Novel Splicing Driven Gene Regulation Platform for Precise Dose Responsive Control of Gene and Cell Therapies

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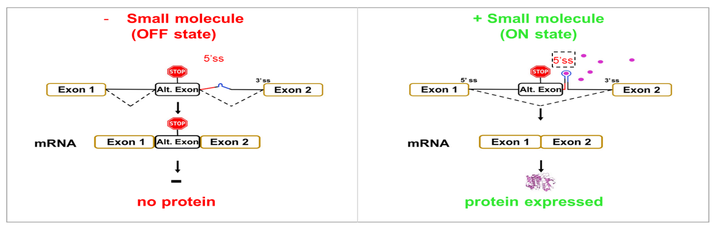
Abstract

Precise control of delivered transgenes allows *in vivo* delivery of therapeutic biologics. However, gene regulation systems that have clinical applicability have been lacking. Here, by linking aptamers to an alternative splicing-based gene expression platform, we have created a robust riboswitch-based gene regulation system that controls gene expression via bespoke small molecule inducers. Using these novel riboswitches and inducers, we have regulated multiple therapeutic genes including hormones, incretins, antibodies and chimeric antigen receptors (CARs). We observed the robustness of our riboswitch system in controlling Epo expression in anemic animals and in regulating AAV-vectorized, anti-HER2 antibody. In Diet-Induced Obesity (DIO) animals, riboswitch-controlled expression of incretins resulted in significant body weight loss and improved glucose tolerance. CAR-T cells containing riboswitch were remotely controlled *in vivo* by orally administered inducers, showing superior anti-tumor activities when compared with CAR-T cells expressing constitutive CAR. Attributable to the uniquely high dynamic range of our riboswitch and the safety and oral bioavailability of the small molecule inducer, our riboswitch-based gene regulation platform enables precise control of therapeutic genes to advance the development of gene and cell therapies for a broad range of human diseases, including large indications with unmet needs such as metabolic disease, oncology and autoimmune disease.

Aptamer-modulated alternative splicing riboswitch regulates multiple therapeutic genes



Aptamer is inserted in the downstream intron of an Intron-AltExon-intron cassette within the cDNA of transgene.

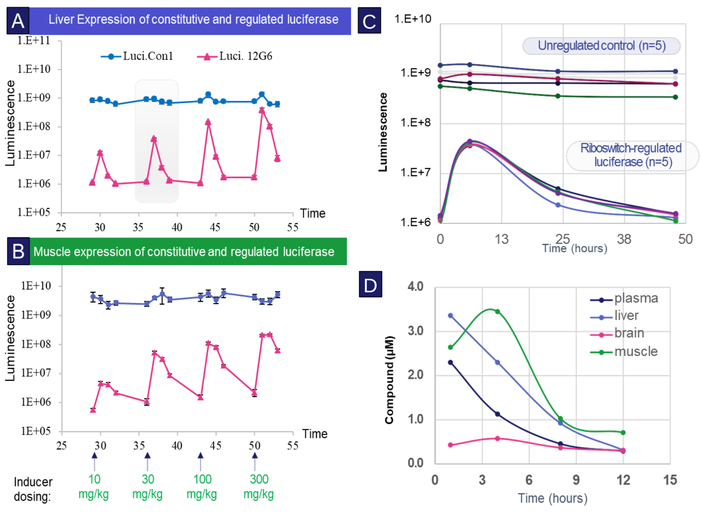
In the absence of small molecule (left panel):	In the presence of small molecule (right panel):
Alt. exon 5' ss is accessible	Alt. exon 5' ss is sequestered
Alt. exon is included	Alt. exon is skipped
No protein expressed	Protein expressed

In vivo delivered, Riboswitch-regulated transgene is precisely controlled by orally administered small molecule inducer

- **Luci.Con1**: constitutive expression – no regulation cassette
- **Luci.12G6:** identical construct to Luci. Con1 but with regulation cassette

