The CRE Luc Reporter Mouse Model
A transgenic bioimaging mouse model
to assay ligand activation of GPCRs

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The CRE Luc mouse model
background and objectives

CRE-luciferase reporter system
- CRE promoter is responsive to the activation of CREB via the cAMP or PLC pathway
- Luciferase reporter expression is modulated to reflect GPCR activity through a transcriptional readout
- Assay can be used for all 3 GPCR classes: Gs, Gi and indirectly Gq

Bioimaging
- Real-time in vivo imaging utilizes the light emitted by a bioluminescent reporter gene (luciferase) expressed in vivo
- Allows for quantification of the signal non-invasively
- Temporal and spatial data can be collected from the same animal which reduces variation and allows each animal to be its own control
The CRE Luc mouse model

*background and objectives*

- **Model goal:** Combine a GPCR reporter system with real-time in vivo bioimaging to assay GPCR ligand receptor interactions in primary cells, tissues or live animals.
- Same reporter system utilized for both in vitro and in vivo assays
- Profiling of compounds selected from in vitro assays for rapid PK/PD
- CRE Luc mouse models support rapid application to ligand receptor pharmacological assays in vitro
- GPCR ligand interactions can be assayed in a native system avoiding difficult to transfect primary cells and engineered cell lines

- **Model application:** The CRE Luc model has broad applications to GPCR ligand and receptor interactions.
- Addresses the transition from cells to animal model profiling of leads in GPCR drug development
Starting with a variety of luciferase expression profiles, pilot studies defined the model's potential impact on drug development projects.

Typical pilots started with CRE Luc primary cell responses followed by in vivo experiments.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Tg lines for model applications</th>
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<tbody>
<tr>
<td>Liver</td>
<td>187</td>
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<td>Kidney</td>
<td>175</td>
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<tr>
<td>Adipose</td>
<td>219</td>
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<tr>
<td>CNS</td>
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CRE Luc Reporter Mouse Model
Application Strategy

Feasibility profiles: T, B, Macs, Pancreas, Lung, CNS.
Studying the GPCR cAMP signaling pathway using CRE Luc mouse

- Baseline imaging
- Compound dosing
- Re-imaging

- Compound dosing
- Tissue homogenates
- Luciferase assay

- IVIS bioimaging
  - Whole live animal imaging
  - Simple, quick
  - Limited resolution

- Microplate reader
  - Sensitive, accurate
  - Better organ resolution
  - Time-consuming

Next set of slides demonstrates this diversity of data with isoproterenol
Isoproterenol in vivo response in CRE Luc

Response to isoproterenol in line 187 with CNS predominate luc expression
- Treatment: isoproterenol, 10MPK, ip
- Imaging at T=0 and 5 hours
- Statistically significant increase in quantitative CNS response over baseline

Brain and Spinal Cord imaging comparison between baseline and treatment groups.
Compound induced changes in luciferase levels in brain slices can be detected and quantified by bioimaging.

- Gi agonist: Isoproterenol signal is diminished by Gi agonist AMN087
- Strategy to identify the region specific expression of the transgene and drug interaction
Isoproterenol response of CRE Luc primary neurons (and Gs or Gi agonist profiles)

- Gs: ADR, isoproterenol
  - DMSO
  - F/100nM
  - 1µM

- Gi: CB1, CP55940
  - DMSO
  - F/R 50000
  - 10µM rolipram

- Gi: mGluR7, AMN082
  - DMSO
  - F/R 100nM
  - 10µM rolipram

**Significance:**
- *p < 0.05
- **p < 0.01
- ***p < 0.005
- ****p < 0.0001
Pancreatic specific induction of luciferase by a GLP1 agonist is blocked by streptozotocin treatment due to the destruction of β-cells.
GLP1 agonist induces luciferase expression mainly in the pancreas.

Ex vivo assay on tissue homogenates:
- GLP1R found in multiple tissues, however, compound activity is only seen in pancreas.
- CRE Luc model defines the site of action for a compound in vivo (rapid PK/PD).
- Compound dependent patterns of luciferase expression, suggesting that pancreas-specific activity of the GLP1 agonist is unlikely an transgenic artifact.
- Strong induction in the pancreas by the GLP1 agonist, isoproterenol, and forskolin plus rolipram was observed.

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Ins2Akita is an autosomal dominant mutation that causes early onset hyperglycemia in the absence of obesity, due to a missense mutation resulting in mis-folding of proinsulin and death of β cells. Crossed CRE Luc with Ins2Akita (FVB/N background) to see if CRE-Luc induction is correlated with β cell function in this T1DM model. 8-week old mice were subject to baseline imaging on day 1 and treatment with GLP1 agonist (0.1mpk, sc) followed by re-imaging at 4 hr on day 2.

- Decreased CRE Luc induction by the GLP1 agonist (0.1 mpk, sc, 4 hrs) in the highly diabetic male mice. This effect was not significant in the less diabetic female littermates.
- In vivo signals were confirmed by ex vivo luciferase assay in a subset of animals.
From initial studies, we have demonstrated the utility of the CRE Luc model to profile compounds in whole animals, tissue extracts, slices, and primary cells in vitro.

Profiling responses for various GPCRs have been tested in the following combinations:

**Gs agonists:**
- In vitro with microglia, neurons, cardiomyocytes, MEFs and brain slices
- In vivo in the pancreas, brain, spinal cord

**Gs antagonists:**
- In vitro: microglia, neurons, and T cells
- In vivo: brain, spinal cord

**Gi agonists:**
- In vitro: neurons, T cells, brain slices

**Gi antagonists:**
- In vitro: neurons, T cells, brain slices
Characterization of the CRE Luc lines

- Details of the profiling assays with the CRE Luc transgene have been summarized in a single table (available upon request).
- Eight CRE Luc lines are available through Taconic Biosciences.

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<th>Line</th>
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<th>Profiling Assays</th>
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![Images of CRE Luc lines](images)
Acknowledgements and Model Availability

- **Immunology Experimental Pharmacology**
  - Holly Dressler (PTL, model generation, development, and applications)
  - Fernando Camacho (psoriasis)
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  - Andy Giovanni (brain slices)
  - Sarah Favara (lineage profiling, CNS)
  - Zhen Pang (diabetes, Metabolism)
  - Nancy Wu (diabetes, Metabolism)

- **CRE-Luc model information**
  - Greg Polites: greg.polites@sanofi-aventis.com or gopolites3@gmail.com

- **CRE-Luc model availability**
  - Taconic Biosciences
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