The CRE-Luc Reporter Mouse Model: A transgenic bioimaging mouse model that can assay ligand activation of GPCRs

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Abstract and Introduction

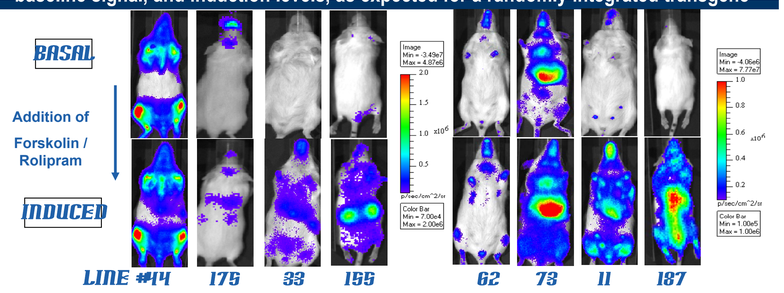
Abstract

Numerous bioassays have been developed to investigate the interactions between GPCRs and their ligands. Reporter based assays using the cAMP response element (CRE) coupled with bioluminescence from a luciferase reporter has been used extensively *in vitro* with high-throughput screens (HTS) of large compound libraries. We have generated a transgenic mouse model (CRE-Luc) with a luciferase reporter under the control of a synthetic promoter containing six copies of CRE, which supports real-time bioimaging in whole animals, tissues, or cells of GPCR ligand activity in a native environment. Assays with the CRE-Luc mouse will be presented to demonstrate the wide application of this model to GPCR drug development. We have crossed a CRE-Luc line expressing luciferase in the pancreas with the Akita pancreatic mutant mouse and demonstrated a significant decrease in the luciferase signal that is proportional to the ablated tissue. A chemically induced psoriasis model was generated by the application of Imiquimod to a CRE-Luc line and luciferase intensity quantitatively correlates with the severity of the induced psoriasis. Finally, we have completed several assays with primary neuronal cells, *in situ* brain slices, and whole animals to demonstrate the consistency of the luciferase reporter in these different cellular formats and ligand receptor interactions. Access to the CRE-Luc mouse model is available through an exclusive licensing agreement between ***Sanofi*** and ***Taconic***.

Introduction

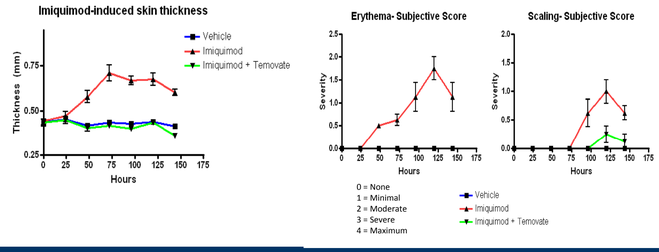
The interaction between GPCRs and their extracellular ligands has proven to be an attractive point of interference for therapeutic agents. For this reason, the pharmaceutical industry has developed biochemical drug discovery assays to investigate these ligand GPCR interactions. Here, we describe the generation and application of a transgenic mouse model that contains six cAMP response elements (6X CRE) upstream of a luciferase cDNA. The transgene enables the specific monitoring of G protein dependent signaling via molecular bioimaging. Molecular imaging techniques can be performed in the intact organism with sufficient spatial and temporal resolution to study biological processes *in vivo*. Furthermore, the CRE-luc mouse can also used a source of cells and tissues to support parallel native cellular GPCR assays performed *in vitro* or *ex vivo* which cam lead to a more realistic profile of ligand and receptor interactions.

Luciferase expression varies amongst individual lines with respect to tissue pattern, baseline signal, and induction levels, as expected for a randomly integrated transgene

