DEVELOPMENT OF AN ANALYTICAL PLATFORM FOR DELIVERY OF RECOMBINANT ONCOLYTIC VIRUSES

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Over the past two decades, a number of modified DNA and RNA viruses have shown great promise in cancer therapy. Their tumor specific cytotoxic activity involves several mechanisms. These include preferential uptake and replication of the virus in cancer cells, followed by infection and lysis of these cells (oncolysis). In addition, oncolytic viruses (OV), especially those engineered with appropriate transgenes, have been shown to induce tumor specific immune responses, thereby opening up an exciting area of cancer immunotherapy.

One OV, containing a recombinant Herpes Simplex Virus (HSV) with a Granulocyte Macrophage Colony Stimulating Factor (GMCSF) transgene insert, was approved in 2015 for treatment of refractory melanoma. While this drug, known as T-Vec or Imlygic, is administered intratumorally, several other OVs are in pre-clinical and clinical development with intravenous (IV) administration as an objective. The IV route potentially offers the advantages of accessing metastatic sites and eliciting systemic immunotherapeutic response. A recombinant form of the Newcastle Disease Virus (rNDV) has been recently developed that also incorporates GMCSF as a transgene. NDV is a single stranded, negative sense, enveloped RNA virus and, in native form, is not a human but an avian pathogen. Genetic engineering was performed to reduce avian pathogenicity and increase immunotherapeutic potential against cancer [1]. In addition to lysis of cancer cells produced by viral infection, rNDV has demonstrated induction of cytokine and chemokine responses, cell infiltration and long - term suppression of tumor growth in animal models.

Production of recombinant viruses requires selection of appropriate host cells, based on considerations of yield and quality of the product. In recent years, regulatory agencies have been open to use of human cell lines as host, including some tumor derived cells, such as HeLa. An advantage offered by HeLa cells is potential incorporation into the virus of host proteins known as regulators of complement activation (RCA). This allows a longer half-life of the virus in circulation. However, impurities derived from host cells as well as process conditions must be kept to the lowest levels possible. A thorough analytical and biophysical evaluation of the purified virus particles includes measurements of (a) infectivity, (b) morphology, (c) size distribution, (c) genetic integrity, and (d) major viral proteins. In addition, levels of contaminants such as residual host cell proteins and DNA must be evaluated, including sizes of residual DNA fragments. Robust and accurate measurement of infectious titer is especially important as this determines doses of an OV to be delivered to patients. It also provides a measure of virus stability and is critical to supporting process development. We have optimized a Fluorescent Focus Assay (FFA) that produces concordant results with Plaque Assay (PA) over several orders of magnitude of infectious titers [2]. FFA provides a faster turn-around in a higher throughput format compared to PA. These and additional structure-function assays were developed to support process development and deliver a well-characterized OV for cancer immunotherapy.

Acknowledgment: Contributions of Benjamin S Rush, Melissa L Coughlin and Jenny Sun of MedImmune to analytical development of rNDV are gratefully acknowledged.

References:

[1]. Cheng, X., Wang, W., Xu, Q., Harper, J., Carroll, D., Galinski, M.S., Jin, H*. 2016. Generation of a potent nonvirulent Newcastle disease virus for cancer therapy. J. Virol. 90, 5343–5352.

[2]. Rush, B.S., Coughlin, M.L., Sanyal, G*. 2018. *In vitro* infectivity of oncolytic Newcastle Disease Virus: Correlation between plaque and fluorescent focus assays. J. Virol. Methods 251, 69–74.

[1]Includes work performed at Biopharmaceutical Development, MedImmune LLC, Gaithersburg, MD, USA