Evaluating Gene Transfer Products for Gene Expression in Mouse Nasal Epithelium

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Overview.

- We aim to develop a gene therapy for Cystic Fibrosis (CF).
- Core facilities have been established within the UK CFGT Consortium to identify candidate products for clinical trials.
- Reporter gene (luciferase) screening in the readily accessible mouse nose provides the first *in vivo* test.
- The mouse nasal epithelium consists of ciliated and non-ciliated airway epithelial cells, the principal targets of CF gene therapy.
- We have used pDNA delivered alone (naked) or complexed with GL67 to begin to evaluate:

  a) the optimum formulation (ratio of DNA to GTA in 100μl), using pCIKLux as reporter vector (CMV promoter-driven *luciferase* gene cassette).

  b) the peak expression time point.

  c) the effect of increased contact time.

- This information will be used subsequently for the evaluation of GTAs in CF functional assays (including epithelial ion transport) in the mouse nose.

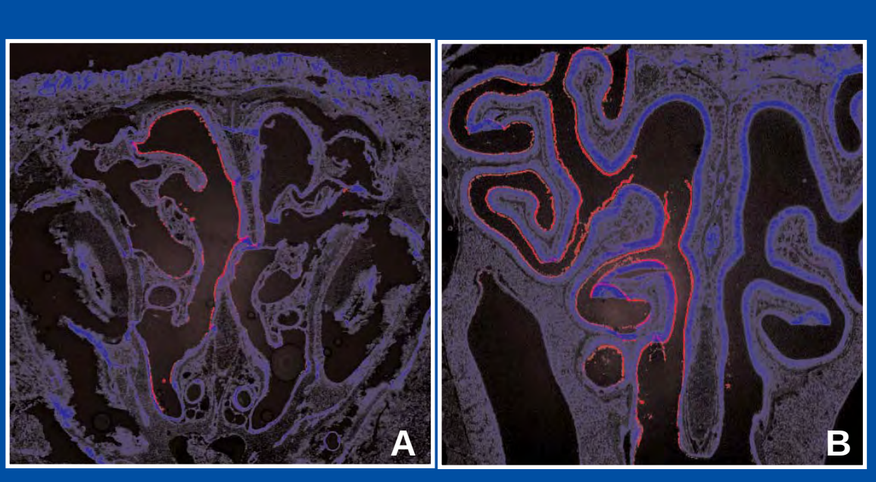
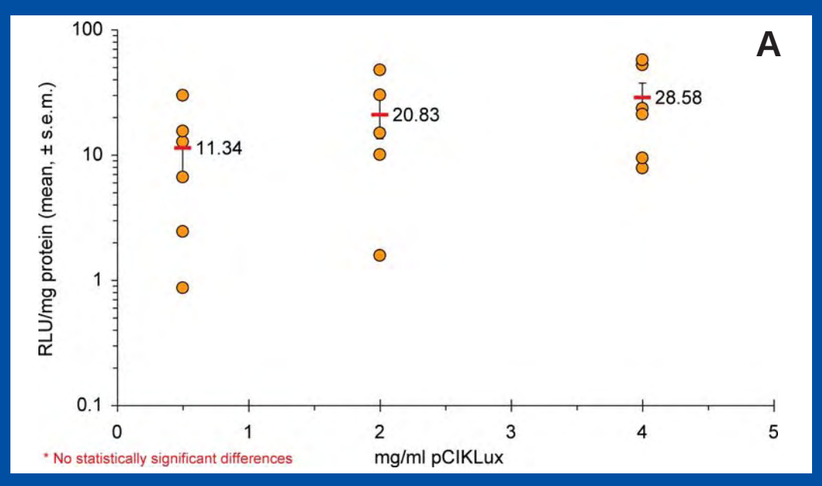


Figure 1: Perfusion of nasal cavity.

All studies were carried out in female C57BL6 mice, 7-10 weeks old. Mice were anaesthetised by i.p. injection of Domitor/Ketaset, placed on their backs, and the GTA perfused through a thin catheter inserted 2.5mm deep into the left nostril only. Nasal septum and all attached tissue were harvested and analysed for luciferase activity.

