Introduction:
• Transduction of resident airway stem or progenitor cells using an integrating gene vector has clear potential for enabling long lasting CFTR gene correction for cystic fibrosis (CF) airway disease.
• Gene correction of basal cells, some of which will be stem/progenitor cells, allows the gene correction to be passed on to the daughter cells, including terminally-differentiated airway cells and most importantly ciliated cells, a primary target for correcting CF airway disease.
• Here we tested if human airway basal cells grown in culture can be effectively transduced with our human-based (HIV-1) lentiviral vector (LV) and whether the amount of time cultured cells are exposed to the vector influences the resulting gene expression levels.

Methods:
• Normal Human Primary Bronchial Epithelial Cells (LONZA CC-2540S) were seeded, expanded and passaged (as per manufacturer’s instructions) in Bronchial Epithelial Growth Medium (BEGM, CC3170). Once 90% confluent at passage 2, cells were harvested and seeded into 6 well plates at 2.5 x 10^5 cells per well (~70% confluence).
• Untreated control cells were used for immunohistochemistry staining against keratin 5 (KRT5) to confirm basal cell identity.
• To test the efficiency of our LV vector (1.4 x 10^9 TU/ml) the lacZ reporter gene was utilised. The vector was applied to cells 2 hours after seeding at multiplicity of infections (MOI) of 100, 10 and 1 (n=3 per group) and left overnight.
• To test the effect of vector residence time on cells, vector with the lacZ reporter was applied at an MOI of 10 (n=3) and cells were washed 3 times to remove the vector at; 1 minute, 30 minute, 3 hr or 6 hr post application.
• LacZ expression in both studies was assessed 3 days after applying LV to cells using standard Xgal processing.
• Level of gene expression was quantified in both studies by analysing 5 random fields of each replicate to obtain the means and standard deviation.

Results:
• KRT5 staining showed that the cells at passage 2 were predominantly (>90%) human airway basal cells (Fig 1d).
• The human airway basal cells show essentially complete LacZ transduction at an MOI of 100: 99.9% and at an MOI of 10: 97.7%. At an MOI of 1 47.4% of cells were transduced (Fig 1a-c).
• Only brief vector residence time was required to produce high levels of expression using an MOI of 10: LacZ gene expression was 75.1% after 1 minute, 95.4% at 30 minutes, 98.4% at 3 hours and 99.4% at 6 hours (Fig 2).

Conclusion and Discussion:
• Human airway basal cell cultures can be efficiently transduced with our LV gene vector at a low MOI in vitro.
• High levels of lacZ expression resulted after as little as 30 minutes of vector exposure. Although transduction was lower after 1 minute of vector exposure, the 75% cell transduction level remains substantial, indicating that in the absence of other factors that influence expression levels, this vector may be able to produce high levels of basal cell transduction in vivo, with only brief exposure lengths.