LENTIPRO STABLE PRODUCER CELLS: DELIVERING SCALABLE AND RELIABLE LENTIVIRAL VECTOR MANUFACTURING

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Lentiviral vectors are one of the most currently used viral vectors for gene and cell therapies. Their use in clinical protocols has significantly increased in the past 5 years with the approval of several gene therapeutic products relying on lentiviral vector gene delivery. Capable of transducing non-dividing cells and presenting safer integration profiles as self-inactivating vectors, lentiviral vectors have progressively undertaken gammaretroviral vector use in gene therapies. However the knowledge on lentiviral vector manufacture is far more immature than that of gammaretroviral vectors. While the production of gammaretrovirus rely on stable producer cell lines and perfusion systems, enabling high cell density and longer term productions, most of the bioprocesses for lentiviral bioproducts rely on transient transfections and short term batch productions.

At the upstream process, many of the challenges lentiviral bioproducts present in their 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 carried out the challenge of establishing a constitutive stable lentiviral producer cell line. To surpass the challenges we proposed to eliminate or reduce the cytotoxicity of the lentiviral vector expression components[1]. Several strategic novelties were introduced in the development of the cell line namely: (i) the use of a modified gag-pro-pol, (ii) introduction of all the third generation lentiviral expression cassettes by chemical transfection instead of viral transduction and (iii) performing only one clone screening step (enabling the use on the 'Single step cloning screening' protocol developed by our group[2]). After establishing a stable producer cell line the culture conditions were developed with the main aim of extending bioreaction culture time and viral vector total yields.

A lentiviral producer cell line constitutively producing infective titers above 10^6 $TU.mL^{-1}.day^{-1}$ was established. Moreover the new protocol to generate the cell line enabled its development in less than six months. 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[1]. These results validate the transition to continuous or perfusion large-scale production systems qualifying the strengths and advantages of the strategies followed.

This work to be presented will discuss the challenges on the manufacture and scale-up of lentiviral vectors as well the strategies and novel technologies to be adopted to enable effective upstream processes.

[1]Tomás et al. (2018) 'LentiPro26: novel stable cell lines for constitutive lentiviral vector production' Sci Rep. 8(1):5271

[2] Rodrigues et al. (2015) 'Single step cloning-screening method: a new tool for developing and studying high-titer viral vector producer cells' Gene Ther. 22(9):68