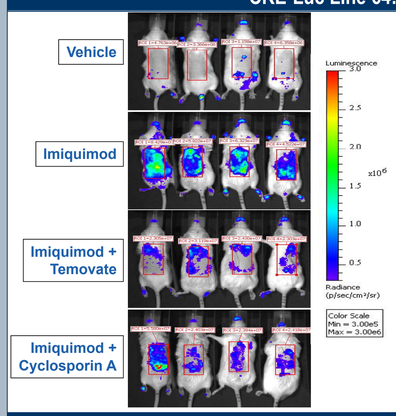


The Imiquimod psoriasis model induces thickening of skin, erythema, and scaling

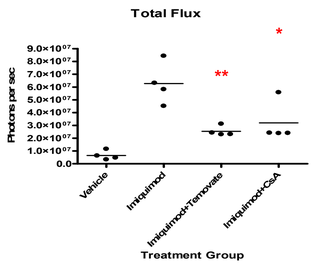
- Imiquimod (IMQ) is a ligand for TLR7 and TLR8 and can exacerbate psoriasis in patients with topical treatment, both locally and at distant sites (side effect)
- IMQ-induced psoriasis is mediated via IL-23 and IL-17
- Skin on back of mice was folded and measured using digital calipers
- Imiquimod group showed a significant increase in skin thickness
- Increase in skin thickness was completely blocked by Temovate



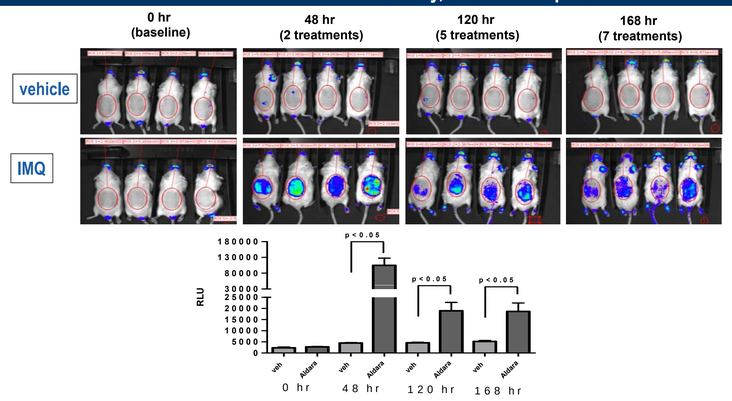
CRE-Luc Line 64: 24 hour treatment



- Significant reduction in bioimaging signal is observed in both temovate and CsA treated groups
- One animal in CsA treated group is an outlier



CRE-Luc Line 64 in an IMQ study, time course profile

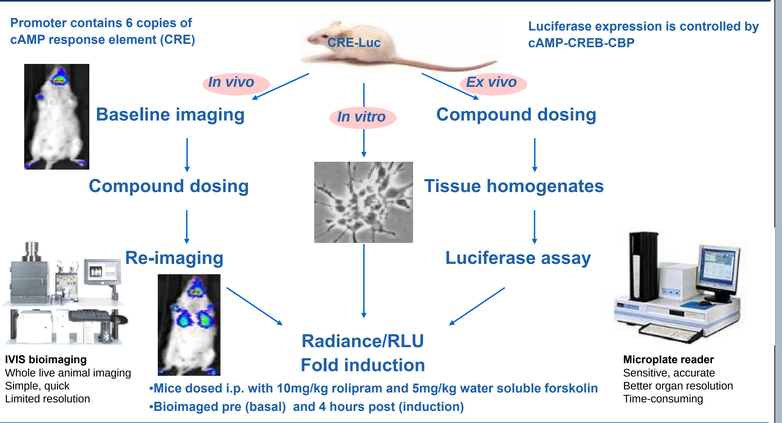


Methods

The transgene used to generate this model contains the following elements which in combination produce a high frequency of functional lineages: MAR (matrix attachment regions); to generate position independent expression, 6X CRE; a response element represented by CRE-cAMP repeated six times, HSV TKmin; a simplex virus thymidine kinase minimal promoter, LUC 2; a luciferase cDNA optimized for mammalian expression , and a hGH polyA element which contains the human growth hormone minigene with the poly A tail to enhance transgene expression.

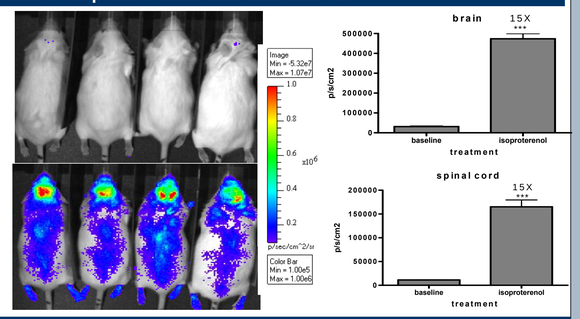
The initial screen for luciferase expressing founders was performed by dosing IP with a combination of 5mg/kg forskolin and 10mg/kg rolipram followed by bioimaging with an IVIS Lumina. Detectable levels of luciferase was measured in 87 of the 112 DNA positive lineages. Tissue expression profiles were used to select the optimal lineages.

