The Trials And Tribulations Of The UK Cystic Fibrosis Gene Therapy Consortium

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Things I Think You Know…

• **CF, CFTR & ENaC**
  - CF Is Caused By Mutations In The CFTR Gene (>1600)
  - CFTR Is Expressed At Low Level In The Conducting Airway
  - CFTR Is An Epithelial Chloride Channel
  - CFTR Downregulates ENaC The Epithelial Sodium Channel

• **CF, CFTR & Lung Pathophysiology**
  - In CF There Is Reduced Epithelial Chloride Secretion
  - In CF There Is Increased Epithelial Sodium Absorption
  - In CF These Altered Ion Fluxes Result In Reduced ASL Height
The height of fluid on the surface of the Airways is crucial to Lung Clearance.
Normal Airway Epithelia Maintains ASL Height By A Combination Of Sodium Absorption & Chloride Secretion

Normal ASL Height

ASL Height Is Actively Maintained

T=0 h

T=6 h

T=48 h

Normal ASL Height Is Required For Cilia Function
CF Conducting Airways Fail To Maintain ASL Height

CF
ASL Height

CF ASL Height Is Excessively Reduced

T=0 h

T=6 h

T=48 h

Na\(^+\) absorption

Reduction In ASL Height Leads To Cilia Dysfunction
Mucus Transport Is Defective In CF

Non-CF

CF

Images Kindly Provided By Ric Boucher, UNC
Cystic Fibrosis Gene Therapy?

- Gene Repair
  Repair Only The Defective Genetic Material - $\Delta F508$ 3bp
Cystic Fibrosis Gene Therapy?

• Gene Repair
  Repair Only The Defective Genetic Material - ΔF508 3bp

• Gene Inhibition
  Use siRNA / Antisense RNA To Downregulate ENaC / Mucins…
Cystic Fibrosis Gene Therapy?

- **Gene Repair**
  Repair Only The Defective Genetic Material

- **Gene Inhibition**
  Use siRNA / Antisense RNA To Downregulate ENaC / Mucins…

- **Gene Replacement**
  Add Back An Additional Copy Of The CFTR Gene
Only A Small Proportion Of Cells Need To Express CFTR To Correct The Chloride Defect

Johnson et al. 1992
Nature Genetics 2:21
Gene Replacement Therapy: How Do We Get The CFTR cDNA Into The Cells Of The Lung?

- Modified Virus Vectors
  - CFTR cDNA Inserted Into Viral DNA
  - Compacting Viral Proteins And Lipids
  - rAd, rAAV, rPIV, rLentiVirus...
Gene Replacement Therapy: How Do We Get The CFTR cDNA Into The Cells Of The Lung?

- Modified Virus Vectors
  CFTR cDNA Inserted Into Viral DNA
  Compacting Viral Proteins And Lipids
  rAd, rAAV, rPIV, rLentiVirus…

- Non-Viral (Synthetic) Vectors
  Synthetic DNA Circle Carrying CFTR cDNA
  Compacting Liposomes Or Polymer
Non-Viral Gene Transfer Leads To The Correction Of The CF Defect In CF Mice

Hyde et al. 1993
Nature 362: 250
CF Gene Therapy: Clinical Experience

- Multiple Clinical Trials
  - Adenoviral Vectors
  - Adeno-Associated Virus
  - pDNA / Cationic Liposomes
CF Gene Therapy: Clinical Experience

• Multiple Clinical Trials
  Adenoviral Vectors
  Adeno-Associated Virus
  pDNA / Cationic Liposomes

• Broadly Similar Results
  Modest Gene Transfer
  Transient Correction Of Some CF Related Defects
CF Gene Therapy: Clinical Experience

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Caplen et al 1995 Nat Med 1: 39
Porteous et al 1997 Gene Therapy 4:210
Gill et al 1997 Gene Therapy 4:199
Hyde et al 2000 Gene Therapy 7:1156
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Non-Viral Lung Trial

Transient Correction (~25%)    Mild Flu-Like Symptoms

Δ PD (mV)

