

Clinical translation of CF lung gene therapy has been constrained because:

A) CF mice do not exhibit human-like lung disease.

Solution:

- We have established an Adelaide-based CF rat colony.
- The first rats with lung disease were born in June 2017.
- See C. McIntyre in Concurrent Session 3B (Tuesday 11:00-12:30) and poster for details.

B) Current measurement techniques cannot rapidly and accurately pinpoint and measure changes in local lung disease.

- Lung *function* tests typically produce a single measure of how the whole lung is functioning.
- Lung *structure* is visualised with Computed Tomography (CT), but structural changes are not always predictive of function / disease.
- Both techniques have limited sensitivity and specificity to detect early disease, small changes in disease, or their origin within the lung.

Solution:

- Computed Tomographic X-Ray Velocimetry (CTXV) gathers information on lung motion, during normal breathing.
- CTXV quantifies information on lung function in any airway in the lung, allowing us to detect, quantify and follow changes in regional lung function over time.
- We have validated CTXV in β -ENaC mice and littermate controls.
 - CTXV can identify regions of reduced airflow, and the locations and effects of airway obstructions (Fig 1).
 - β -ENaC animals had a significantly higher level of lung heterogeneity than littermate controls, as measured by lung hysteresivity ($p < 0.0001$, t-test).

Project Aim

- We will 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 2).

Methods

- HIV-1 lentiviral (LV) CFTR gene vector delivered into rat lungs.
- Analyses will include CTXV, lung function testing (Forced Oscillation Technique; FOT), and immunohistochemical and molecular analyses.
- CTXV studies will be performed at the Australian Synchrotron.
- We will also test the effectiveness of gene therapy treatments delivered in the presence of lung bacterial infections that are common in CF patients.
- We will determine whether co-treatment with inhaled antibiotics improves gene transfer effectiveness in pre-infected CF rat lungs.

Progress

- CF rat breeding and LV gene vector production are underway.
- Rat CTXV imaging and FOT protocols have been developed and tested in normal rats 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 lentiviral CFTR gene addition therapy on the progression and treatment of CF lung disease.

Acknowledgements

- CTXV data processing performed by Dr Freda Werdiger, Monash University.
- Project funding provided by Fay Fuller Foundation, Cure4CF Foundation, & NHMRC.
- The founder rats were generated via injection of guide and Cas9 RNA into oocytes through the CRISPR service of the Australian Phenomics Network (APN) at Monash University (www.australianphenomics.org.au).
- CF rat colony start-up funding provided by CF South Australia.

