Gene Therapy for Cystic Fibrosis Lung Disease

MTERMS 2012
To develop a clinical gene therapy for CF lung disease
CFTR Gene Replacement Therapy

• Clinical programme
• Development & selection of GL67A/pGM169 non-viral formulation
• Early development of a new viral product
CFTR Gene Replacement Therapy

- Non-viral vectors (synthetic)
- Plasmid DNA compacted with proteins, polymers & lipids
- Versatile
- Minimal immune response

- Viral vectors
- Evolved to be highly efficient
The Problem with Viral Vectors…

- Unable to repeatedly administer to the airways without loss of efficacy
UK CF GT Consortium Strategy

- Use ‘best’ Non-Viral Vector

- Keep searching for Viral Vector candidate
Best Non-Viral Vector

- Screened 20-30 non-viral formulations
- Selected plasmid DNA/cationic lipid GL67A
- Most efficient for lung aerosol delivery

- Patients show flu-like symptoms
- Transient gene expression

- Improve plasmid DNA:
  - Circular, double stranded DNA
  - Carries gene of interest
  - Manufactured in bacteria
Animal Models for Lung Gene Delivery

Aerosol delivery to mouse lung
Animal Models for Lung Gene Delivery

Aerosol delivery to sheep lung
Response to GL67/Plasmid Complexes in Lung

Transient expression
- Change promoter

Pro-inflammatory Cytokines
& Neutrophil influx
- CpG Response?

BALB/c
n=6
Instillation
GL67 vs
Naked DNA
Host Response to CpG Motifs in Plasmid DNA

Endosomal Compartment

- CpG Plasmids
- TLR9
- Neutrophil Recruitment

NFκB

- IκB
- PI

TNF-α, IFN-γ, IL12, etc

Plasmids
• CG dinucleotides (CpGs)
• Negative impact on transgene expression

• CpGs common in bacterial DNA - rare in mammalian DNA
• CpGs unmethylated in bacteria - tend to be methylated in mammalian DNA

• Plasmid DNA recognised by host cells via TLR9
• Activates innate immune system

• Exploited - In vaccination protocols (pDNA/Virus Prime/Boost)
• Avoid in gene therapy?
Strategy to Re-design Plasmid DNA

- Reduce CpG content of plasmid
- Improve duration of gene expression
- Appropriate for clinical use in patients
Evolution of Non-Viral Expression Plasmids: 
*First Generation* Have Many CpGs

pDNA Backbone similar to Phase I Trials in 1990’s
Evolution of Non-Viral Expression Plasmids: Second Generation Have Fewer CpGs

First Generation
317 CpG

Second Generation
193 CpG

pDNA Backbone similar to Phase I Trials in 1990’s

CpG Free Antibiotic Resistance Region
Minimal Col E1 Origin
Second Generation Plasmids: Screen Promoters for High Sustained Expression
Second Generation Plasmids Do Not Reduce GL67A Mediated Lung Inflammation

Mann Whitney
$P > 0.05$

n=10 BALB/c
Lung Instillation
GL67A/pDNA
pDNA <5EU/mg
Evolution of Non-Viral Expression Plasmids: Third & Fourth Generation Have No CpGs

Third Generation  
Zero CpG

Fourth Generation  
Zero CpG

Based on InvivoGen CpG-Free Backbone  
pCpGLacZ

UK CF Gene Therapy Consortium CpG-Free Backbone  
FDA Compliant  
Hyde 2008 *Nature Biotechnology* 26:549
Third & Fourth Generation Plasmids Abolish Lung Inflammation

**Cells**
- First Generation 317 CpG
- Second Generation 193 CpG
- Third Generation 0 CpG
- Naive

**IFN-γ**
- 317 CpG pDNA
- 193 CpG pDNA
- 0 CpG pDNA
- Naive

**IL-12**
- 317 CpG pDNA
- 193 CpG pDNA
- 0 CpG pDNA
- Naive

**TNF-α**
- 317 CpG pDNA
- 193 CpG pDNA
- 0 CpG pDNA
- Naive

n=10, BALB/c, Lung Instillation, GL67A/pDNA, pDNA <5EU/mg
Fourth Generation Plasmids: Screen CpG-Free Promoters for High Sustained Expression

hCEFI
Few CpG-Free Promoters Available

- Promoters used in clinical trials

<table>
<thead>
<tr>
<th>Transgene Promoter</th>
<th>Gene Therapy Clinical Trials (1991-2011)</th>
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<tr>
<td>CMV</td>
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<td>RV LTR</td>
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<td>PGK</td>
<td>5</td>
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<tr>
<td>Others</td>
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</tr>
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<td>Unclear</td>
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Limited selection tested in clinic

Mainly viral promoters

No CpG-free promoters

Build database to search for naturally occurring CpG-free promoters
New CpG-Free Promoters Identified

CpG-Free Promoter Activity
Day 1 Post-Dosing

CpG-Free Promoter Activity
Day 14 Post-Dosing
Effect of Bacterial Genomic DNA - Lung Inflammation

- Plasmid DNA preparations are contaminated with bacterial genomic DNA

- Significant correlation between level of bacterial genomic DNA & cytokines
Effect of Bacterial Genomic DNA - Vector mRNA

- Trend for higher vector mRNA with LOW levels of bacterial chromosomonal DNA

![Graph showing the relationship between number of CpGs delivered and percentage of vector transgene/endoogenous CFTR mRNA.]