In addition, cryosections from the nose of a mouse perfused with biotin-labelled lycopersicon esculentum lectin (Vector Labs) in water, showed complete coverage of the nasal epithelium by the GTA in the dosed nostril. Typical sections are shown in Figure 1, at a depth of (A) 3mm and (B) 7mm . The lectin was detected using streptavidin conjugated to Alexa Fluor594 (Molecular Probes), in red, and nuclei were stained with DAPI, in blue.



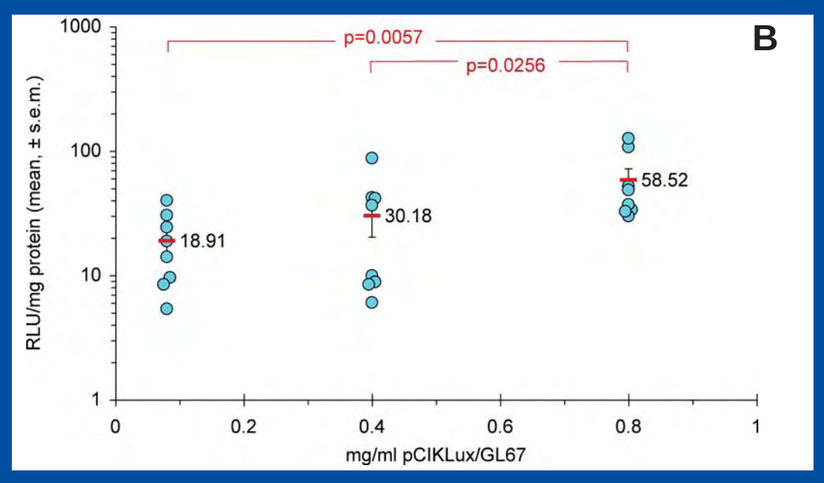
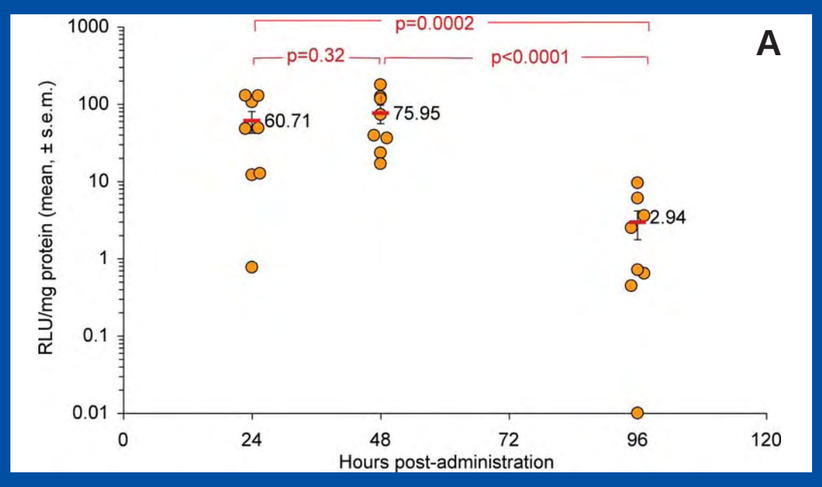


Figure 2: Reporter gene expression after perfusion of pCIKLux or pCIKlux/GL67 complexes.

Mice were perfused with (A) 100μl pCIKLux at 0.5, 2 and 4mg/ml or (B) 100μl pCIKLux/GL67 complexes at 0.08, 0.4 or 0.8mg/ml over 15'. Nose tissues were harvested after 24 hours.