Liver Expression: tail vein injection AAV-Luciferase

Muscle Expression: direct I.M. injection of AAV-Luciferase



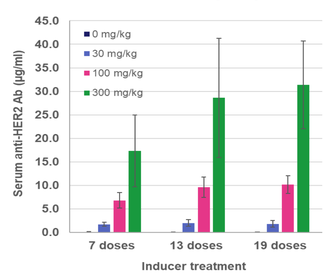
- **Figures A and B** Single oral dose of small molecule results in dose responsive expression of Luciferase from Luci. 12G6 in comparison to constitutive expression with Luci con1 in both tissues.
- **Figure C** is a blow up of the individual mice in each cohort at 30mg/kg dose in Figure A – indicated by the gray box in Figure A. This illustrates the restriction of expression with small molecule dosing between individual mice. On a mouse-by-mouse basis there is about 0.4 log range of expression between the 5 mice from constitutively active unregulated control construct. In contrast, induced expression in response to oral inducer is tightly controlled, expression is limited by the dose of the oral small molecule such that each mouse receiving the same oral dose expresses the same level of luciferase.
- **Figure D** shows the differential tissue distribution of the small molecule inducer when delivered orally. The different shapes of the induced luciferase curves in Fig. A (liver) and Fig. B(muscle) precisely reflect the different tissue biodistribution to liver and muscle. A sharp peak for liver (blue) vs. slow accumulation and then exit from muscle (green). Tissue distribution of orally delivered small molecule inducer shows short term accumulation in muscle, whereas clearance from liver is linear. This is directly reflected in the different profiles of regulated luciferase expression in the liver and muscle (Figures A and B)

Riboswitch-controlled therapeutic genes

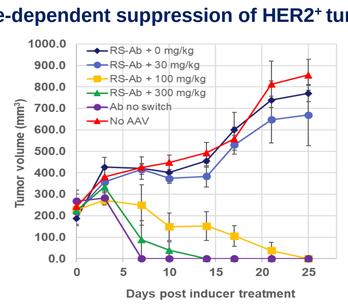
Therapeutic Antibodies	Cell Therapy	Therapeutic Hormones / Cytokines / Peptides	Gene/RNA Editing Nucleases
• Anti-PCSK9	• RiboCAR:	• Epo	• Cas9
• Anti-VEGFR2 (eye)	• Anti-CD19	• hGH	• CasRx
• Anti-Amyloid	• Anti-PSMA	• Gut peptide combinations: GLP1, GIP, PYY, Glucagon, Amylin	
• Anti-IL-17, Anti-IL4Ra	• Anti-mesothelin		
• Anti-PD1, Anti-Myostatin	• Anti-HER2		

Riboswitch-controlled, AAV-vectorized anti-HER2 antibody halts tumor progression *in vivo*

Dose-dependent anti-HER2 antibody expression in serum

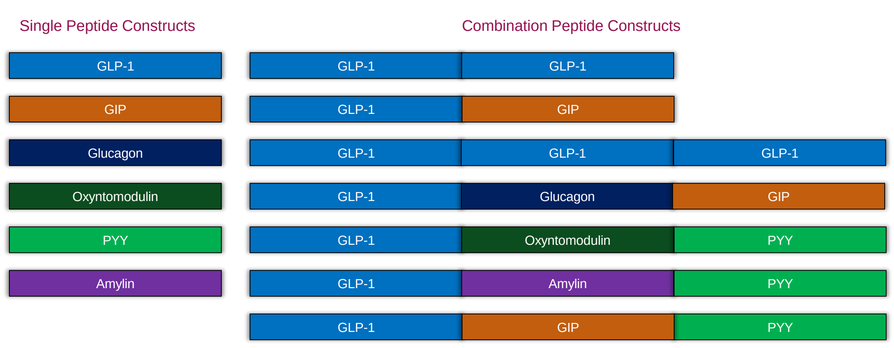


Dose-dependent suppression of HER2⁺ tumor



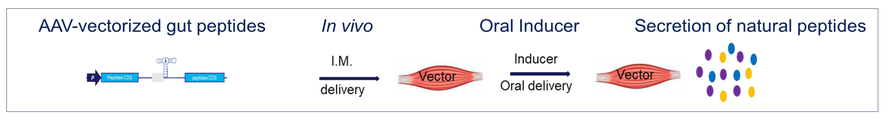
Riboswitch-controlled incretins reduces body weight and improves glucose tolerance in DIO mice

In vivo delivery of natural gut peptides

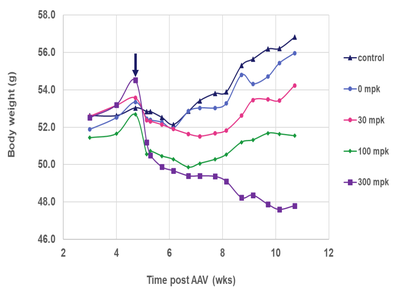


- Expressing gut peptides has been challenging
- MeiraGTx has achieved high expression of natural gut peptides, alone or in combination
- The riboswitch platform provides tight and controlled expression of unmodified, wild-type peptides
- Delivery of multiple combinations of peptides can be achieved in a single vector. These can be constructed and tested rapidly head to head to provide fast *in vivo* proof of concept of efficacy and benefit on muscle mass, metabolism, and feeding as well as behavior and CNS impact.

