



Optimising Gene Transfer Products For Evaluation In The Mouse Nasal Epithelium

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► Overview.

The UK Cystic Fibrosis Gene Therapy Consortium has established a core research facility to identify non-viral gene transfer agents (GTAs) with enhanced airway gene transfer.

A wide range of cationic polymer, cationic lipid and cationic peptide-based GTAs have been screened for reporter gene (luciferase) activity in the mouse nose which consists of ciliated and non-ciliated airway epithelial cells, the principal targets of CF gene therapy.

These reporter gene studies inform subsequent wide-ranging CFTR-based assays (epithelial ion transport, airway surface liquid height, bacterial adherence and goblet cell number) in the nose of CF mice.

► Methods.

We have studied a range of commercially-available and proprietary GTAs to evaluate:

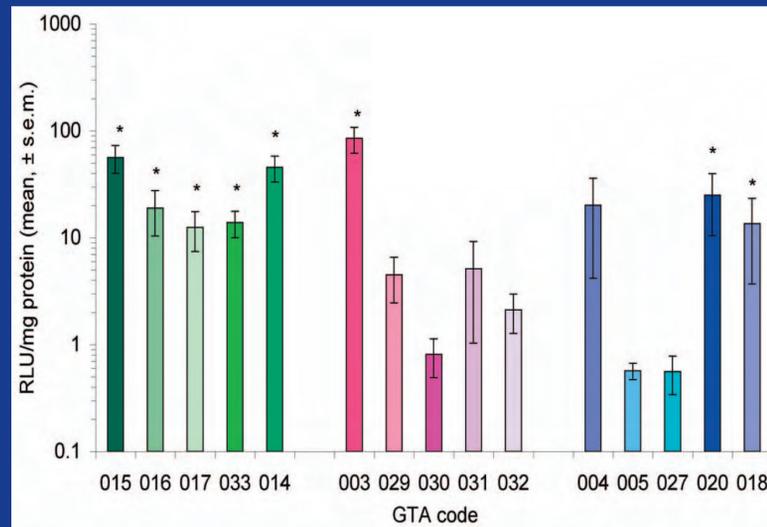
- a) optimum formulation - ratio of GTA to plasmid pCIKLux (CMV promoter-driven luciferase reporter gene) (1).
- b) peak expression time-point
- c) effect of increased contact time
- d) effect of plasmid modifications on longevity

For intellectual property reasons, the identity of several GTAs cannot be revealed at this time. For ease of illustration the GTAs are identified only by internal three-digit GTA codes (e. g. GTA001).

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

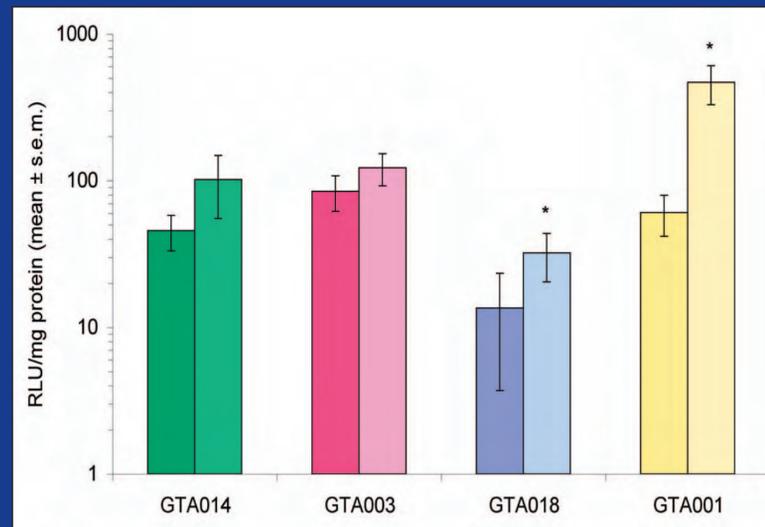
(1) Gill D.R. *et al.* (2001) Increased persistence of lung gene expression using plasmids containing the ubiquitin C or elongation factor 1a promoter. *Gene Therapy* 8, 1539

► Results.



In Vivo Reporter Gene Expression From Different Classes Of GTAs.

To date >30 GTA formulations have been assessed. Green, pink and blue bars indicate gene transfer activity mediated by representative cationic polymers, lipids and peptide-based GTAs respectively. Murine nasal epithelium (n=6-8) was perfused with 100µl of the indicated GTA formulated at the maximum feasible dose (MFD). Nasal tissue was harvested 24 hours post-treatment. Samples from untreated mice would typically have ~0.5 RLU/mg protein. (*) indicates p<0.05 compared to untreated animals (ANOVA with Fisher's PLSD on log-normalised data).