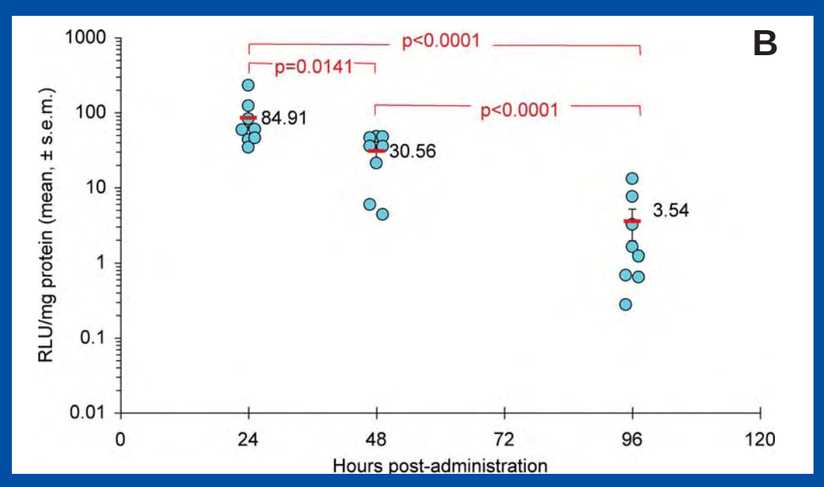
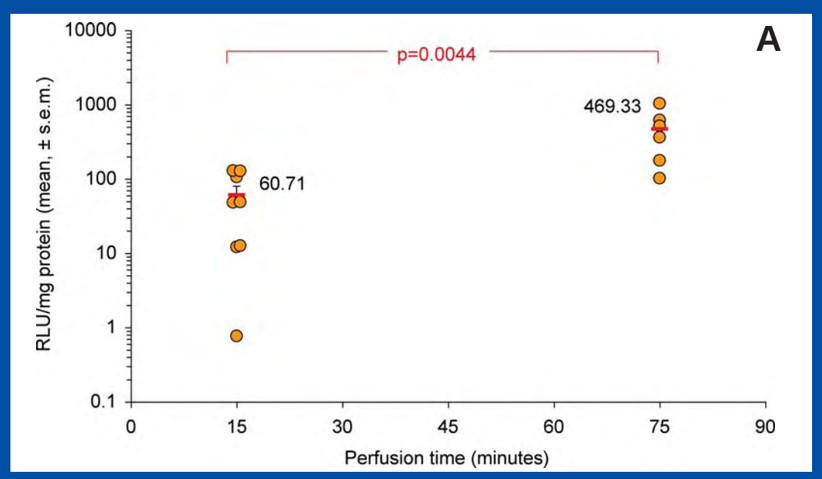


Figure 3: Time course of reporter gene expression after perfusion of pCIKLux or pCIKLux/GL67 complexes.

Mice were perfused with (A) 100μl pCIKLux at 4mg/ml or (B) 100μl pCIKLux/GL67 complexes at 0.8mg/ml over 15'. Nose tissues were harvested after 24, 48 or 96 hours.



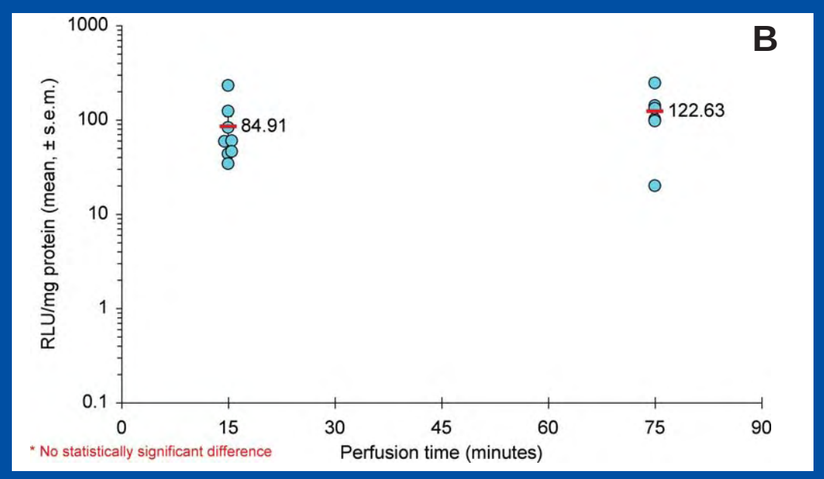


Figure 4: Effect of increased contact time on reporter gene expression.

Mice were perfused with (A) 400μg pCIKLux over 15' (100μl) or 75' (500μl), or (B) 80μg pCIKLux/GL67 complexes over 15' (100μl) or 75' (500μl). Nose tissues were harvested after 24 hours.

Conclusions.

- A core facility has been established to provide information on the optimum formulation for each GTA tested.
- Dose response experiments are used to ascertain the most effective ratio (DNA:GTA) and dose. In general the maximum feasible dose (highest concentration of DNA achievable in 100μl) is the most effective.
- Time course experiments are used to ascertain the time point for maximal gene expression. In general day 1 (or 2) generates peak expression.
- Novel GTAs are now being evaluated.

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