Studying the GPCR cAMP signaling pathway using CRE-Luc transgenic mouse



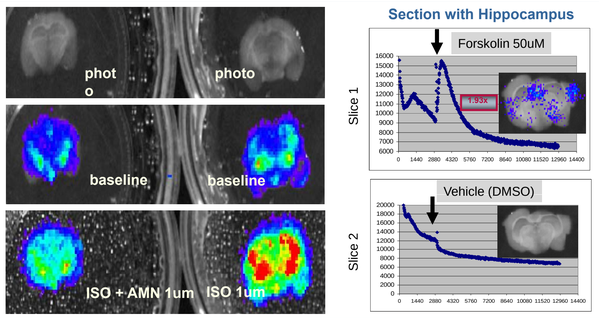
Profile of isoproterenol *in vivo* with line 187

- Treatment: hours isoproterenol treatment, 10MPK, ip
- Imaging at T=0 and 5 hours
- Statistically significant increase in CNS response over baseline



Profile of isoproterenol and its antagonist AMN082 with brain slices

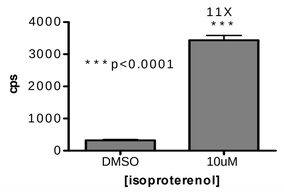
- Compound induced changes in luciferase levels in brain slices can be detected, and quantified by bioimaging
- Potential use to identify the region specific expression of the transgene and drug interaction



Luciferase levels are increased by Gs agonists ADβR (isoproterenol) and dopamine in primary neuronal cells

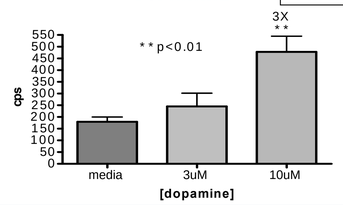
•E18, D3 CORTICAL NEURONS
•T-TEST VS DMSO
•1 HOUR TREATMENT

GS: ADRβ1/2, isoproterenol



GS: DRD, DOPAMINE

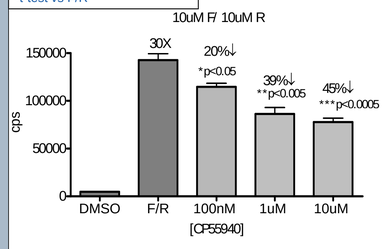
•E14, D4 STRIATAL NEURONS
•T-TEST VS MEDIA
•5 HOUR TREATMENT



Luciferase levels are reduced by Gi agonists for CB1 and mGluR7 in primary cortical neuronal cells treated with forskolin plus rolipram

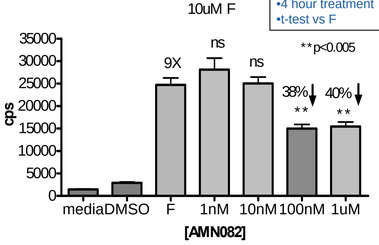
•E18, d3 cortical neurons
•10μM forskolin/ 10μM rolipram
•8 hour treatment
•t-test vs F/R

GI: CBI, CP55,940



GI: MGLUR7, AMN082

•E18, d3 cortical neurons
•10μM forskolin
•4 hour treatment
•t-test vs F



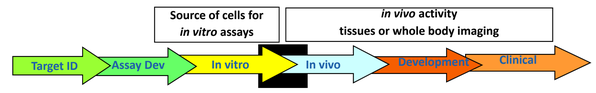
Summary

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*

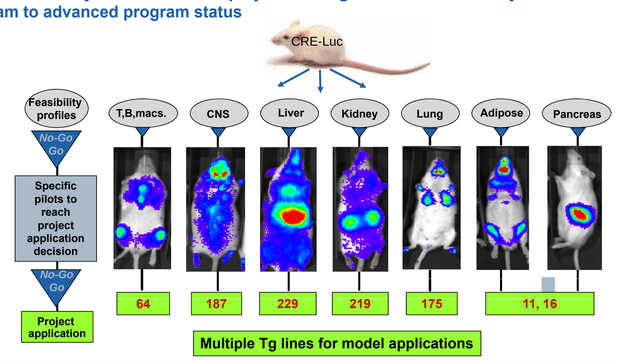
GPCR	agonists	antagonists
Gs	*in vitro*: microglia, neurons, T cells, cardiomyocytes, MEFs, brain slices *in vivo*: pancreas, brain, spinal cord	*in vitro*: microglia, neurons, T cells *in vivo*: brain, spinal cord
Gi	*in vitro*: neurons, T cells, brain slices	*in vitro*: neurons, T cells