Pre           Post                 Non-CF

p < 0.05

 transient correction (~25%) Mild Flu-like Symptoms

Alton et al 1999 Lancet 353:947
UK Cystic Fibrosis Gene Therapy Consortium

Combined Research Programme Of UK Groups Who Have Previously Performed CF Gene Therapy Clinical Studies

• Edinburgh
  Chris Boyd Alastair Innes
  David Porteous

• London
  Eric Alton Jane Davies
  Uta Griesenbach

• Oxford
  Deborah Gill Stephen Hyde

www.cfgenetherapy.org.uk
Combined Research Programme Of UK Groups Who Have Previously Performed CF Gene Therapy Clinical Studies

- **Wave 1**
  - Identify Best Current Available GTA
  - Extend Duration Of Expression
  - Reduce Flu-Like Symptoms
  - Develop Novel Clinical Assays
  - Evaluate Clinical Efficacy

- **Wave 2**
  - Identify Best Possible GTA
  - Evaluate Clinical Efficacy

- **Move Towards Phase III Studies**

www.cfgenetherapy.org.uk
UK CF Gene Therapy Consortium
Clinical Trials Programme

• Tracking Study
  Evaluate Performance Of Novel Biomarkers During Exacerbation

• Wave 1 Single Dose Trial
  Establish Safety & Gene Expression Profile Of Best Currently Available GTA
  Low Numbers (27) Short Duration (~1 Month)

• Run-In Study
  Longitudinal Assessment Of Biomarker Assays
  Extensive Pre-Treatment Baseline For Wave 1 Multiple Dose Trial

• Wave 1 Multiple Dose Trial
  Determine Clinical Benefit Of Best Currently Available GTA
  Large Numbers (~100) Long Duration (~12 Months)
UK CF Gene Therapy Consortium: Biomarkers Statistical Power & The Tracking Study

- Traditional Trial Designs Not Helpful For Evaluation Of GT Safety (Phase I) Irrelevant Without Efficacy (Phase III)

- **Proof-Of-Principle** / Therapeutic Trial Design
  Therapeutic Trials Require New Primary Endpoints
  \( \Delta 5\% \text{ FEV}_1 > 80\% \) Power: \( n = 130 / 4\text{yr} \) \( n = 172 / 2\text{yr} \) \( n = 1100 / 1\text{yr} \)
  Therapeutic Trials Require Long-Term Expression/Dosing

- Tracking Study
  Model The Effects Of Successful Gene Transfer
  Evaluate Biomarker Assays During IV Antibiotics
UK CF Gene Therapy Consortium Tracking Study

46 patients recruited

5 excluded

41 subjects in study

Exclusions:

3 = on oral steroids
2 = excessive interval between end treatment and follow-up

Subjects Evaluated At Start & End Of IV Antibiotics & At Follow-up >12 Years Brompton & Edinburgh Clinics
UK CF Gene Therapy Consortium
Tracking Study

Nasal Potential Difference
Sputum
  Inflammatory Markers: Luminex Multiplex Cytokines, ELISA, Metals, Cells, NE
  Properties: Viscoelasticity, W:D wt, DNA
24 Hour Volume & Microbiology
Blood / Serum
  WBC, CRP, inflammatory cytokines, UNC Markers
Urine
  N-Term Lobe Transferrin
EBC
  pH, Ammonia, Nitrites, Glucose
CT
Lung Clearance Index (LCI) & Lung Function (PIKo & Spirometry)
Symptom Score Sheet

Subjects Evaluated At Start & End Of IV Antibiotics & At Follow-up
>12 Years  Brompton & Edinburgh Clinics
FEV₁
Validated UK English CFQ - Quittner et al 2005 Chest 128:2347
CRP

P = 0.0002

P = 0.15

Visits

mg/L
Pseudomonas aeruginosa

Log$_{10}$ CFU P. aeruml

p<0.01
n=15

p=0.7
n=11
<table>
<thead>
<tr>
<th>Sputum Markers</th>
<th></th>
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<tbody>
<tr>
<td><strong>Unchanged</strong></td>
<td><strong>Changed</strong> *</td>
</tr>
<tr>
<td>IL6, IL8, IL10,</td>
<td>IL1b   (p&lt;0.05)</td>
</tr>
<tr>
<td>TNFa, RANTES</td>
<td>24 hrs weight (p=0.06)</td>
</tr>
<tr>
<td>Solid content</td>
<td>Total DNA (p=0.06)</td>
</tr>
<tr>
<td>Viscosity/elasticity</td>
<td>Calprotectin (p=0.07)</td>
</tr>
<tr>
<td>Extracellular DNA</td>
<td></td>
</tr>
</tbody>
</table>

