Gene Therapy for Cystic Fibrosis: Finally Turning the Hype into Clinical Progress

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www.cfgenetherapy.org.uk
“The first example of gene therapy having a meaningful effect on lung function in patients with cystic fibrosis”
Cystic Fibrosis (CF)

Recessive fatal genetic disorder

100,000 people affected worldwide

Multi organ disease affecting epithelial surfaces

Caused by mutations in CFTR (>1900)
CFTR is an Epithelial Chloride Channel

Functions in the airways to keep lungs clear & hydrated
CFTR Maintains Airway Liquid Homestasis

- Mucus
- Peri-ciliary liquid (PCL)
- Ciliated Cells
- Pseudostratified Epithelia
- Basal Cells
CFTR Maintains Airway Liquid Homestasis

Airway liquid interface cultures

PCL height is actively regulated

Normal PCL Height

0 hours

6 hours

48 hours
Loss of Regulation in CF Airways

1. Increased Na absorption in CF
2. PCL height correction overshoots
3. Reduced mucociliary clearance
Cystic Fibrosis Lung Disease (CF)

Loss of CFTR function
  - Imbalanced fluid homestasis
  - Impaired mucociliary clearance

Dehydrated sticky mucus

Chronic infection & inflammation

Progressive airway destruction

Median age of death 25
Does restoring CFTR undo the damage of CF?
G551D Mutation – 4% of CF Patients

Gating mutation, protein gets to surface but doesn’t open

Kalydeco (Ivacaftor/VX-770) from Vertex

10% changes in lung function (FEV1) within 2-3 weeks

Whiting et al., 2014. Pubmed ID 24656117
Gene Therapy will Treat All Mutations

Over 1900+ mutations

dF508 CFTR in 66% of patients

Restore CFTR function regardless of genotype
CF Gene Therapy should be easy?

CFTR is expressed at a very low level (1-2 copies mRNA per cell)

Topical delivery to the airway is achievable by aerosol

Very well studied disease with dedicated clinics/specialist centres

Patient population is highly motivated
CF Gene Therapy should be easy?

Only a small % of CFTR expression would be required to correct CF

Some transgenic mice have <10% normal expression

Rare human mutations with low levels of activity (male infertility)
Overcoming CF lung disease

Cells of the respiratory epithelium are terminally differentiated
Not known how long they survive (6 months+)
Very little known about airway stem cells

Repeated delivery of gene therapy vector will likely be required

Mouse models do not develop lung disease

The ferret and pig models develop really severe lung disease

CFTR protein is awful to work with
27 Other Gene Therapy Trials for CF 1993-2007

Viral and non-viral vectors

- Adenovirus 12
- AAV2 6
- Non-viral 9

Generally small numbers of patients

More than half were nose only

Only two considered repeat administration

Only three involved aerosolisation of the vector
Proof of concept but no evidence of clinical benefit

Hyde et al., 2000
Nose only trial
12 patients with repeat administration
DC-Chol/DOPE
Positive results in molecular assays
Safe

Huge effort went into these small trials

Hyde et al., 2000, Pubmed ID 10918483
UK CF Gene Therapy Consortium – 2001

Oxford
- 2 previous clinical trials
- Molecular biology
- Vector development
- Aerosol delivery & characterisation

Roslin Institute, Edinburgh
- Large animal delivery model

Brompton, London & Western General, Edinburgh
- Clinical assay development
- Clinical teams
- 3 Previous clinical trials
UK CF Gene Therapy Consortium – 2001

Oxford
   2 previous clinical trials
   Molecular biology
   Vector development
   Aerosol delivery & characterisation

Roslin Institute, Edinburgh
   Large animal delivery model (Sheep!)

