeted since the concentration step itself allows to reduce the burden on process and improve the transduction efficiency.

To address the high doses requirements we will report an improved oncolytic adenovirus purification process for phase I and II clinical trials and present a case on the use of Polysorb 20 as a replacement for Triton X-100 during cell lysis. Product recovery, potency, purity and the effect of manufacturing holding points will be discussed.

Results and discussion

A lentiviral producer cell line constitutively producing titers above 10^6 TU.mL^{-1}.day^{-1} was established. The cell line showed to be stable, consistently maintaining vector productivity over one month in the absence of antibiotics. At the bioreaction process it was possible to maintain the cells continuously producing over 10 days. At downstream we implemented scalable protocols for lentiviral and adenoviral vectors that is easy to transfer to GMP environment, combining microfiltration, anion-exchange, and ultrafiltration membranes technologies toward maximization of infectious virus recovery, allowing generation of clinical-grade viral vectors without the need for cleaning validation in a cost-effective manner.

Herein we will present and discuss the challenges on the biomanufacturing of lentiviral as well as adenoviral virus, the strategies and novel technologies to be adopted in order to enable a faster development of novel vectored vaccine candidates focusing on several case studies, supported by process technology innovation.