Gene Therapy for Lung Diseases

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Who am I?

What do I do?

- crash course in genetics
- cystic fibrosis lung disease
- gene therapy

- anything else?
Genetics - A 5 Minute Crash Course

- Humans are made from ~100 trillion cells
  ($=100,000,000,000,000$)
- Different parts of the body are made from different sorts of cells

- Red Blood Cells
- Nerve Cells
- Lung Cells
- Muscle Cells
- Play-Doh Cells
Genetics - A 5 Minute Crash Course

- Chromosomes are found in the nucleus of every cell*
- Chromosomes are made of DNA “The Chemical of Life”

*Except Red Blood Cells
• Every cell has 23 pairs of chromosomes
Genetics - A 5 Minute Crash Course

• Every cell has 23 pairs of chromosomes
• You inherit 1 copy of each chromosome from each parent
Genetics - A 5 Minute Crash Course

- The DNA in human chromosomes encodes 25,000 "genes"
- Differences in our genes is what make us different
Differences in one gene causes albinism - monogenic disorder

~75%/25%
Differences in several genes control eye colour - polygenic
CF Genetics - A 5 Minute Crash Course

- Cystic Fibrosis
- Monogenic
- Gene encodes CFTR protein
- 1900 mutations found

- ~1:25 People in UK is a CF carrier
- Carriers usually healthy
- No knowledge they carry CF mutation

- If 2 carriers have children
- 1:4 chance of inheriting 2 mutated copies of CFTR
Cystic Fibrosis - CF

• CF is the most common life threatening genetic disease in the UK
  Every week 5 babies are born with CF
  Every week 2 young lives are lost to CF

• CF affects ~9K people in the UK & ~70K worldwide

• CF affects many parts of the body
  Lungs & digestive system get clogged with sticky mucus
  Hard to breathe & digest food

• Only half of those with CF live past their late 30s
  • There is no cure
Current CF Medicines Only Treat CF Symptoms

One Months Supply Of Medicines For A Typical CF Patient
Current CF Medicines Only Treat CF Symptoms

A Typical CF Patient Spends Several Hours Everyday Taking Medicines And Performing Treatments
Gene Therapy: Putting a Working Copy of the CF Gene Back into Cells of the Lung
How Will the CF Gene Help?

Normal Airway

Obstructed CF Airway
The CF Gene is Needed to Clear Mucus from the Lung
Cilia Keep the Airways Clear of Mucus

- Mucus traps particles & bacteria
- Cilia beat to clear mucus
- The CF lung is dehydrated
- The CF mucus is dehydrated
- Cilia cannot clear the lung
CF Gene Encodes CFTR Protein

- CFTR is located in cell membrane
- CFTR is a Chloride channel - keeps lung hydrated
Some Good News

- New drug - ivacaftor - corrects mutations in CFTR & improves lung function

- Only ~4% of CF patients
- Gene therapy should work for all mutations
Gene Therapy only Needs to Correct 10% Cells

CFTR Function

% Cells Expressing CFTR

CF is a good candidate for gene therapy
How to ‘do’ Gene Therapy…

- Find a (genetic) disease
- Match disease and gene delivery vector
Gene Therapy: How to Put the Gene into the Lung?

- Modified virus
  - CFTR gene inserted into a safe virus
  - Virus infects Cells of the lung
  - Delivers the CFTR gene...
Gene Therapy: How to Put the Gene into the Lung?

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- Synthetic ‘virus-like’ liposome
  DNA circle carrying CFTR gene
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- Modified virus
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- Synthetic ‘virus-like’ liposome
  DNA circle carrying CFTR gene
  Mixed with pharmaceutical liposomes
Gene Therapy: How to Put the Gene into the Lung?

- Modified virus
  - CFTR gene inserted into a **safe** virus
  - Virus infects cells of the lung
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- Synthetic ‘virus-like’ liposome
  - DNA circle carrying CFTR gene
  - Mixed with pharmaceutical liposomes
  - Make a virus-like particle...
Gene Therapy: How to Put the Gene into the Lung?

