Development of protocols for mouse GLP-toxicology studies

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Introduction: We have developed a Simian Immunodeficiency Virus (SIV)-based lentiviral vector pseudotyped with the Sendai-virus envelope glycoproteins (F/HN) (rSIV.F/HN) that is effective at transducing pulmonary epithelium *in vivo*. To prepare for a first-in-man clinical trial, we have developed protocols that can be used in a mouse GLP-toxicology study to efficiently transduce the nose or lungs. In addition, we have used a radiopharmaceutical as a surrogate for the viral vector, to quantify dose deposition in target organs.

Mouse nose transduction protocol: We aimed to efficiently target gene transfer to the murine nose with minimisation of vector loss to non-target organs, including the lung. We compared our standard 100 µL bolus administration ("nasal sniffing") to slow perfusion of 100 µL of the vector via a catheter (6.7 µL/min) and to pipetting of small volume doses (2 x 5 µL). All animals were treated with 1e7 TU/mouse of rSIV.F/HN expressing luciferase (n=6/group). Animals were culled 10-12 days after transduction, and all methods led to similar gene expression in the nasal cavity. Catheter-based delivery led to significant ($p<0.05$) spill-over into the lung and was discontinued. However, in contrast to administration via nasal sniffing, pipetting of small volumes prevented gene expression in the lungs. Technetium radiotracer (5MBq of ^{99m}Tc-DTPA/mouse) showed that 98±1% of the pipetted dose was retained in the head. In contrast, nasal sniffing retained only 36±12% in the head, with the rest of the radioactivity dispersing into lungs (34±16%) and the rest of the body (30±5%). To increase vector delivery to the nasal epithelium further, we used up to 10 x 5 µL doses (at 5 min intervals) to a maximum of 2.4e8 TU/mouse, n=5/group. We observed a dose-related increase in gene expression ($P<0.0001$, $r2=0.87$). Importantly, even after 10 x 5 µL doses the majority of the radio-tracer (90±3%) was retained in the head.

Mouse lung transduction protocol: We aimed to preferentially target only the lung comparing nasal sniffing to an oropharyngeal (OP) delivery method (1e7 TU/50 µL dose for both techniques). There was no significant difference in gene expression within the lung. We further compared volume distribution using technetium as a radiotracer (5MBq of ^{99m}Tc-DTPA/mouse, n=5-6/group). Mice were culled 10 mins after administration and radioactivity was measured in lung, head and rest of the body. No significant differences in quantities of radioactivity were detectable in lungs using either delivery method (sniffing: 40±18%, OP: 37±11%). However, nasal sniffing is able to deliver double the volume of that for OP.

Conclusions: We have developed protocols suitable for GLP toxicology studies in the murine nose and lung to optimise vector delivery to the target organ. By defining dose distribution with a radiotracer we are better able to calculate effective viral titres and overages compared to proposed clinical doses in future toxicology studies.