Brompton, London & Western General, Edinburgh
   Clinical assay development
   Clinical teams
   3 Previous clinical trials
UK CF Gene Therapy Consortium – Aim

Develop a clinical gene therapy for CF lung disease

1. Must be aerosolisable

2. Repeat administration must be possible
Ciliated Airway Epithelium

- Mucus
- Peri-ciliary liquid (PCL)
- Ciliated Cells
- Pseudostratified Epithelia
- Basal Cells
No Viral Vectors

Neutralising antibodies against viral vectors (rAd and rAAV)

At most viral vector administration can work twice

rAAV is too small for CFTR cDNA

Repeat administration of rAAV5/5 to mouse lungs at 8-week intervals. Female BALB/c, n = 5 to 6 per group

Sumner-Jones et al., 2007, Pubmed ID 17855531
Non Viral Gene Transfer Agents

GTA + Plasmid DNA

Very few GTAs are suitable for lung airway gene transfer

1. Must be able to make enough *(volume)*

2. Must be able to repeat administer *(toxicity)*

3. Must be aerosolisable *(sheer forces)*
UK CF Gene Therapy Consortium

Develop a clinical gene therapy for CF lung disease

1. Must be aerosolisable

2. Repeat administration must be possible

3. No favourites – take an unbiased approach to selection
1. 22kDa linear PEI
2. 25kDa branched PEI
3. BGTC
4. Compacted poly-L Nanoparticles
5. Galactose-PEI
6. Genethon Peptides
7. GL67A
8. GSK Lipids
9. ICH LID
10. M760001
11. Mannose-PEI
12. ML Peptides
13. Naked pDNA
14. Nano-Dendrimers
15. SecR-LPD
16. TAT

McLachlan et al., 2011, Pubmed ID 21512505
Standardised Product Testing Pipeline

1. Mouse nose/lung basic efficacy
2. Mouse lung aerosol delivery aerosol suitability
3. Sheep lung aerosol delivery clinically relevant delivery
Positive in Mouse Lung Aerosols

1. 22kDa linear PEI
2. 25kDa branched PEI
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McLachlan et al., 2011, Pubmed ID 21512505
Positive in Mouse Lung Aerosols

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2. 25kDa branched PEI GeneMedicine Group
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McLachlan et al., 2011, Pubmed ID 21512505
Sheep Lung Testing

Vector mRNA normalised to sheep CFTR (% V/E)

GL67A was consistently higher than PEI or nanoparticles

McLachlan et al., 2011, Pubmed ID 21512505
GL67A Selected for Clinical Trial

The leading non viral GTA for aerosol administration

Two previous clinical trials – full toxicology package

McLachlan et al., 2011, Pubmed ID 21512505
GL67A Selected for Clinical Trial

Two previous clinical trials – full toxicology package

McLachlan et al., 2011, Pubmed ID 21512505
Optimisation of the Plasmid

In vivo activity
Promoter
UTRs
Transgene sequences

Production
Resistance genes
Transgene
Optimisation of the Plasmid

Modular Plasmid

Number of Clinical Trial Publications

Pringle et al., 2012. Pubmed ID 22767241
Optimisation of the Plasmid

Very few promoters have been used

By far the most common is CMV

Why would it be optimal?
Heart
Lung
Tumours
Muscle

Pringle et al., 2012. Pubmed ID 22767241
We Optimised Everything

Single most expensive component of the programme

Only going to it once

Every base has to be justified
Reducing CpGs to Reduce Inflammation

Hyde et al., 2008, Pubmed ID 18438402
Reducing CpGs to Reduce Inflammation

= 1 CpG Motif

Hyde et al., 2008, Pubmed ID 18438402
Inflammatory Response in the Mouse Lung

Cells

IFN-γ

IL-12

TNF-α

BALB/c
n=10
GL67 Instillation
24 hours

Hyde et al., 2008, Pubmed ID 18438402
Clinically Suitable Plasmids

CpG-free plasmids

Minimal backbone (1100 bp)

High yields still possible

No shortage of CpG-free promoters

EFI   hTSHB
mCEFI  hTDOX
hEFI   hCBOX
GZB    mFABP

Pringle et al., 2012. Pubmed ID 22767241
Inflammation Reduced to Background Levels

**Cells**

- Total Cells per ml BALF
- Mann Whitney P>0.05

**IFN-γ**

- PG IFN-gamma per ml BALF
- P>0.05

**IL-12**

- PG IL-12 per ml BALF

**TNF-α**

- PG TNF-alpha per ml BALF

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BALB/c
n=10
GL67 Instillation

Mann Whitney
P>0.05

Hyde et al., 2008, Pubmed ID 18438402
Do we really need zero CpGs?