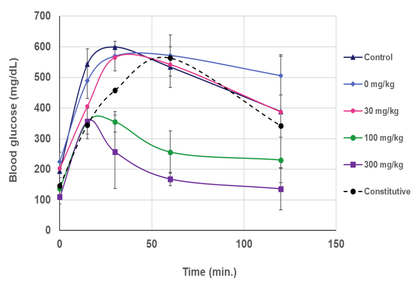
In vivo delivery of GLP-1 and GIP and Glucagon cause weight loss



weight loss in DIO mice



glucose control in DIO mice



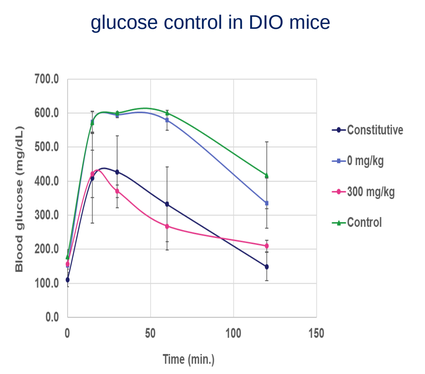
- Untreated DIO show persistent weight gain over 10 weeks (black line, control)
- The regulated construct in the absence of the small molecule (blue line, 0mg/kg) shows no difference in weight gain from the DIO mice
- A low dose of the small molecule delivered orally daily every day including weekends (pink line) results in some initial weight loss.
- Increased weight loss is seen at a higher dose of small molecule (green line)
- When the daily oral small molecule dose is further increased (purple line), persistent and significant weight loss is observed
- In this experiment the animals received a single oral dose of the small molecule every day including weekends

- Untreated DIO animals show poor glucose control post the glucose challenge (black line, control)
- The regulated construct in the absence of the small molecule (blue line, 0mg/kg), and the animals treated with the low dose of small molecule (pink line) shows no difference from the DIO mice in glucose control
- At increasing doses of the oral small molecule a dose response is seen with respect to glucose control with the higher dose giving the most rapid glucose control (purple line). This experiment was carried out following 6 weeks of *in vivo* daily delivery of GGG via oral small molecule induction
- In contrast, animals with persistent GGG activity showed complete failure of glucose control (dotted line)

In vivo delivery of GLP-1 and GIP causes weight loss

weight loss in DIO mice

glucose control in DIO mice



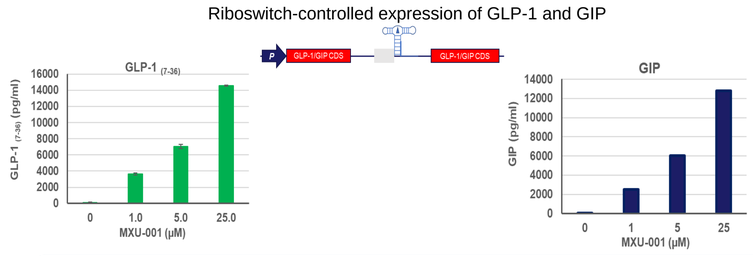
- Untreated DIO animals gain weight persistently over the 15 weeks of the experiment (dark blue line)
- *In vivo* delivery of GLP1-GIP from the constitutive vector (light blue line) results in reduced weight compared to control DIO animals (dark blue line)
- The regulated construct in the absence of the small molecule (pink line) shows no difference from the control DIO mice
- A low dose of the small molecule delivered orally daily on weekdays does not result in meaningful weight loss (green line)
- Daily oral dosing of the small molecule at an increased dose (olive green line) results in rapid and persistent weight loss with the DIO mice reaching lean weight (black line).The zigzag line reflects that the animals were only dosed on weekdays (not on weekends), indicating that GLP1-GIP production diminishes in the absence of the small molecule

- In control untreated DIO animals (green line) poor glucose control following a glucose challenge is observed
- No improvement in glucose control is observed in animals with the regulated GLP1-GIP construct in the absence of oral dosing of the small molecule (light blue line 0mg/kg)
- Glucose control is clearly improved when GLP1-GIP is constitutively present (dark blue line)
- When animals receive GLP1-GIP via the daily dose of the small molecule rapid glucose control is seen in these animals (pink line)

