Inducible systems for gene therapy medicines

Graham Whyteside,
Principal Scientist
Enabling the development of new gene medicines
Center of Excellence for Gene Regulation

<table>
<thead>
<tr>
<th>Promoters for current products</th>
<th>Promoters for future gene medicines</th>
</tr>
</thead>
<tbody>
<tr>
<td>i Constitutive Expression</td>
<td>ii Regulated Gene Control</td>
</tr>
<tr>
<td>Tissue-selective</td>
<td>Pharmacologically responsive</td>
</tr>
<tr>
<td>Variable strength</td>
<td>Oral Control</td>
</tr>
<tr>
<td>Multi-tissue control</td>
<td>Inducible</td>
</tr>
<tr>
<td>Size to specification</td>
<td>Repressible</td>
</tr>
<tr>
<td></td>
<td>Tissue selective</td>
</tr>
<tr>
<td></td>
<td>Safety switch</td>
</tr>
<tr>
<td></td>
<td>iii Intelligent Design for</td>
</tr>
<tr>
<td></td>
<td>Greater Gene Control</td>
</tr>
<tr>
<td></td>
<td>Pharmacologically responsive</td>
</tr>
<tr>
<td></td>
<td>Oral Control</td>
</tr>
<tr>
<td></td>
<td>Dually regulated</td>
</tr>
<tr>
<td></td>
<td>Multi-gene control</td>
</tr>
<tr>
<td></td>
<td>iv Autoregulatory,</td>
</tr>
<tr>
<td></td>
<td>Environmental Control</td>
</tr>
<tr>
<td></td>
<td>Biologically responsive</td>
</tr>
<tr>
<td></td>
<td>Adaptive Control</td>
</tr>
<tr>
<td></td>
<td>Self-regulated</td>
</tr>
<tr>
<td></td>
<td>Temporally regulated</td>
</tr>
<tr>
<td></td>
<td>Physiologically regulated</td>
</tr>
</tbody>
</table>

Enable the development of new gene medicines

Promoters for current products
- Constitutive Expression
  - Tissue-selective
  - Variable strength
  - Multi-tissue control
  - Size to specification

Promoters for future gene medicines
- Regulated Gene Control
  - Pharmacologically responsive
  - Oral Control
  - Inducible
  - Repressible
  - Tissue selective
  - Safety switch
- Intelligent Design for Greater Gene Control
  - Pharmacologically responsive
  - Oral Control
  - Dually regulated
  - Multi-gene control
  - Pathway control
- Autoregulatory, Environmental Control
  - Biologically responsive
  - Adaptive Control
  - Self-regulated
  - Temporally regulated
  - Physiologically regulated
Tissue specific inducible promoters
POC of design model: Liver specific induction

- Liver specific
- Small molecule inducible
  - GRAS/FDA
- Modularised enhancer
POC of design model: Liver specific induction

Liver specific

Tissue expression level of inducible TF

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Protein</th>
<th>mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>[Liver expression level]</td>
<td>[Liver expression level]</td>
</tr>
<tr>
<td>Kidney</td>
<td>[Kidney expression level]</td>
<td>[Kidney expression level]</td>
</tr>
<tr>
<td>Brain</td>
<td>[Brain expression level]</td>
<td>[Brain expression level]</td>
</tr>
<tr>
<td>Heart</td>
<td>[Heart expression level]</td>
<td>[Heart expression level]</td>
</tr>
<tr>
<td>Spleen</td>
<td>[Spleen expression level]</td>
<td>[Spleen expression level]</td>
</tr>
<tr>
<td>Muscle</td>
<td>[Muscle expression level]</td>
<td>[Muscle expression level]</td>
</tr>
</tbody>
</table>

Small molecule inducible

Modularised Enhancer

Inducer

iTF

Enhancer → TATA-Box → GOI
POC of design model: Liver specific induction

- Identified 4 different enhancers responsive to small molecule
- Tested inducibility of each enhancer
  - Huh7 model
- Different levels of induction for each enhancer
- Dose responsive
- Further tested in primary hepatocytes
POC of design model: Liver specific induction

Enhancer 1 was chosen as our lead candidate
POC of design model: Liver specific induction

- Created combinations of enhancer 1 with different minimal promoters
  - Inducibility
  - Strength
  - Background
- Level of induction and strength dependent on MP
- >5-fold range from single enhancer
POC of design model: Liver specific induction

- Enhancer 1
  - Self-contained 51-bp module
  - Test modularity

![Graph showing luciferase activity](image-url)
POC of design model: Liver specific induction

- Lead candidates
- Robustness of promoter
- Expression of EPO

**SYN-PIND-01**

**SYN-PIND-06**

<table>
<thead>
<tr>
<th>Enhancer</th>
<th>MP1-4</th>
</tr>
</thead>
</table>

**EPO**

---

**Graphs:**

1. **[EPO] mIU/mL**
   - SYN-LIND-01:
     - No Drug: [ ]
     - Conc 1: [ ]
     - Conc 2: [ ]
     - Conc 3: [ ]

2. **[EPO] mIU/mL**
   - SYN-LIND-01:
     - No Drug: [ ]
   - SYN-LIND-06:
     - Conc 1: [ ]
   - CMV_IE: [ ]
POC of design model: Liver specific induction

- Are the promoters specific?
  - Tested in HEK293

- Promoters show no activity in HEK293 cells

- Indicates initial analysis of specificity was correct
POC of design model: Liver specific induction

- Lead candidates
- Cloned into AAV vector
- Effect of ITR’s
  - Inducibility and background
POC of design model: Liver specific induction

- Can the promoters be switched off?
- Removal of drug after 24hrs
- Drug removal returns activity to baseline
- Progressed to in vivo POC
POC of design model: Liver specific induction

Specific

12-24hr induction

>45-fold induction

Inducible

SYNP-LIND-01  SYNP-LIND-06  CMV-IE

Luciferase expression (photons/second)

Fold change

Days after injection

Luciferase expression (photons/second)

Days after injection

0  9  24  48

SYNP-LIND-01  SYNP-LIND-06
POC of design model: Liver specific induction

- Enhancer optimisation
  - 3 component parts
  - Optimised each part
  - Improved activity
    - Enhanced in vivo performance?