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Hyde et al., 2008, Pubmed ID 18438402
Effect of Single CpG Motif in Mouse Lung

Cells

IFN-γ

IL-12

TNF-α

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Pringle et al., 2012. Pubmed ID 22767241
Most Non Viral Vectors are Poor

![Graph showing RLU per mg Total Lung Protein vs. Days Post Dosing. The graph indicates a decline in RLU over time, with a peak around 0 days post dosing. The x-axis represents days post dosing, ranging from 0 to 28 days. The y-axis represents RLU per mg Total Lung Protein, ranging from 0 to 1000 RLU. The graph includes points for Most Promoters, UbC, and hCEFI, with a significant decrease in RLU for Most Promoters.]

Hyde et al., 2008, Pubmed ID 18438402
Tested Many Different Promoters in Aerosols

![Graph showing RLU per mg Total Lung Protein against Days Post Dosing with data points for Most Promoters, UbC, and hCEFI. The graph includes error bars to indicate variability.](image)

Hyde et al., 2008, Pubmed ID 18438402
hCEFI Promoter – Persistent High Level Activity

Hyde et al., 2008, Pubmed ID 18438402
hCEFI Promoter

Enhancer sequence from human CMV

Promoter from human Elongation Factor 1alpha

Expression for > 5 months in mouse lung (unpublished 12 months)

Since 2008 we have not found anything better

Why is it so good?
Clinical Trial Plasmid – pGM169

- GL67A/pGM169
- Naive

Days Post Aerosol

% pGM169/mCFTR mRNA

No Detection

Alton et al., 2013, Pubmed ID 1973824
Non Viral Gene Therapy is Not Effective

BUT........

We made a vector that can express for a long time

It's very safe

Repeat administration
Clinical Trial Plasmid – pGM169

Alton et al., 2013, Pubmed ID 1973824
Consortium Clinical Trial Programme

Manufacture GL67A & plasmid pGM169 to cGMP (70 g)

Mouse & Sheep toxicology studies

Developed clinical nebuliser delivery method
(1) The Run In Study

Great spectrum of CF

How do you differentiate between patients?

We optimised the plasmid and the lipid, optimise the patients too.
(1) The Run In Study

Followed a group of patients (~200) for a year

Repeated clinical measurements to see how stable they were
  Clinical examination
  Sputum collection and sputum induction
  Lung function measurements
  Blood tests and urine samples
  Completion of a Quality of Life Questionnaire (CFQ-UK)
  Collection of Exhaled Breath Condensate (EBC)
  Lung Clearance Index (LCI)
  Shuttle Exercise Test
  Computed Tomographic (CT) scanning of the chest
  Mucociliary Clearance (MCC) scan (in Southampton)
  Bicycle Exercise Tests
  Completion of a symptom score card
(1) The Run In Study

Who are the correct patients?

What are the correct endpoint assays?

Define a population suitable for a clinical trial
   Stable CF
   Want to be able to see a small improvement
Technetium Deposition Scans (CT)

Able to deliver

Able to measure

Too Good
Technetium Deposition Scans (CT)

- Able to deliver
- Able to measure

Too Good
Technetium Deposition Scans (CT)

Able to deliver

Able to measure

Too Good

Too bad
Technetium Deposition Scans (CT)

Able to deliver

Able to measure

Too Good

Too bad

Ideal
Technetium Deposition Scans (CT)

50% to 90 % FEV1

Able to deliver

Able to measure

Too Good
Too bad
Ideal
(2) Single Dose Study - 2011

Safety

Establish a maximum dose

35 CF patients

20 ml, 10 ml & 5 ml GL67A/pGM167 aerosol

Also served to fine tune SOPs
  Pharmacy procedures
  Sample collection
  Lab assays
Selecting a Safe Single Dose

Alton et al., 2015, Pubmed ID 26623687
5 ml (13 mg pDNA) to Avoid Unblinding Patients

Spearman’s p=0.001
ANOVA p<0.01

Alton et al., 2015, Pubmed ID 26623687
Modest improvement in LCI

Alton et al., 2015, Pubmed ID 26623687
(3) Multi Dose Clinical Trial 2012-2014

Phase 2B, double blind placebo controlled trial

140 CF subjects (12+), 50% < FEV1 < 90%

Any combination of CF mutations

12 monthly 5 ml aerosols (13 mg pGM169)
(3) Multi Dose Clinical Trial 2012-2014

Phase 2B, double blind placebo controlled trial

140 CF subjects (12+), 50% < FEV1 < 90%

Any combination of CF mutations

12 monthly 5 ml aerosols (13 mg pGM169)