*Changed P<0.10...
Serum Markers

Serum IL-6

Visit 1  Visit 2  Visit 3

p < 0.0001  p = 0.14

p = 0.04

No change in serum: IL1β, TNFα, RANTES, IL8
Lung Clearance Index

Inert Gas Washout. Thought Better Than FEV$_1$ For Measuring Small Airway Disease

Historically Resident N$_2$ Washed Out By Breathing 100% O$_2$

Newer Methods Based In Breathing In 0.2% SF$_6$ To Equilibrium The Measuring Wash-Out With Medical Air

Quantitation Of SF$_6$ Faster & More Reliable Than N$_2$
Lung Clearance Index

Gas signal

Flow signal

Innocor

SF₆
Lung Clearance Index
Lung Clearance Index

Horsley et al 2008 Thorax 63:135
Lung Clearance Index

![Graph showing Lung Clearance Index pre and post with a significant difference indicated by p=0.0012.]

**Lung Clearance Index**

- **Pre**
- **Post**

*p=0.0012*
CT Scans: Wall Thickness

Pre-treatment

Post-treatment
CT Scans: Large Mucus Plugs

Pre-treatment

Post-treatment
CT Scans: Small Mucus Plugs

Pre-treatment

Post-treatment
LCI Correlates With HRCT Gas Trapping

More abnormal gas mixing (LCI)

Pre-Treatment
$r=0.61$, $p=0.0012$

Post-Treatment
$r=0.70$, $p<0.0001$

More gas trapping on HRCT
UK CF Gene Therapy Consortium Tracking Study

- Multiple Biomarkers Report ‘Improved’ Status In Response To IV Antibiotics During Exacerbation
- Novel Biomarker Changes Typically More Sensitive Than $\Delta FEV_1$
- Biomarker Changes Generally Not Correlated With Patients Perception Of Changes In Symptoms
- HRCT & LCI Correlation Supports Use Of Composite Scoring
  May Need To talk To More Mathematicians…
UK CF Gene Therapy Consortium
Clinical Trials Programme

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UK CF Gene Therapy Consortium
Wave 1 Pre-Clinical Research Programme

- Find The “Best Buy” Currently Available Gene Therapy Vector For The Lung
UK CF Gene Therapy Consortium
Wave 1 Pre-Clinical Research Programme

• Find The “Best Buy” Currently Available Gene Therapy Vector For The Lung

• The Initial Screen (Wave 1)
  Test Commercial & Academic Vectors For Improved Activity
  Viral & Non-Viral Vectors Tested In Extensive Mouse & Sheep Studies
Human Air Liquid Interface Cultures

• In Vitro Model Of Human Airway Epithelium
  Ciliated, Mucus & Basal Cells
  Polarised Epithelium
  Beating Cilia Transporting Mucus Rafts

• Useful Model For GTA Evaluation

• Generated From Non-CF Nasal Polyps By Epithelix Sarl

• Supply Agreement For CF Cultures

• Struggling To Obtain CF Nasal Polyps…
First Generation pDNAs Have A High CpG Content

pDNA Similar To That Used In Multiple Phase I Trials In Mid 1990’s
First Generation pDNAs Have A High CpG Content

- CpG - CG Dinucleotides
  Rare & Methylated In Mammals
  Common & Unmethylated In Bugs

pDNA Similar To That Used
In Multiple Phase I Trials In Mid 1990’s

pDNA CGs Recognised By TLR9
TLR9 Activates Inflammatory Cascade
Fourth Generation pDNAs Have Zero CpGs

First Generation
317 CpG

Fourth Generation
Zero CpG

pDNA Similar To That Used In Multiple Phase I Trials In Mid 1990’s

Minimal Zero CpG Backbone Smallest CFTR pDNA’s Of All Time 6.2kb
Fourth Generation pDNAs Abolish Flu-Like Symptoms And Lung Inflammation