For further model information contact *Greg Polites* at: greg.polites@sanofi-aventis.com or hgpolites3@gmail.com
CRE-luc mice are available from *Taconic* contact: info@Taconic.com

CRE-Luc reporter mouse model application strategy



Starting with a variety of *in vivo* luciferase expression profiles, our goal for the CRE-Luc model was to more efficiently transition GPCR projects through their traditional major hurdle from pre-program to advanced program status



The induction of luciferase levels in the pancreas by the GLP1 agonist, AVE0010, is reduced by streptozotocin treatment

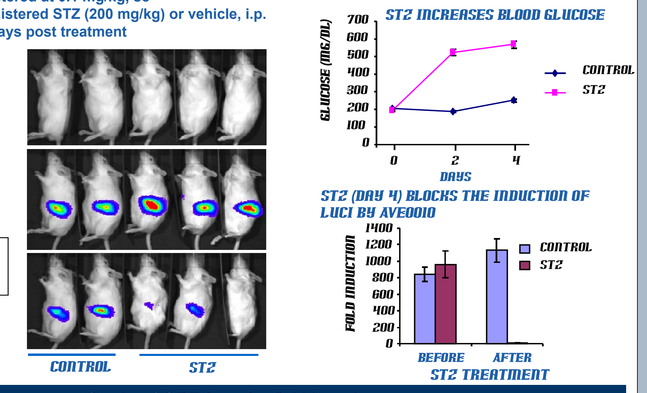
- Streptozotocin is toxic to the insulin-producing beta cells of the pancreas
- AVE10 administered at 0.1 mg/kg, sc
- Animals administered STZ (200 mg/kg) or vehicle, i.p.
- Bioimaged 4 days post treatment

BASAL

INDUCTION BY AVE0010

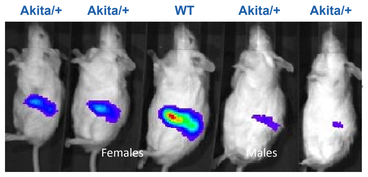
INDUCTION BY AVE0010, 4 DAYS POST STZ OR VEHICLE TREATMENT

CONTROL STZ

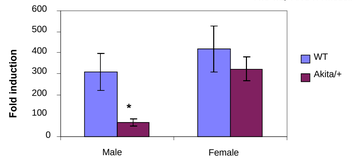


Study of CRE-Luc Ins2 Akita mice

- *Ins2^{Akita}* is an autosomal dominant mutation that causes early onset hyperglycemia
- CRE-Luc crossed with *Ins2^{Akita}* to determine correlation of βcell function in this T1DM model
- 8-week old mice were treated with GLP1 agonist (0.1mpk, sc) imaged at 4 hr on day 2



*P < 0.05, Akita/+ vs WT
Two-way ANOVA model.

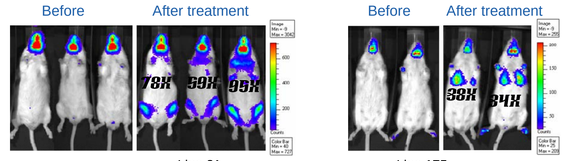


- Decreased CRE-Luc induction by the GLP1 agonist (0.1 mpk, sc, 4 hrs) was observed in the highly diabetic male mice.

The ADRβ3 receptor agonist CL316,243 induces luciferase expression in adipose, lung, and small intestines

•CL316,243(1mg/kg) was administered by i.p. injection
•Bioimaged 4 hours post dosing, then tissue was harvested

Bioimaging results



Luci enzyme assay in tissue extracts

Tissue	Line 31 fold-induction	Line 31 induced level	Line 175 fold-induction	Line 175 induced level
Adipose	22.3	58.3	20.0	10.3
Intestine	13.3	7.8	2.7	2.3
Pancreas	7.5	1.5	0.9	0.5
Spleen	10.4	0.5	1.6	0.8
Lung	35.9	20.8	49.5	1702.5
Brain	0.7	100.5	1.0	89.3