Over 2100 patient visits
5 per day per site
(3) Multi Dose Clinical Trial 2012-2014

28 day interval

**Pre visit**  Baseline assays

**Delivery 1**  5 ml Aerosol

**Delivery 1+n**  Assays in the morning
Next aerosol in the afternoon
Always 28 days between assays and delivery

**Post visit**  Final assays
Outcome Measures

Primary outcome FEV1*
Pre V Post 12 doses

Multiple secondary outcomes
Lung clearance index
CT scan
QoL questionnaire

* Vol exhaled in 1s of a forced expiration from total lung capacity
**Patient Groups Were Well Balanced**

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Active</th>
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</thead>
<tbody>
<tr>
<td>Age</td>
<td>26 (12-64)</td>
<td>23.6 (12-57)</td>
</tr>
<tr>
<td>Male/female</td>
<td>29/25</td>
<td>31/31</td>
</tr>
<tr>
<td>White/other</td>
<td>51/3</td>
<td>61/1</td>
</tr>
<tr>
<td>Edin/London</td>
<td>24/30</td>
<td>22/40</td>
</tr>
<tr>
<td>Height (SD) cm</td>
<td>164.4 (10.3)</td>
<td>164.5 (10.5)</td>
</tr>
<tr>
<td>Weight (SD) kg</td>
<td>60.7 (15.5)</td>
<td>60.6 (14.6)</td>
</tr>
<tr>
<td>BMI</td>
<td>22.2 (4.3)</td>
<td>22.2 (4.1)</td>
</tr>
<tr>
<td>FEV1%</td>
<td>69.0</td>
<td>69.9</td>
</tr>
</tbody>
</table>
Results: Safety – No Safety Concerns

Independent data safety monitoring board

No serious adverse events related to treatment

No significant changes in biochemistry, haematology, histology, exacerbations, lipid accumulation, CFTR immune response

Alton et al., 2015. Pubmed ID 26149841
Results: Primary Endpoint FEV1

Percent change in FEV1 from baseline – everyone starts at 0

Alton et al., 2015. Pubmed ID 26149841
Results: Primary Endpoint FEV1

Placebo group gradually declined over 14 months

Alton et al., 2015. Pubmed ID 26149841
Results: Primary Endpoint FEV1

Active group showed stabilised lung function relative to Placebo

Treatment Effect
3.7%
p=0.046

Alton et al., 2015. Pubmed ID 26149841
Results: Summary of Secondary Outcomes

Spirometry

CT measurements

QoL questionnaire

Safety

Alton et al., 2015. Pubmed ID 26149841
Results: Summary of Secondary Outcomes

Secondary outcomes not powered

All favour the treatment effect

Alton et al., 2015. Pubmed ID 26149841
Results: Summary of Secondary Outcomes

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All favour the treatment effect

<table>
<thead>
<tr>
<th>Primary Endpoint</th>
<th>Placebo/Active</th>
</tr>
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<tbody>
<tr>
<td>FEV₁</td>
<td>54/56</td>
</tr>
<tr>
<td>Lung Function</td>
<td></td>
</tr>
<tr>
<td>FVC</td>
<td>54/56</td>
</tr>
<tr>
<td>MEF₂₅-₇₅</td>
<td>54/56</td>
</tr>
<tr>
<td>LCI</td>
<td>51/59</td>
</tr>
<tr>
<td>CT</td>
<td></td>
</tr>
<tr>
<td>Bronchiectasis Extent</td>
<td>54/51</td>
</tr>
<tr>
<td>Bronchiectasis Severity</td>
<td>54/51</td>
</tr>
<tr>
<td>Wall Thickness</td>
<td>54/51</td>
</tr>
<tr>
<td>Large Airway Mucus Plugs</td>
<td>54/51</td>
</tr>
<tr>
<td>Small Airway Mucus Plugs</td>
<td>54/51</td>
</tr>
<tr>
<td>Gas Trapping</td>
<td>54/81</td>
</tr>
<tr>
<td>Quality of Life</td>
<td></td>
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<tr>
<td>QoL Physical</td>
<td>54/51</td>
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<tr>
<td>QoL, Respiratory</td>
<td>54/51</td>
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<tr>
<td>Safety</td>
<td></td>
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<td>CRP</td>
<td>48/55</td>
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<tr>
<td>ESR</td>
<td>54/80</td>
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<tr>
<td>WBC</td>
<td>54/51</td>
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<tr>
<td>KCOc</td>
<td>51/51</td>
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<tr>
<td>TLCOc</td>
<td>51/51</td>
</tr>
<tr>
<td>VA</td>
<td>52/51</td>
</tr>
<tr>
<td>Sputum 24 Hour Weight</td>
<td>27/22</td>
</tr>
</tbody>
</table>

