Scale-up challenges – an academic view

Author Steve Hyde, University of Oxford

While laboratory production of lentiviral vectors has been well described, commonly used approaches utilise methods such as calcium-phosphate-mediated transfection and ultra-centrifugation-based concentration that are poorly suited to cGMP manufacturing approaches necessary for clinical development. Thus, a clear, rate limiting issue with the timely development of lentiviral gene transfer vectors is the availability of high-titre purified stocks of vector with product characteristics similar to those anticipated from cGMP manufacturing efforts. To that end, we have focused our academic-housed vector production and purification efforts on developing methods that mimic those approaches adopted by leading lentiviral CDMOs.

Initially, our production of rSIV.F/HN vectors (a novel, lentiviral gene transfer vector specifically pseudotyped for efficient airway cell transduction) was established in a serum-dependent, calcium phosphate-mediated, transient transfection process. Subsequently, we have developed a scalable, animal-free, cGMP-compliant suspension cell culture based upstream production (USP) method. Serum-free media screening identified a suspension of cell growth conditions that support efficient (>70%) multiple plasmid transient transfection of HEK293T cells with a range of cGMP-compliant liposomal and polymeric gene transfer agents. Small volume shake flask studies identified robust growth and transfection conditions that yield ≥5e6 TU/mL of unpurified rSIV.F/HN vectors. These parameters supported transition to 1L and 5L scale WAVE bioreactor cultures, where virus yield is maintained and plasmid DNA usage (a high proportion of the cGMP cost of goods) has been reduced. Crucially, increasing production scale beyond 5L appears facile. F/HN pseudotype specific DNA removal and virus activation steps are incorporated into the downstream purification (DSP) method which relies on anion-exchange membrane virus capture and hollow-fibre UF/DF for vector purification and final formulation. In more than 100 independent pH-controlled WAVE bioreactor cultures, purified yields typically exceed 2.0e9 TU/L for a wide range of promoter/transgene constructs. rSIV.F/HN vectors manufactured using this approach direct abundant in vivo airway gene transfer, transducing 14.1% epithelial cells in the murine lung ($p<0.001$;8e8 TU rSIV.F/HN hCEF EGFP) exceeding the presumed therapeutic transduction threshold. While these CDMO and

regulator friendly USP/DSP approaches have been tuned to yield highly purified and potent rSIV.F/HN vectors, the methodology is readily transferrable to other pseudotype configurations.