Non-viral CFTR Gene Delivery To The Lungs Of Cystic Fibrosis Patients

Stephen Hyde
UK Cystic Fibrosis Gene Therapy Consortium
Radcliffe Department of Medicine
University of Oxford
Oxford, UK
Cystic Fibrosis - CF

- Most Common Serious Genetic Disease In UK
  In The UK: ~1:20 Carriers
  Worldwide: ~70,000 Affected

- Disease Of The Epithelia
  Many Organs Affected
  Lungs Accumulate Sticky Mucus
  Sticky Mucus Encourages Bacterial Infections
  Chronic Bacterial Infections Lead To Lung Destruction

- Cystic Fibrosis Transmembrane Conductance Regulator (CFTR)
  CFTR Controls The Movement Of Salt (& Hence Water) Across Epithelia
  CFTR Is An Epithelial Chloride Channel
  CFTR Regulates ENaC The Epithelial Sodium Channel
The Height Of Fluid On The Surface Of The Airways Is Crucial To Lung Clearance
Only a small proportion of cells need to express CFTR to correct the chloride defect.
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 In 90s
  Adenoviral Vectors
  Adeno-Associated Virus
  pDNA / Cationic Liposomes
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- Broadly Similar Results
  Evidence For Modest Gene Transfer
  Transient Correction Of CF Ion Channel Defect
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Caplen 1995 Nat Med 1: 39
Porteous 1997 Gene Therapy 4: 210
Gill 1997 Gene Therapy 4:199
Porteous 1997 Gene Therapy 4: 210
Hyde 2000 Gene Therapy 7:1156
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  Evidence For Modest Gene Transfer
  Transient Correction Of CF Ion Channel Defect ~ 1 Week
  Mild To Moderate Flu-Like Symptoms Following Lung Delivery

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

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

• Edinburgh
  Chris Boyd
  David Porteous
  Alastair Innes

• London
  Eric Alton
  Jane Davies
  Uta Griesenbach

• Oxford
  Deborah Gill
  Stephen Hyde

www.cfgenetherapy.org.uk
UK Cystic Fibrosis Gene Therapy Consortium

Combined Research Programme Of UK Groups Who Had 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

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• Translational Programme
  - From Phase I/IIa Biomarker Studies
  - To Phase IIb/III Clinical Benefit Studies

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Wave 1 Pre-Clinical Research Programme: GL67A

• Target GTA Product Profile
  ✔ Manufacturable To cGMP
  ✔ Potent After Aerosol Delivery
  ✔ Easily Prepared Under Pharmacy Conditions

• Target Efficacy
  ✔ Transgene mRNA: 5% of Endogenous CFTR mRNA Levels
  ✔ Transfection Efficiency: 5% of Conducting Airway Cells
  ✔ Duration Of Gene Expression: >2 Weeks

• Target Safety
  ✔ Effective After Repeated Administration
  ✔ Reduction / Elimination Of Flu-Like Symptoms
UK CF Gene Therapy Consortium
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Easily Prepared Under Pharmacy Conditions: Scalable Near-To-Bedside Mixing

- pDNA & Non-Viral GTA Often Have To Be Freshly Prepared

- Tissue Culture / *In Vivo* IV Rodent Studies
  - Dilute pDNA & GTA (µg…)
  - Prepared By Mixing Small Volumes (µl…)

- *In Vivo* Aerosol Studies
  - Highly Concentrated pDNA & GTA
  - Inefficient Aerosol Delivery Methods Require Large Volumes

- Standard Pre-Clinical pDNA/GL67A Aerosol Dose 10mL
  - 5mL 8mM pDNA (26.5mg pDNA)
  - 5mL 6mM GL67 (143.1mg Combined Lipid)
Easily Prepared Under Pharmacy Conditions: Inconsistent Mixing At Large (10mL) Scale
Easily Prepared Under Pharmacy Conditions: Static Mixer Devices
Easily Prepared Under Pharmacy Conditions: LMD-2 & Static Mixer
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 Manufactured In Bugs...

pDNA CGs Recognised By TLR9
TLR9 Activates Inflammatory Cascade
Leading To Flu Like Symptoms

pDNA Similar To That Used
In Multiple Phase I Trials In Mid 1990’s
Fourth Generation pDNAs Have Zero CpGs

First Generation
317 CpG

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

Fourth Generation
Zero CpG

Minimal Zero CpG Backbone Licence For Production From Invivogen
Fourth Generation pDNAs Reduce Flu-Like Symptoms And Lung Inflammation

