CALCULATING THE PERCENTAGE OF CELLS TRANSFECTED FOLLOWING NON-VIRAL AEROSOL DELIVERY TO THE RESPIRATORY EPITHELIUM

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4 Introduction

The UK CFGTC have commenced clinical trials for the treatment of Cystic Fibrosis (CF) lung disease

Genzyme Lipid 67 complexed with the plasmid pGM169 is being aerosolised to the lungs/nose of CF patients

Following gene transfer to CF patients or large animal models we would like to:

- Quantify total plasmid mRNA in a tissue sample
- Quantify the percentage of positive cells (PPC) in a tissue sample that express plasmid derived mRNA

We aim to use the novel PPC assay following gene transfer to the respiratory epithelia of CF patients/animal models

4 3. Cell Culture Model to Verify PPC Assay

1. 293T cells were transfected with pGM169/22KDa Linear-PEI (Polyplus)
2. Transfected cells and naive controls were harvested at 24 hours and stored in RNALater for 1 month at 4°C
3. The following groups were created:
   - Neat: pGM169 transfected cells @ 1 cell/µl
   - Dilution 1: Neat diluted 5 fold with Naive Control
   - Dilution 2: Neat diluted 25 fold with Naive Control
   - Naive Control: Untransfected 293T cells @ 1 cell/µl
4. 80 x 10 µl replicates were dispensed from each group and Cells-to-Ct lysis buffer added to create PPC lysates
5. Samples were analysed by Taqman Q-RT-PCR to detect pGM169 mRNA

4. Long Term Storage of Samples

1. Human nasal brushings (HNBr) and HEK293T cells were stored, intact, in RNALater for 1 month at 4°C
2. Cells were diluted to 1 cell/µl to make 40 x 10 µl replicates
3. Replicates were lysed with Cells-to-Ct lysis buffer (ABI) and analysed by Q-RT-PCR for hGAPDH mRNA

4 Result

After 1 month storage 38/40 HNBr replicates were positive for hGAPDH
40/40 293T cell replicates were positive for GAPDH
Long term storage of samples has no effect on detection of mRNA by Q-RT-PCR

4 Conclusion

- pGM169 mRNA can be detected by novel PPC Taqman approach
- The 5 fold reduction in PPCmin in Dilution 1 matches the fold dilution of positive cells with naive cells
- PPC can be used to determine the transfection efficiency in a sample of transfected cells

4 Future Plans

- Clinical samples from our trial are currently being processed and evaluated
- Compare the efficiency of different aerosolisable non-viral GTAs in large animal model