# How do we know if cystic fibrosis lung gene therapy works? The importance of the right animal model, and the right measurement method

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## Cystic fibrosis

- The most common fatal genetic disease in the Caucasian population – 1 in 25 people carry a defective CFTR gene.
- Currently affects more than 3,000 Australians and 70,000 people worldwide.
- Affects many organs, but most of the mortality and morbidity is caused by lung disease.
- Patients can spend a lifetime struggling to breathe and half die before they reach 40.
- The introduction of new CFTR mutation-specific modulator drugs such as Kalydeco and Orkambi offers hope that, for some patients, lung disease might be prevented if treatment begins early and is continued daily for the patient's lifetime.
- However, addition of a properly-functioning CFTR gene into affected CF airway cells, before disease is established, is the only biologically rational way to prevent or treat CF airway disease for all classes of CF mutation.

## Lentiviral airway gene-addition therapy

- 2nd / 3rd Generation HIV-1 VSV-G Pseudotyped LV vector, produced in adherent or serum-free suspension cultures.
- Prior to LV delivery we condition the airway with lysophosphatidylcholine (LPC) to transiently permeabilise airway tight-junctions. This provides vector access to the basolateral region of the airway epithelium, the location of the airway surface cell receptors and the cells of the airway stem cell niche, to achieve effective and extended airway gene expression.
- Demonstrated *in vivo* acute and long term effectiveness of single-dose LV airway reporter gene transfers in mice, and the ability to transduce the conducting airways in sheep, marmosets and ferrets.
- LPC conditioning enhances functional CFTR correction in CF mouse nasal airways – the only site of measurable CF airway pathophysiology in CF mice. Improvements in CFTR function lasted for at least 12 months after a single gene vector dose.

## Clinical translation of CF lung gene therapy has been constrained by the:

### 1) Absence of a good small animal model with human-like CF lung disease

- CF mice do not exhibit human-like lung disease, so we have established an Adelaide CF rat colony.
- CRISPR-Cas9 gene-editing was used to create founder animals with the Phe508del mutation.
- Animals with 3 genotypes were chosen for breeding: Phe508del/WT, 506frameshift/WT (mosaic Phe508del mutation and a C-T silent point mutation), and N505IYX/WT (premature termination).
- In May 2017 one round of breeding has been completed to produce heterozygote breeder pairs.
- The first CF animals will be available for study by mid-2017.

***Figure 1:*** *CF rats exhibit slowly-progressing human-like lung disease.*

### 2) Inability to rapidly and accurately pinpoint and measure changes in local lung disease

- Changes in CF lung *function* are normally measured using lung function tests that produce a single measure of how the whole lung is functioning. Changes in lung *structure* are normally measured with Computed Tomography (CT). Both have limited sensitivity and specificity to detect early disease, small changes in disease, or their origin within the lung.
- Can now utilise a new X-ray imaging technique called Computed Tomographic X-Ray Velocimetry (CTXV), which gathers information on lung motion, rather than structure, during normal breathing.
- CTXV gives us quantitative information on lung function in any airway or region in the lung, allowing us to detect, quantify and follow changes in regional lung function over time.
- We have already used CTXV to demonstrate heterogeneous lung function in β-ENaC mice, and identify the locations and effects of airway obstructions (Fig 2).

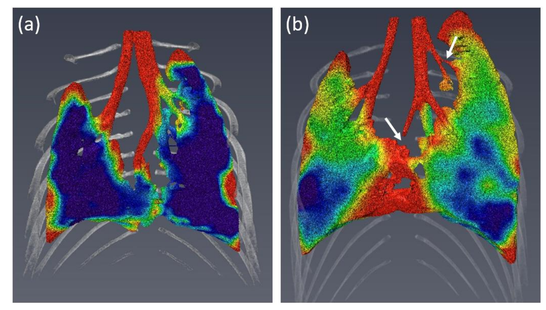


***Figure 2:*** *Lung tissue expansion map from (a) a typical healthy littermate control mouse and (b) a β-ENaC mouse (right). Aeration is uniform in the littermate, but airway obstruction restricts airflow in the β-ENaC. The locations of the obstructions have been highlighted by arrows.*

## Project Aim

- Use CTXV to determine whether our CFTR gene therapy protocol can prevent the onset and/or the progression of airway disease in the CF rat (Fig 3).

## Methods

- Analyses for CF gene-addition studies in CF rats will include CTXV, lung function testing (Forced Oscillation Technique), and immunohistochemical and molecular analyses.
- All CTXV studies will be performed at the Australian Synchrotron.
- CTXV will also be used to test the effectiveness of gene therapy treatments delivered in the presence of lung bacterial infections that are common in CF patients.
- We will also determine whether co-treatment with inhaled antibiotics improves gene transfer effectiveness in pre-infected CF rat lungs .

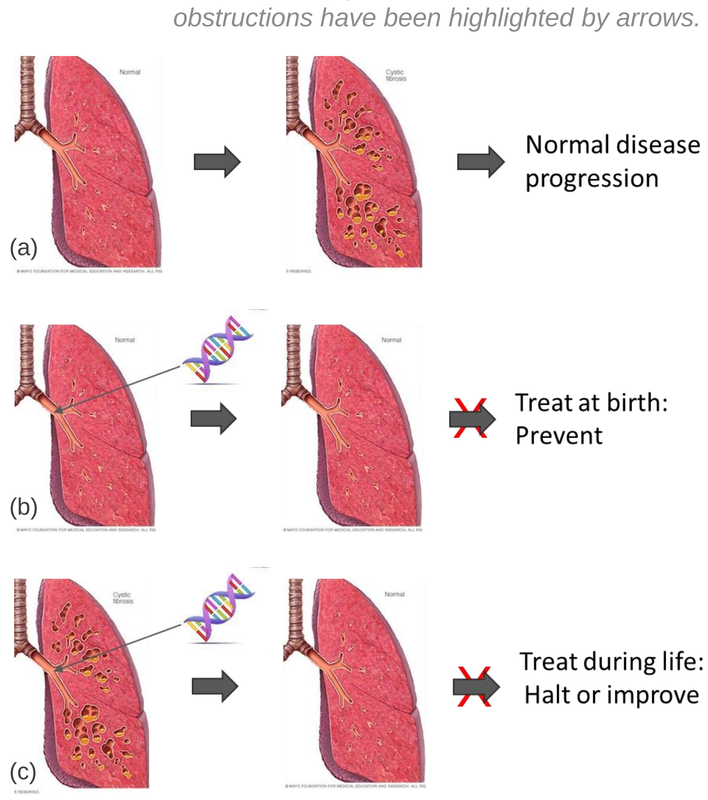


***Figure 3:*** *Key experiments. (a) Establish the normal progression of CF lung disease in the Phe508del rat. (b) Determine whether CFTR gene therapy soon after birth prevents lung disease establishing. (c) Test whether treating later in life can halt or reverse lung disease.*
*Lung diagram adapted from http://www.mayoclinic.org*

## Progress

- CF rat breeding and LV gene vector production are underway.
- Rat CTXV imaging protocols developed and tested at the Australian Synchrotron.

## Conclusions

- The availability of CF rats as well as CTXV makes this the first opportunity worldwide to examine the effects of LV-CFTR gene therapy on the progression and treatment of CF lung disease.

## Acknowledgements

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- The founder rats were generated via injection of guide and Cas9 RNA into ooctyes through the CRISPR service of the Australian Phenomics Network (APN) at Monash University (www.australianphenomics.org.au).
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