Translating Research Ideas Into Clinical Trials

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Regulatory
Translating Pre-Clinical Ideas Into Clinical Trials: Why Does It Take So Long?
Guidance Overload: GMP Manufacturing


Guidance Overload: ICH Guidance For Just About Everything

- International Conference on Harmonisation of technical requirements for registration of pharmaceuticals for human use

- Worldwide Consensus

<table>
<thead>
<tr>
<th>Q</th>
<th>S</th>
<th>E</th>
<th>M</th>
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<tbody>
<tr>
<td>&quot;Quality&quot; Topics, i.e., those relating to chemical and pharmaceutical Quality Assurance (Stability Testing, Impurity Testing, etc.)</td>
<td>&quot;Safety&quot; Topics, i.e., those relating to in vitro and in vivo pre-clinical studies (Carcinogenicity Testing, Genotoxicity Testing, etc.)</td>
<td>&quot;Efficacy&quot; Topics, i.e., those relating to clinical studies in human subject (Dose Response Studies, Good Clinical Practices, etc.)</td>
<td>&quot;Multidisciplinary&quot; Topics, i.e., cross-cutting Topics which do not fit uniquely into one of the above categories (MedDRA, ESTRI, M3, CTD, M5)</td>
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Guidance Overload: EMEA Guidance For Everything Else

- **EudraLex**
  Every EU Guidance On Products & Clinical Trials On One (Free) CD
  Mostly Harmonised With FDA...

- Doesn’t Replace, But Adds To The Orange Guide & EP
CFTR Gene Delivery To Treat Cystic Fibrosis
Gene Transfer Formulation Development
pGM169/GL67A

• **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
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• **Target GTA Product Profile**
  - Manufacturable To cGMP
  - Potent After Aerosol Delivery
  - Easily Prepared Under Pharmacy Conditions
Manufacturing & Stability
11.53 Normally the first three commercial production batches should be placed on the stability monitoring program to confirm the retest or expiry date. However, where data from previous studies show that the API is expected to remain stable for at least two years, fewer than three batches can be used.

11.54 Thereafter, at least one batch per year of API manufactured (unless none is produced that year) should be added to the stability monitoring program and tested at least annually to confirm the stability.

11.55 For APIs with short shelf-lives, testing should be done more frequently. For example, for those biotechnological/biologic and other APIs with shelf-lives of one year or less, stability samples should be obtained and should be tested monthly for the first three months, and at three month intervals after that. When data exist that confirm that the stability of the API is not compromised, elimination of specific test intervals (e.g. 9 month testing) can be considered.

11.56 Where appropriate, the stability storage conditions should be consistent with the ICH guidelines on stability.
How To Deliver The Formulation To The Lung?

Jet Nebuliser

Pressured Air Supply
How Does A Jet Nebuliser Work?

- Liquid Placed In Reservoir
- Air Flow Breaks Up Liquid Into Droplets
- Suitable For Large Volumes (10ml)
- Droplets 2-5µm
Jet Nebuliser - Constant Output
Droplet Size Is Crucial to Delivery

- Need To Target Delivery To Appropriate Lung Area

- Implications For:
  - Efficiency
  - Specificity
  - Toxicity

- CF Patients 3-4µm Droplets
How To Measure Droplet Size

Compressed air cylinder

Nebuliser

NGI

Next Generation Impactor

Vacuum pump
How to Measure Droplet Size

Compressed air cylinder

Nebuliser

NGI

Vacuum pump
<table>
<thead>
<tr>
<th>Stage</th>
<th>Range</th>
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<tbody>
<tr>
<td>1</td>
<td>&gt;14.1 µm</td>
</tr>
<tr>
<td>2</td>
<td>8.61 - 14.1 µm</td>
</tr>
<tr>
<td>3</td>
<td>5.39 - 8.61 µm</td>
</tr>
<tr>
<td>4</td>
<td>3.29 - 5.39 µm</td>
</tr>
<tr>
<td>5</td>
<td>2.07 - 3.29 µm</td>
</tr>
<tr>
<td>6</td>
<td>1.35 - 2.07 µm</td>
</tr>
<tr>
<td>7</td>
<td>0.97 - 1.35 µm</td>
</tr>
<tr>
<td>8</td>
<td>0 - 0.97 µm</td>
</tr>
</tbody>
</table>

- Aerosol droplets are ‘sieved’ by the Impactor
- Collect samples from each tray
- Quantify % droplets in each size range
GL67A Generates Large Droplets Compared With Standard Salbutamol Solution
Droplet Size Produced By Range Of Nebulisers
Trudell AeroEclipse II

- Selected For pGM169/GL67A
  - Ideal Droplet Size
  - High Fine Particle Fraction
- Breath Activated
  - Inspiration Triggered
  - No Formulation Waste
- Trudell Very Helpful
  - Supplied Devices At No Cost
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: Static Mixer Devices
Easily Prepared Under Pharmacy Conditions: LMD-2 & Static Mixer
pGM169/GL67A Phase IIb Trial Preparation
Mouse Biodistribution

• Study Design
  2 x UK Based CROs Operating To GLP
  12 x Nose-Only Aerosol Doses At 2-Weekly Intervals

• 4 Dose Groups
  Low Dose (LD): 0.5hr pGM169/GL67A Exposure Per Dose*
  Medium Dose (MD): 2hr pGM169/GL67A Exposure Per Dose*
  High Dose (HD): 6hr pGM169/GL67A Exposure Per Dose
  No Dose (AC): 6hr Air Only

