# PRODUCTION OF A FUSOGENIC ONCOLYTIC RVSV-NDV VIRUS: CELL-LINE SCREENING AND PROCESS DEVELOPMENT IN SMALL-SCALE SUSPENSION CULTURES

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Fusogenic oncolytic viruses represent a novel class of immunotherapeutics, which offer hope for the treatment of otherwise incurable cancers. Their enhanced intratumoral spread through syncytia formation allows for a potent mechanism of tumor cell death and induction of antitumor immune responses [1]. While the ability of these viruses to induce cell-cell fusion reactions offers numerous beneficial properties, it also presents unique challenges for large-scale clinical-grade manufacturing. Infected cells rapidly fuse with surrounding cells, resulting in large multinucleated syncytia, which quickly die before high titers of the virus can be produced or released [2]. Here, we evaluated the production of a novel hyper-fusogenic hybrid of vesicular stomatitis virus and Newcastle disease virus (rVSV-NDV) in four different suspension cell lines. Cell growth, metabolism, and virus productivity were characterized for each candidate respectively. Permissiveness was evaluated based on extracellular infectious virus titer and cell-specific virus yields (CSVY). For the purpose of process intensification, virus adaptation, and multiplicity of infection (MOI) screenings were conducted in small-scale and confirmed in a 1 L bioreactor. BHK-21 and HEK293SF were identified as promising candidates for rVSV-NDV production, yielding infectious titers at infection cell concentrations of 2.0 E06 cells/mL of up to 3.0 E08 $TCID_{50}$/mL and 7.5 E07 $TCID_{50}$/mL, and CSVYs of 153 and 9, respectively. Oncolytic potency was not affected by production in suspension cultures compared to the reference stock produced in adherent AGE1.CR.pIX cultures. Overall, promising suspension cell substrates were identified for a highly efficient and scalable production process of this fusogenic rVSV-NDV. This paves the way for an efficient large-scale manufacturing process, which can be further intensified towards high cell density production in order to provide sufficient virus material for conducting a phase I clinical trial of oncolytic VSV-NDV in cancer patients.

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