- Modified virus
  - CFTR gene inserted into a safe virus
  - Virus infects cells of the lung
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- Synthetic ‘virus-like’ liposome
  - DNA circle carrying CFTR gene
  - Mixed with pharmaceutical liposomes
  - Make a virus-like particle...
  - Which ‘infests’ cells of the lung
  - Delivers the CFTR gene...
How to ‘do’ Gene Therapy…

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• Match disease and gene delivery vector

• Show your idea can work in a model system
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• Engineer gene therapy for human use
• Manufacture in large quantities
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• Test in patients ‘Proof of Principle’
Proof of Principle for CF Gene Therapy

- Correct chloride defect in CF mice
- Clinical trials in the nose
- Clinical trials in the lung
- UK CF Gene Therapy Consortium (2001)
Synthetic DNA and Liposomes

- No viral proteins
- Minimise immune responses
- Engineer plasmid DNA (Gene) and Liposome
Engineer Gene for Patients

- Previous trials reported
  - flu-like symptoms
CFTR Gene Therapy in CF Patients

- Manufactured plasmid & liposome for human use
- Mix plasmid and liposome together
- Safety tests
CFTR Gene Therapy in CF Patients

- Manufactured plasmid & liposome for human use
- Mix plasmid and liposome together
- Safety tests
- Aerosol delivery (inhaler)
- Recruited CF patients
Showing Gene Therapy Can Work

Staining the cells for CFTR protein

Non-CF

CF

CF: Treated
Showing Gene Therapy Can Work

- Measure CFTR function in the nose
CF Patient

PD
mV

Time

-20
-15
-10
-5
0
5
10
1 16 31 46 61 76 91 106 121 136 151 166 181 196 211 226 241 256 271 286
Non-CF

![Graph showing PD changes over time for pre and non-CF conditions.](image-url)
CF Patient after Gene Therapy  
2 Weeks
CF Patient after Gene Therapy

4 Weeks

Time

PD mV

pre
d14
d28
non-CF
CF Patient Gene Therapy

6 Weeks

PD
mV

Time
CF Patient Gene Therapy

9 Weeks

Functional CFTR in humans for many weeks after 1 dose
Showing Gene Therapy Can Work

CFTR function
In the lung
CFTR Function in the Lung

Towards Non-CF

7/8 Change Towards Non-CF
Summary of Clinical Progress

- 1989 CF Gene Discovered
- 1992 First proof of principle in model systems
- 1999 First proof of principle in the lung

Safety
Efficiency
Ethics
Manufacturing

- 2009 Consortium gene therapy trial - Single Dose (35 patients)
- 2012 Consortium gene therapy trial - Multi Dose starts (12) monthly doses
- 2014 Consortium gene therapy trial - Multi Dose finished (116 patients)

Results soon
Developing New Viral Vector for Lung Delivery

• **Non-Viral Vectors**
  - Safe
  - Versatile
  - Low immunogenicity

• **Viral Vectors**
  - Increased efficiency?
Lentivirus for Lung Gene Delivery

- Long-lived gene expression
- Can be repeated
- Does not infect lung cells
New Improved Lentivirus - SIV F/HN

- Remove original coat proteins
- Replace with coat proteins from virus which is efficient in lungs

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<th>Expression</th>
<th>Tropism</th>
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<td>No airway</td>
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New Efficient Viral Vector

- Green Fluorescent Protein - from Jelly Fish

Respiratory epithelium
GFP positive cells (Green)
Nuclei (Blue)
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Respiratory epithelium
GFP positive cells (Green)
Nuclei (Blue)

14% GFP positive cells
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- Find a (genetic) disease
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- Show your idea can work in a model system
- Engineer gene therapy for human use
- Manufacture in large quantities
- Test in patients ‘Proof of Principle’
- Improvements? Apply to other diseases?
Scientists Doctors Nurses Physiotherapists Vets Technicians Pharmacists Administrators Project Managers Patients & Families
Important Questions

- Which diseases?
- Is it safe/ethical?
- Can we afford it?