- Manufacturing needs to further reduce bacterial chromosomal DNA
Fourth Generation Plasmid Expressing CpG-Free CFTR: Mouse Lung - Day 56
Fourth Generation Plasmid Expressing CpG-Free CFTR: Sheep Lung - Day 1

Naïve (-ve control)

G449 anti-CFTR antibody shows human CFTR

Arrows indicate apical staining in positive cells
Fourth Generation Plasmid Expressing CpG-Free CFTR: Sheep Lung - Day 56

**Naïve (-ve control)**

G449 anti-CFTR antibody shows human CFTR

Arrows indicate apical staining in positive cells
Fourth Generation Plasmid Expressing CpG-Free CFTR: CF Patients

- Plasmid pGM169:
  - CpG-Free Fourth Generation
  - Synthetic CpG-free hCEFl promoter
  - CpG-Free, codon optimised CFTR cDNA

- Complexed with GL67A
- Aerosol delivery

- Phase I Single Dose clinical study - completed 2011
- Phase 2b Multiple Dose clinical study - initiated April 2012
What We Have Learned About Non-Viral Vectors

• Plasmid design can boost gene expression
• Promoter screening is important
• CpG-free plasmids can reduce inflammation
• Minimise CpGs in plasmid preparation
Viral Vectors for Cystic Fibrosis Gene Therapy

- We would like:
- Highly efficient transduction of airways
- Persistent gene expression
- Ability to repeatedly administer without loss of efficacy

  - Adenovirus
  - Adeno-Associated Virus
  - Sendai Virus
Sendai Virus Expressing LacZ in Airways

Cytoplasmic RNA virus

Affinity for airway epithelial cells

Efficient

Transient

No repeat administration
Lentivirus: Pseudotyped with Sendai Virus Coat Proteins

- Collaboration with DNAVEC in Japan
- Combine:
  - Efficient airway transduction of Sendai Virus
  - Long-term expression & low immunogenicity of Lentivirus

Lentivirus

Pseudotyped Lentivirus
F & HN coat proteins
SIV-F/HN
Co-Transfection of 5 Plasmids - SIV F/HN

- **Plasmid SIV1**
  - Vector Genome

- **Plasmid SIV2a & 2b**
  - Packaging
  - Gag/Pol & Rev

- **Plasmid SIV3a & 3b**
  - Coat Proteins
  - F & HN

- **pGTV-EGFP (+cPPT, +WPRE)**

- **pPV-3rd**

- **pCAGGS-Fct4**

- **pRev**

- **pCAGGS -Envct-HN**
SIV-F/HN in Mouse Airway Cells

~5% cells transduced
~69% of which are ciliated epithelial cells

Ciliated AEC

SIV-F/HN-EGFP
4x10^8 TU / mouse @ d30
SIV-F/HN Directs Sustained Airway Gene Expression

SIV-F/HN-EGFP
4x10^8 TU / mouse
N= 10 mice
SIV-F/HN Can Be Repeatedly Administered in Mouse Nasal Epithelium

SIV-F/HN-EGFP
or
SIV-F/HN-Lux
$1 \times 10^7$ TU /mouse @ d28
SIV-F/HN Can Be Repeatedly Administered in Mouse Nasal Epithelium

SIV-F/HN-EGFP or
SIV-F/HN-Lux
1x10^7 TU /mouse @ d28
Human Air Liquid Interface Cultures

- Model for human lung epithelium
  - Human cells
  - Grown at Air Liquid interface
  - Differentiated
  - Cilia beating
  - Tight junctions
  - Active ion transport
Human Air Liquid Interface Cultures

Maintain cultures for several months
Lentiviral Transduction of Human ALI Cultures

Stable Luciferase expression in human airway cells

IVIS100
N=6/group
Single donor

100µl virus
1.7x10^8 TU/ml IC
Lentiviral Transduction of Human ALI Cultures

- Assess:
  - Transduction efficiency
  - Cell type specificity
  - Duration of expression

- Investigate:
  - Integration site profile
  - Risk of genotoxicity in airway cells
  - Integrase-deficient vectors
Use SIV-F/HN as Lung Delivery Platform

- To deliver any gene to airway cells
- To knockdown lung genes via RNAi
- Combine both approaches in one vector to treat CF…
CFTR & ENAC Balance Airway Surface Liquid
Combine CFTR Delivery with ENaC Knockdown

- Loss of CFTR leads to increased ENaC activity.

**Normal**

- $\text{Cl}^-$ $\text{Cl}^-$
- $\text{Na}^+$ $\text{Na}^+$

**CF**

- $\text{Cl}^-$ $\text{Cl}^-$
- $\text{Na}^+$ $\text{Na}^+$
Combine CFTR Delivery with ENaC Knockdown

- Loss of CFTR leads to increased ENaC activity
- Simultaneous CFTR expression
Combine CFTR Delivery with ENaC Knockdown

- Loss of CFTR leads to increased ENaC activity
- Simultaneous CFTR expression & ENaC knockdown may be synergistic
To identify a potent and safe F/HN-pseudotyped Lentivirus that directs robust CFTR expression suitable for early phase clinical trials

- We need:
  - pharmacopoeial compliant vectors
  - investigate risk of genotoxicity
  - assess alternative promoters
  - develop non-integrating vectors
  - generate CFTR vectors
  - evaluate performance & safety
Acknowledgements

- Gene Medicine Research Group
- UK Cystic Fibrosis Gene Therapy Consortium
- Cystic Fibrosis Trust

www.GeneMedResearch.ox.ac.uk
Lentiviral Transduction of Human ALI Cultures: Integrase Competent & Deficient

Significant decline in expression over time P=0.0011 (Pearson Correlation)

IVIS100
N=6/ group
Single donor
100µl virus
1.7x10^8 TU/ml IC
0.2x10^8 TU/ml ID