Title: Rapid Determination Of rSIV.F/HN Lentiviral Vector Titre

Authors: **Catriona C. Conway**; Toby Gamlen.; Mariana F. A. Viegas Rebecca J. Dean; Kamran M. Miah; Stephen C. Hyde; Deborah R. Gill

Address: Radcliffe Department of Medicine, University of Oxford, Oxford, United Kingdom

Our laboratory is developing non-viral and viral vectors for the treatment of a range of airway disorders. We have previously demonstrated that a recombinant simian immunodeficiency virus (rSIV) vector, pseudotyped with the fusion (F) and hemagglutinin-neuraminidase (HN) envelope proteins from Sendai virus (together termed rSIV.F/HN), has high tropism for multiple cell types found within both the conducting airway epithelium and the gas exchanging alveolar regions of the mouse, sheep and human lung. Historically, we have routinely used two titre assays to assess vector preparations and to normalise vector dose between studies: (i) a viral particle assay (reported as VP/mL) is derived from the concentration of viral RNA and is evaluated using qRT-PCR, (ii) a transducing assay (reported as TU/mL) is derived from the concentration of vector-derived DNA within cells transduced with a viral preparation and is evaluated using qPCR. Both approaches are widely used within the field, and accepted by multiple competent authorities. However, both approaches are relatively slow, taking serval hours (VP/mL) or several days (TU/mL) to report. We are routinely producing a large number (multiple lots per week; target >1e10 TU) of highly purified (anion-exchange/TFF), high-concentration (target >1e9 TU/mL) preparations of rSIV.F/HN. To refine this process and to produce vector lots with more consistent vector concentration, we are seeking a very rapid titre-dependent assay that could be incorporated into the downstream purification workflow that would allow an assessment of vector yield during the final stages of vector formulation. To this end, we have evaluated the utility of a simple hemagglutination assay in which sialic acid receptors on the surface of red blood cells (RBC) bind to the hemagglutinin-neuraminidase found on the surface of rSIV.F/HN. This creates a lattice structure of interconnected RBC and virus particles that prevents RBCs from settling under gravity. While a standard rSIV.F/HN-EGFP vector preparation demonstrated a consistent HA value (the highest virus dilution inhibiting RBC settling) of 800±0 (n=18), similar vector preparations pseudotyped with VSV-G or GP64 failed to show any evidence of hemagglutination 0±0 (n=8 and 12, respectively; $p<0.0001$ for both). Importantly, for multiple vector preparations, HA values remain essentially unchanged as RBC preparations are replaced ($p>0.9999$) or used repeatedly over 4 weeks ($p>0.9999$). As anticipated, HA values between virus preparations vary with VP/mL and TU/mL. In summary, the HA assay is rapid (<1 hour), inexpensive, requires no specialist equipment, training or interpretation, and allows vector concentration information to be incorporated into the choice of final vector formulation volume without extending the vector processing time. Using this approach will narrow variations in final vector concentrations and thus allow greater consistency in dosing volumes between vector lots - a

particularly important property for rSIV.F/HN vectors where *in vivo* administration of a high concentration is anticipated.