Alton et al., 2015. Pubmed ID 26149841
Results: % Gas Trapping from CT Scan

All Subjects

Increased gas trapping due to airway obstruction

Treatment Effect
3.0%
p=0.031

Treatment Effect -3.49
p-value 0.048

Alton et al., 2015. Pubmed ID 26149841
Any Patterns in the Data?

Do any cohorts perform better?

Stratify the data by FEV1 (roughly 50:50)

50% - 70%  70% - 90%

Alton et al., 2015. Pubmed ID 26149841
70% - 90% Baseline FEV1

No difference between treatment and placebo

Alton et al., 2015. Pubmed ID 26149841
50% - 70% Baseline FEV1

Doubling of treatment effect

Alton et al., 2015. Pubmed ID 26149841
50% - 70% Baseline FEV1

Doubling of treatment effect

Treatment Effect 6.4%

Alton et al., 2015. Pubmed ID 26149841
Patients with Worse Lung Function Do Better?

We don’t really know why

Not simply a trick of the maths (relative changes)

More concentrated delivery to the affected airways

50%-70% 70%-90%

Alton et al., 2015. Pubmed ID 26149841
How does it compare to other products?

Vertex clinical trial of their two drugs for dF508

1108 patients

Ivacaftor (potentiator) & Lumacaftor (corrector)

Daily combinations for 2 years

Wainwright et al., 2015. Pubmed ID 25981758
Similar Differences in FEV1

Broadly similar changes to lung function

Gene therapy once a month

Still doesn’t get all mutations

Wainwright et al., 2015. Pubmed ID 25981758
Concerns?

None

- The trial met its primary endpoint
- None of the less convincing assays were powered
- Waste of money

A match for leading drugs in development as well
Molecular Data

Vector expresses for a long time
Repeat administration boosts activity

Any evidence that this is happening in patients? DNA & mRNA

![Graph showing % pGM169/mCFTR mRNA over days post aerosol and monthly aerosol doses of GL67A/pGM169.](chart.png)
Molecular Data

No mRNA positive
  Small number of samples (14 active & 7 placebo)
  Not every patient
  Single timepoint

Why no positive?
1. Bronchial brushing samples are difficult to get mRNA out of
2. Assay is not powered
3. Bronchoscope not going down far enough
Molecular Data

No mRNA positive
  - Small number of samples (14 active & 7 placebo)
  - Not every patient
  - Single timepoint

Why no positive?
1. Bronchial brushing samples are difficult to get mRNA out of
2. Assay is not powered
3. Bronchoscope not going down far enough
4. Vector does not persist in human bronchial epithelium
The FEV1 data was positive
The FEV1 data was positive
Summary

Well-tolerated/no safety issues after repeat dosing

Statistically significant change in primary outcome  FDA recognised

Secondary outcomes also supportive  Not powered

First evidence of clinical benefit for CF gene therapy

Could stabilise lung function in CF patients
Treatment Effect is Modest

Treatment Effect is modest & heterogeneous

Compares favourably with other drugs in development
What next?

Can we improve on this?

Increase the dose to 10 ml

Increase the dosing frequency to 2 weeks

Plans under way for a further (larger) clinical trial
Thanks

All the patients and their families

Oxford
Steve Hyde & Deborah Gill

London
Eric Alton, Jane Davies & Uta Griesenbach

Edinburgh
David Porteous, Alastair Innes & Chris Boyd

Roslin Institute
Gerry McLachlan & David Collie

NHS
Medical Research Council
National Institute for Health Research

Cystic Fibrosis investing in research to change lives