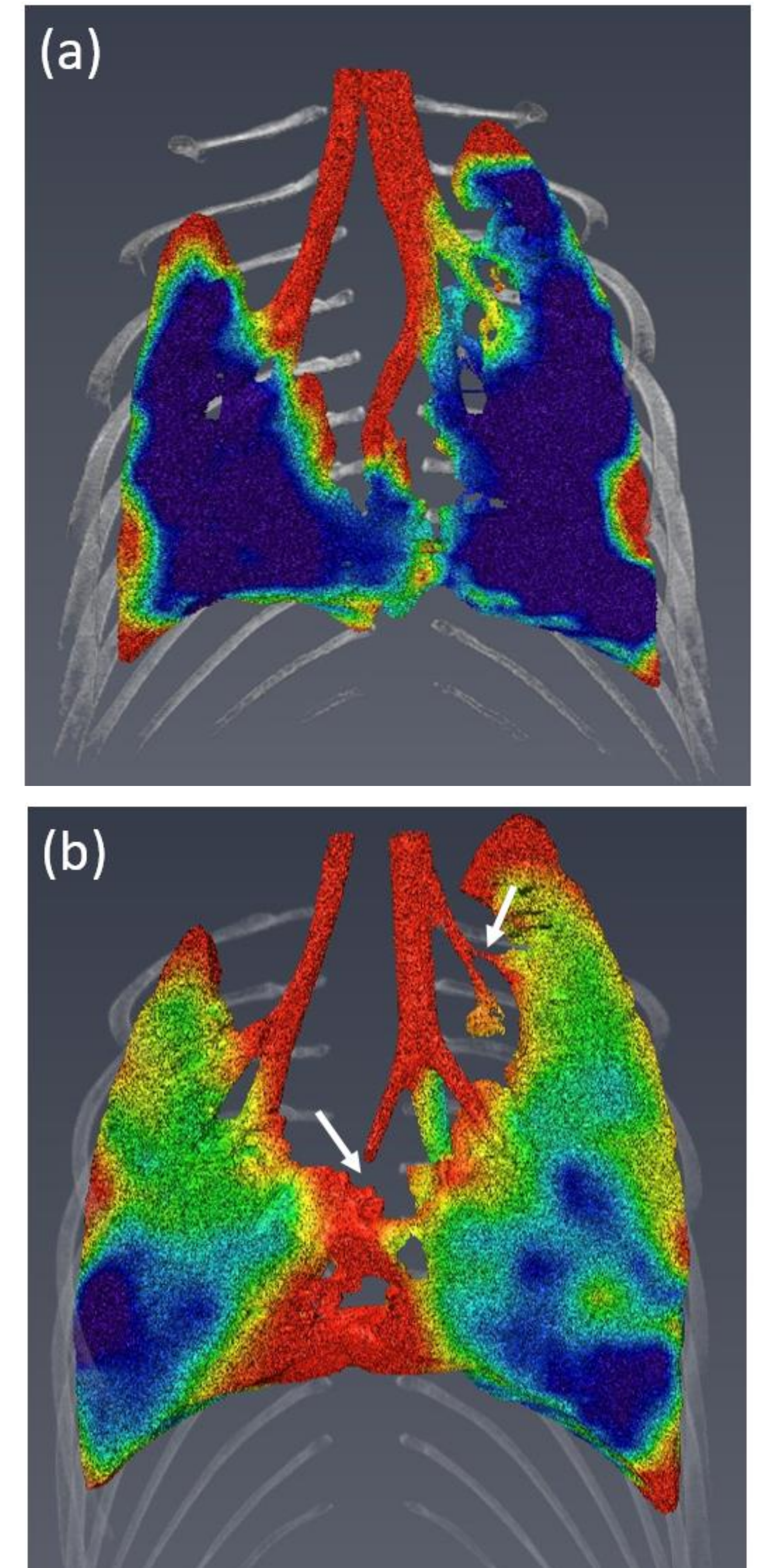


Figure 1: Lung tissue expansion map from (a) a typical healthy littermate control mouse and (b) a β -ENaC CF mouse. Aeration is uniform in the littermate, but airway obstruction restricts airflow in the β -ENaC. Red is low expansion, blue is high. The locations of the obstructions have been highlighted by arrows.

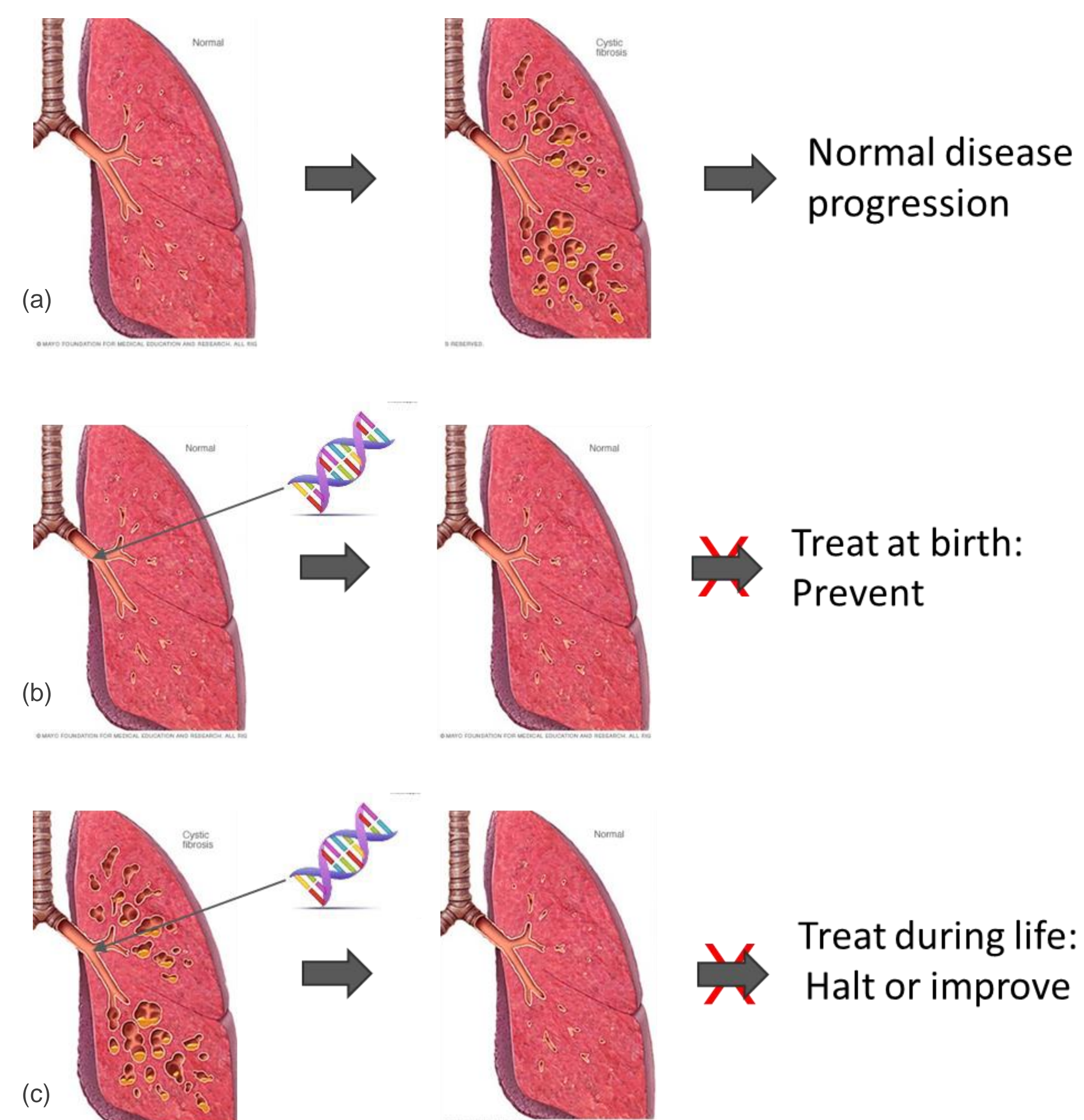


Figure 3: Key experiments. (a) Establish the normal progression of CF lung disease in the Phe508del rat. (b) Determine whether CFTR gene therapy soon after birth prevents lung disease establishing. (c) Test whether treating later in life can halt or reverse lung disease.

Lung diagram adapted from <http://www.mayoclinic.org>