Overview of project.

Existing non-viral gene therapy for CF lung disease is very inefficient.

Electroporation is an efficient method for increasing non-viral delivery.

Lung access makes electroporation technically challenging.

Electroporation enhances reporter gene expression in the mouse lung (Figure 1).

We studied the effect of electroporation on the duration of expression (Figure 2).

Wire electrodes were developed for electroporation in the sheep lung (Figure 3).

Electroporation increased Lux activity in the sheep lung (Figure 4 & 5).

Results.

Duration of Luciferase expression in the mouse lung following electroporation.

Mice were dosed with pCIKLux and pUbLux (luciferase reporter gene expressed from the human polyubiquitin C promoter) (Gill et al., 2001). The left lungs (+) were electroporated (+) and the right lungs (-) served as no electroporation controls. Mice were harvested at 2, 7, 14 & 28 days post-dosing and the levels of Lux activity in the right and left lungs was determined. Error = 1 SEM, n = 6-12.

Results of the procedure were as follows: At day 2, Lux activity from electroporated (+) pCIKLux was 500 fold higher than the no electroporation (-) control, but by day 14 the activity from both conditions had fallen to background levels. Larporation increased the level of Lux activity from pUbLux at each time point of the study. By 28 days post-dosing, the Lux activity from pUbLux (+) was 30 fold higher than the pUbLux (-) at 2 days post-dosing. Therefore while electroporation does not improve the limited duration of expression from the CMV promoter, when appropriate long duration constructs are used, activity may be maintained at elevated levels for at least 1 month.

Figure 3. Teflon insulated wires function as electroporation electrode devices.

Wire electrode test.

Teflon insulated stainless steel wires (350 µm thick) (A-M Systems, Kent Island, WA, USA) were cut into 90 cm sections and electroplated. The sheep were anaesthetised using a BTX ECM830 Electroporator. 24 hours post-dosing the levels of Lux activity were determined. Error = 1 SEM, n = 3 samples per condition.

Conclusions.

Electroporation increased Lux activity for 1 month in the mouse lung.

Wire electrodes are safe and perform as well as needle electrodes.

Electroporation increased Lux activity by 250 fold in the sheep lung.

Successful translation of electroporation to a large animal model.