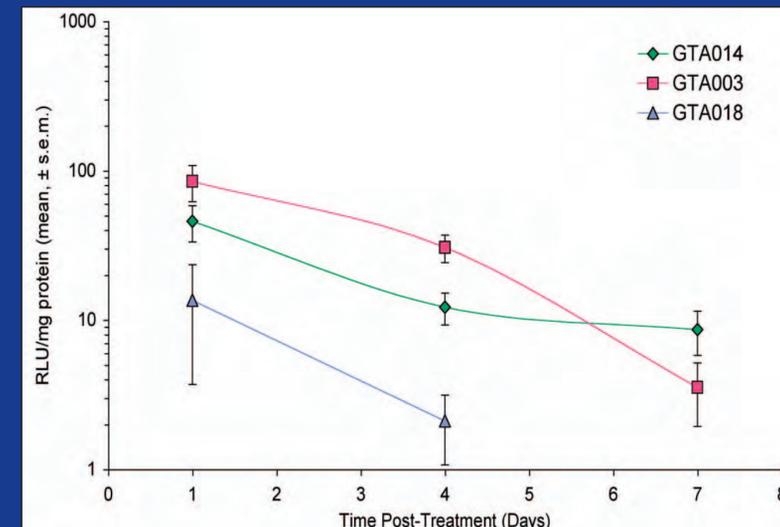


Effect Of Increased Contact Time.

The effect of increasing contact time on reporter gene activity was also evaluated for each GTA. For the majority of GTAs there was less than a two-fold increase in reporter gene activity. Mouse nasal cavity was perfused with 100µl of GTA/pCIKLux formulated at MFD, for 15 minutes (dark bars) or with 500µl of GTA/pCIKLux formulated at MFD, for 75 minutes (light bars). Representative data from GTA014 a cationic polymer (green), GTA003 a cationic lipid (pink) and GTA018 a cationic peptide (blue) are shown along with GTA001 naked plasmid DNA (1mg/ml in water). Nasal tissue was harvested 24 hours post-treatment. (*) indicates p<0.05 compared to 15-minute perfusion group (ANOVA with Fisher's PLSD on log-normalised data).

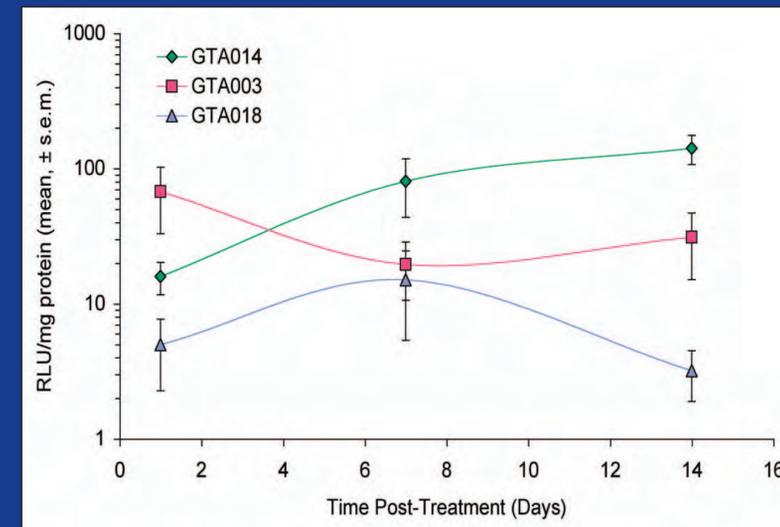
► Conclusions.

- Increasing GTA contact time resulted in little or no increase in reporter gene activity for the majority of GTAs evaluated.
- All GTAs evaluated mediated transient reporter gene expression with a CMV promoter plasmid.
- All GTAs evaluated mediated enhanced duration of airway gene expression with a UbC promoter plasmid.
- The mouse nose can be used to easily compare a range of GTAs.



Duration Of Reporter Gene Expression – CMV Promoter.

The duration of reporter gene expression directed by each GTA complexed with plasmid pCIKLux was also evaluated. Peak reporter gene expression was typically observed 1 to 2 days post-treatment. Disappointingly, all GTAs showed transient gene expression, with reporter gene activity falling towards naïve levels by 7 days post-treatment. Representative data from GTA014 a cationic polymer (green), GTA003 a cationic lipid (pink) and GTA018 a cationic peptide (blue) are shown. Nasal tissue was harvested at the time-points indicated.



Duration Of Reporter Gene Expression – UbC Promoter.

Duration of airway reporter gene expression can be enhanced by replacing the widely used CMV promoter with housekeeping UbC promoter (1). To assess the duration of reporter gene activity mediated by the GTAs, we determined the duration of reporter gene expression when complexed with the UbC promoter plasmid pUbLux (1). Encouragingly, GTAs tended to show enhanced duration of reporter gene expression with the UbC promoter. Representative data from GTA014 a cationic polymer (green), GTA003 a cationic lipid (pink) and GTA018 a cationic peptide (blue) are shown. Nasal tissue was harvested at the time-points indicated.