**TNFα**

- 317 CpG pDNA
- 193 CpG pDNA
- 0 CpG pDNA
- Naive

**IFNγ**

- 317 CpG pDNA
- 193 CpG pDNA
- 0 CpG pDNA
- Naive

**IL-12**

- 317 CpG pDNA
- 193 CpG pDNA
- 0 CpG pDNA
- Naive

**BALF Neutrophils**

- 317 CpG pDNA
- 193 CpG pDNA
- 0 CpG pDNA
- Naive

**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

Lung Luciferase Activity (RLU/mg protein)

Days Post Aerosol Administration

- pG4-hCEFI
- pG2-UbC
- pG1-CMV
UK CF Gene Therapy Consortium
Wave 1 Phase I/IIa Single Dose Clinical Trial

- Extensive Pre-Clinical Programme Identifies Potent Non-Viral Formulation:

  - Zero CpG CFTR Plasmid
  - GL67A Liposomes
  - AeroEclipse II Nebuliser

- Single Dose Open-Label Phase I/IIa Clinical Trial **Completed**
  - n=35 CF Subjects. Nose & Lung Aerosol Administration
  - Pre & Post Bronchoscopic Sampling n=8
  - Safety & Duration Of Expression Endpoints
CF: Pre-Treatment (mean of 3)
Non-CF
CF Post-Treatment: 2 Weeks
CF Post-Treatment: 4 Weeks
CF Post-Treatment: 6 Weeks
CF Post-Treatment: 9 Weeks
Lung Efficacy – Bronchial Potential Difference
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7/8 Change Towards Non-CF
Median Change 1.5mV
p=0.023 Wilcoxon Signed Rank
Lung Efficacy – Lung Clearance Index
Lung Efficacy – Lung Clearance Index

11/14 Change Towards Non-CF
Median Change -0.27
p=0.029 Wilcoxon Signed Rank
UK CF Gene Therapy Consortium
Wave 1 Phase I/IIa Single Dose Clinical Trial

• Safe Effective Clinical Dose Identified
  5mL Aerosol Formulation ~13mg pGM169 ~75mg GL67A

• Biomarkers
  ~33% Subjects No Change In nPD
  ~33% Subjects Modest Correction nPD
  ~33% Subjects Long-Term Correction nPD
  Significant Correction Lung PD

• Lung Function
  Significant Change In Lung Clearance Index After Single Dose
Repeated Aerosol Dose Safety Study

• 12 Aerosol Doses At 2 Week Interval - 2xCROs @ GLP
  3 Dose Groups ~x5, x20 & x60 Fold Over Human Dose
  Air Only - Negative Control

• Pharmacokinetics & Biodistribution
  Clinical & Histopathology
  DNA & mRNA In Lung & Non-Target Organs

• Clinical & Histopathology Results
  No Necropsy Findings
  No Clinical Concerns At Any Dose
  No Treatment Related Effects On Bloods & Chemistry
Biodistribution & Transgene Expression: Transient Low Levels DNA Observed In Non-Target Organs

Liver
Biodistribution & Transgene Expression:
No mRNA Observed In Non-Target Organs
Biodistribution & Transgene Expression: Persistent High Levels DNA Observed In Lung
Biodistribution & Transgene Expression: Increasing mRNA Observed In Lung Upon Repeated Administration
UK CF Gene Therapy Consortium
Wave 1 Phase IIb Multiple Dose Clinical Trial

- Patient Selection
  Longitudinal Biomarker “Run In” Study
  200 CF Patients  3 Years
  Selection Of Appropriate Endpoint – FEV1
  Selection Of Appropriate Patient Demographic
UK CF Gene Therapy Consortium
Wave 1 Phase IIb Multiple Dose Clinical Trial

- **Multiple Dose Lung Gene Therapy Trial** Q2 2012
  130 CF Patients (Age 12+)
  Double Blinded 1:1 Active:Placebo
  12 Months Of 5mL Aerosol Lung Treatment (x1/Month)
  With n=120 Power ≥0.9 For ≥6% Change In FEV1 (n=108 Power ≥0.8)

- **Nasal Subgroup (n=24 2:1 Active:Placebo)**
  Monthly 2mL Nasal Dose
  Nasal PD x3 Pre-Dose 1, After Dose 4/7/10 & x2 Post-Dose 12

- **Bronchial PD Subgroup (n=24 2:1 Active:Placebo)**
  Bronchial PD, Brushings/Biopsies Pre-Dose 1 & Post-Dose 12
UK CF Gene Therapy Consortium
Wave 1 Phase IIb Multiple Dose Clinical Trial

- Safety
  2xDMEC No Safety Concerns
  3xSAEs All Unrelated To Study Drug