Hyde et al., 2008 Nature Biotechnology 26:549

n=10, BALB/c, Lung Instillation, GL67/pDNA, pDNA <5EU/mg
Fourth Generation pDNAs
Direct Sustained Lung Expression
UK CF Gene Therapy Consortium
Single Dose Non-Viral Gene Therapy Trial

- Extensive Pre-Clinical Programme Identifies Potent Non-Viral Formulation:
  - Zero CpG
  - CFTR Plasmid
  - GL67A
  - Liposomes
  - AeroEclipse II
  - Nebuliser

- Single Dose Clinical Trial Started Q1 09
  - N=27 CF Subjects. Nose & Lung Aerosol Administration
  - Pre & Post Bronchoscopic Sampling
  - Safety & Duration Of Expression Endpoints
Non-CF

mV

Time (mins)

amiloride
isoprenaline
Low-chloride

Na^+
Cl^-

0 5 10 15 20
Pre-dose vs post-dose delta ZC

- No change: n=6
- Uncertain: n=2
- Change: n=3*, n=2***
Individual data: 011
Individual data: 011
Individual data: 011
Individual data: 011
Individual data: 011
Individual data: 022
Individual data: 022
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  Extensive Pre-Treatment Baseline For Multiple Dose Trial

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UK CF Gene Therapy Consortium
Clinical Trials Programme

• Run-In Study
  Jan 2008 - December 2010
  200 CF Patients  Age 10+
  ~2 Year  Longitudinal Clinical Measurements
  7 “Super Annual Visits” With Multiple Novel Clinical Assays
  2 Day Stay In Southampton - Lung MCC

Identify Optimal Patients And Assays
For Long Duration Gene Therapy Trial

Can Deliver

Can Measure
UK CF Gene Therapy Consortium
Clinical Trials Programme

• Multiple Dose Gene Therapy Trial     Jan 2011 - July 2012
  Select 100 CF Patients From The Run-In Study (Age 12+)
  50 Gene Therapy Treatment, 50 Placebo
  12 Months Of Gene Therapy Treatment (x1/month)
  Best Clinical Assays (Tracking/Run-In) Each Monthly Visit

Actually Trying To Find Out If Gene Therapy Makes You Better
Currently

1st Treatment

12th Treatment

Run-In

Multiple Dose Trial
Most Get Better Or Stop Getting Worse

• How Will We Get The Drug To The Patients?
  Pharmaceutical Partner
  Large Multi-Centre Clinical Trial
  All UK CF Centres Small & Large Invited To Take Part
  Europe & USA

• What Will Be The Scientific Challenges?
  Need To Manufacture Greater Quantities
  Need To Jump Higher Regulatory Hurdles (More Paperwork…)
Small Or Variable Benefit

• How Much Benefit Is Worthwhile?
  Low Dose Asprin (50mg) - Prevention
  High Dose Asprin (600mg) - Cure

• What Does This Mean For Gene Therapy?
  Low Benefit May = Prevention
  May Need A Higher Benefit (Higher Dose Or Wave 2) = Cure
No Benefit

- Have Done As Much As We Can With Wave 1
  - Learned The Principles
  - Know Where We Stand

- Wave 2 Drug Development
  - Standard Pharmaceutical Company Approach
  - Have Been Doing A Limited Amount Of Research To Facilitate
UK CF Gene Therapy Consortium
Wave 2 Pre-Clinical Research Programme

- Continuing Pre-Clinical Development
  ‘Wave 1’ (Best Currently Available) Product In Clinical Trials
  Identify Novel ‘Wave 2’ Product With Improved Efficacy/Duration
  Viral & Non-Vial Products Under Evaluation.  F/HN Lentivirus Example:

F/HN SIV Mouse Nose Studies In Collaboration With:

Gene expression

F/HN SIV
Cystic Fibrosis Gene Therapy
Where Are We?

• What Has The Consortium Been Doing?
  World’s Biggest Trial For Getting Better

• Why Does It Take So Long?
  Major Problem With Being Cutting Edge

• Spin-Offs
  Improved Understanding Of Lung Pathophysiology
  Increased Understanding Of Biomarkers

• When Will It Be Available?