**Novel variants of LIND promoter**

<table>
<thead>
<tr>
<th>Variant</th>
<th>No drug</th>
<th>Inducer</th>
</tr>
</thead>
<tbody>
<tr>
<td>SYNP-LIND-01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SYNP-LIND-20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SYNP-LIND-21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SYNP-LIND-22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SYNP-LIND-23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SYNP-LIND-24</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Overview

• Synpromics have developed
  • Pipeline for identifying and constructing tissue specific inducible promoters

• Novel liver-specific inducible promoter
  • Single input system
  • GRAS/FDA approved drug

• Small enhancer
  • 51-bp
  • Increases options for packaging in AAV
  • 4x CMV-IE possible from a 200bp promoter

• Modular and configurable
  • Can be multiplied to increase activity
  • Different strengths/background
    • Modify the enhancer
    • Change MP
  • Dynamic range of 16-fold at fully induced activity
Tissue specific repressible promoters
POC: Liver repressible promoters

• Liver specific
• Small molecule inducible
  • GRAS/FDA
• Modularised repressor
POC: Liver repressible promoters

- Identified a liver specific repressible TF
- Modular component
  - 29bp
- Engineered Synpromics Liver specific promoters to contain module
- Modular component confers repressibility on the promoter
- 1 module is adequate for repression

<table>
<thead>
<tr>
<th>SYNP-OFF-01</th>
<th>SYNP-OFF-02</th>
<th>SYNP-OFF-03</th>
<th>SYNP-OFF-04</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVR promoter</td>
<td>LVR promoter</td>
<td>LVR promoter</td>
<td>LVR promoter</td>
</tr>
<tr>
<td>RM</td>
<td>RM</td>
<td>RM</td>
<td>RM</td>
</tr>
<tr>
<td>MP</td>
<td>MP</td>
<td>MP</td>
<td>MP</td>
</tr>
</tbody>
</table>

Base promoter

<table>
<thead>
<tr>
<th>Ratio to CMV-IE</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
</tr>
<tr>
<td>0</td>
</tr>
</tbody>
</table>
POC: Liver repressible promoters

- Optimised expression
  - Analysis of liver transcriptomics
  - Identified 10 5' UTR designs
  - All <50 bp long
- Improved activity of base promoter SYNP-OFF-03
  - 1 promoter design up to 15 fold CMV-IE
- Promoters will be tested in vivo summer 2019
Overview

• Synpromics have developed
  • Pipeline for identifying and constructing tissue specific repressible promoters

• Novel liver-specific repressible system
  • Single input system
  • GRA/FDA approved drug

• Small module
  • 29-bp
  • Increases options for packaging in AAV
  • Can be added to existing promoters to add repressibility

• Discovered Liver 5’ UTRs
  • Small <50bp
  • Can increase dynamic range of promoter (3-fold)
  • Do not interfere with repression
Synpromics
Controlled gene expression in the liver: design of constitutive, inducible and repressible promoters for use in gene medicine
Metabolic, Storage, Endocrine, Liver and Gastrointestinal Diseases II | 798 | Wednesday 1st May, 5 – 6pm

UCL
Development of Novel Promoters for Neurological Gene Therapy
Neurologic Diseases II | 558 | Tuesday 30th April, 5 – 6pm

Solid Biosciences
Identification of Novel Muscle-Specific Promoters for AAV Gene Expression in Skeletal and Cardiac Muscles
Musculo-skeletal Diseases | 822 | Wednesday 1st May, 5 – 6pm

uniQure Biopharma B.V.
Towards AAV5-Mediated Gene Therapy for Hemophilia A with a Factor IX Variant that Functions Independently of FVIII
AAV Vectors and Disease Targets II | 959 | Thursday 2nd May, 11:45am – 12pm

uniQure Biopharma B.V.
Development of an AAV5-Based Gene Therapy for Fabry Disease
AAV Vectors and Disease Targets II | 960 | Thursday 2nd May, 12pm – 12:15pm
Contact Us

If you would like to know more about Synpromics and gene control technology, please get in touch!

info@synpromics.com
www.synpromics.com

Roslin Innovation Centre
Easter Bush Campus
Midlothian, EH25 9RG
Design and layout guidelines

Colours

- #00ADEE
- #F8931F
- #00AD
- #EA222D
- #73BA09
- #9F005C

Fonts

- **Titles** = *Calibri Light (Headings)* 24pt
- **Body** = *Calibri (Body)* 12pt | This can be varied depending on the layout

Tips

- When pasting, paste as plain text, this will help automatically adjust the text with the template

Tabs

- Copy and paste the selected tab onto the slide, they will automatically paste into the correct location