• Sampling: qPCR For pGM169 DNA & Derived CFTR mRNA
  5 Males + 5 Females Harvested At Pre-Specified Time-Points:
  Dose 1: Day 1, Day 56, Day 147
  Dose 6: Day 1
  Dose 12: Day 1, Day 56, Day 147

* All Animals Received 6 Hours Total Exposure For LD & MD, Additional Time Was Air Only
Correlation Between Dose & Levels Of pGM169 DNA In Lungs

Spearman correlation

<table>
<thead>
<tr>
<th>Dose 1 day 1</th>
<th>Dose 6 day 1</th>
<th>Dose 12 day 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>r=0.9434</td>
<td>r=0.849</td>
<td>r=0.9151</td>
</tr>
<tr>
<td>P&lt;0.0001</td>
<td>P&lt;0.0001</td>
<td>P&lt;0.0001</td>
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Persistence Of pGM169 DNA In The Lung

The graph shows the persistence of pGM169 DNA in the lung over different days and conditions. The y-axis represents the concentration of pGM169 DNA in c/g DNA, ranging from $10^1$ to $10^{11}$. The x-axis indicates the time points: day 1, day 56, and day 147. The conditions are labeled as AC and HD for different doses (Dose 1 and Dose 12). The graph also includes a legend for PBNQ and no signal detected.
pGM169 DNA Biodistribution To Blood

Kruskal Wallis P<0.0001; Dunn's multiple comparison test: n/s = P>0.05, * = P<0.05, ** = P<0.01, *** = P<0.001
pGM169 DNA Biodistribution To Liver

Kruskal Wallis $P<0.0001$; Dunn's multiple comparison test: n/s = $P>0.05$, * = $P<0.05$, ** = $P<0.01$, *** = $P<0.001$
pGM169 DNA Biodistribution To Gut

Kruskal Wallis P<0.0001; Dunn's multiple comparison test: n/s = P>0.05, * = P<0.05, ** = P<0.01, *** = P<0.001
**pGM169 DNA Biodistribution To Kidney**

Kruskal Wallis $P<0.0001$; Dunn's multiple comparison test: n/s = $P>0.05$, * = $P<0.05$, ** = $P<0.01$, *** = $P<0.001$
pGM169 DNA Biodistribution To Gonads

Kruskal Wallis P<0.0001; Dunn's multiple comparison test: n/s = P>0.05, * = P<0.05, ** = P<0.01, *** = P<0.001
pGM169 DNA Biodistribution To Spleen

Kruskal Wallis P<0.0001; Dunn's multiple comparison test: n/s = P>0.05, * = P<0.05, ** = P<0.01, *** = P<0.001
pGM169 DNA Biodistribution Summary

The diagram illustrates the distribution of pGM169 DNA over time and dose. It shows the concentration of pGM169 DNA per gram of tissue (c/μg DNA) for different organs and time points. The axes represent log scales for concentration, ranging from 10^1 to 10^11 c/μg DNA. The key indicates the organs tested: Lung, Testes, Ovaries, Spleen, Gut, Liver, Kidney, Lymph Nodes, and Blood. The data points indicate that the concentration varies significantly across different organs and dosing schedules, with some organs showing higher concentrations than others.
pGM169/GL67A Phase IIb Trial Preparation
Biodistribution Summary

• High Levels of pGM169 Detected In Lungs
  Correlation Between Dose Delivered & pGM169 Levels Observed
  (Method Validation)

• Low Levels Of pGM169 Detected In Non-Target Organs
  $10^5$ - $10^8$ Lower Than In Lung
  Complete Clearance Of pGM169 From Gonads
  Clearance To Background By Day 56 Post-Dose In All Except Spleen

• pGM169 CFTR mRNA?
pGM169 DNA Biodistribution Summary

The graph shows the distribution of pGM169 DNA in various tissues over different time points. The x-axis represents the time (day 1, day 56, day 147), and the y-axis represents the concentration of pGM169 DNA (in copies per gram of DNA). The data is categorized by dose (Dose 1 and Dose 12) and includes tissues such as lung, testes, ovaries, spleen, gut, liver, kidney, lymph nodes, and blood. The graph includes markers for each tissue type, with different symbols and colors indicating the dosage and time point.
No Quantifiable pGM169 mRNA In Non-Target Organs
Persistence Of pGM169 CFTR mRNA Expression In Lung

Spearman correlation

- Dose 1: $r = -0.7688$, $P < 0.0001$
- Dose 12: $r = -0.6416$, $P < 0.001$

High Dose
Increasing Numbers Of CFTR mRNA Responders In Lung With Increasing Doses

Spearman correlation:
- D1d1: r=0.7405, P<0.0001
- LD 1d1: r=0.4214, P<0.05
- MD: r=0.2924, P>0.05

* Repeat Analysis Underway
Conclusions

- Biodistribution
  High pGM169 Levels In Lung
  Transient Low Levels pGM169 In Non-Target Organs

- Transgene Expression
  High pGM169 CFTR mRNA In Lung
  Increasing Number Of Doses Increases Consistency Of Expression
  No pGM169 CFTR mRNA In Non-Target Organs

- pGM169/GL67A
  Supports Progression To Repeated-Dose Phase IIb Clinical Study
  Confirms Clinical Strategy To Maximise CFTR Expression
UK CF Gene Therapy Consortium
Single Dose Non-Viral Gene Transfer Study

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

- Single Dose Phase IIa Clinical Trial Started Q1 2009
  N=36 CF Subjects. Nose & Lung Aerosol Administration
  Pre & Post Bronchoscopic Sampling
  Safety & Duration Of Expression Endpoints

- Leading To Repeated Dose Phase IIb Study n=130 Active/Placebo 2012
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pGM169/GL67A

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