Riboswitch-controlled, biologically active expression of natural peptides

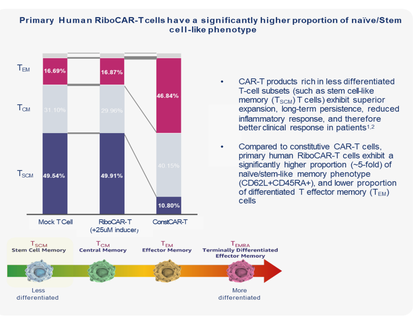
Biologically active GLP-1 and GIP expression

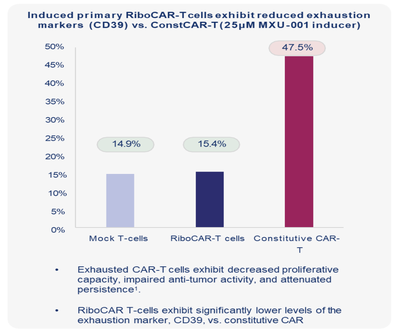
Riboswitch-controlled expression of GLP-1 and GIP



RiboCAR-T cells are more potent than ConstCAR-T cells in anti-cancer activity *in vivo*

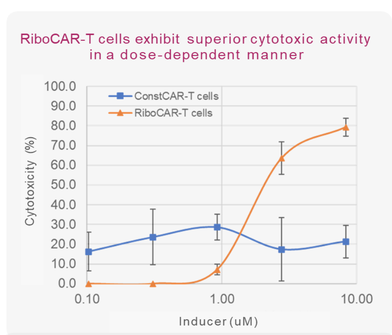
RiboCAR-T Cells Are Enriched in Naïve/Stem Cell-Like Memory Phenotype and Display Reduced Exhaustion Markers

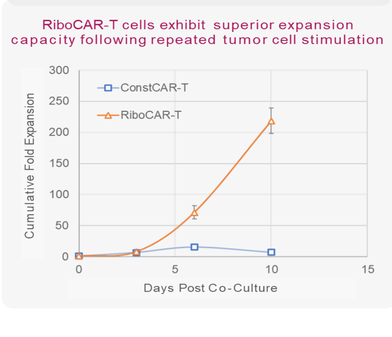




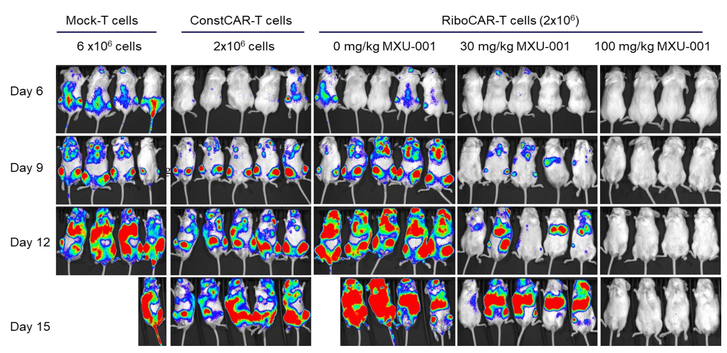
- Expression of CD62L and CD45RA (left) and CD39 (right) on RiboCAR-T and ConstCAR-T cells was measured by FACS 6 days post CRISPR/cas transfection and AAV transduction.
- RiboCAR-T cells have a more naïve/stem cell memory T cell phenotype in culture compared to ConstCAR-T cells.
- RiboCAR-T cells have reduced exhaustion compared to ConstCAR-T cells.

RiboCAR-T cells exhibited superior cytotoxicity and expansion capacity following repeated tumor cell stimulation





- RiboCAR-T cells or ConstCAR-T cells were co-cultured with Raji-ffLuc cells at 2:1 E:T ratio in the presence of various concentration of inducer for 48 hours. Luciferase activity was measured for cytotoxicity assessment.
- CAR-T cells were stimulated with MMC-treated Raji cells at 1:1 ratio in the presence of inducer. Stimulation was repeated every 3 days under the same conditions.



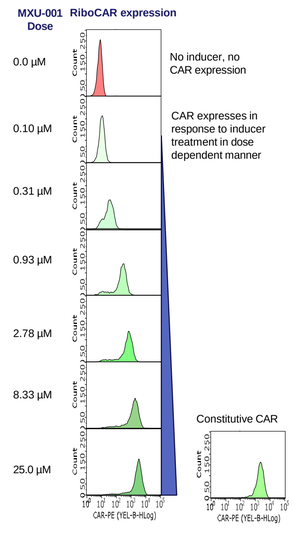
- Raji-ffLuc cells were injected I.V. into NSG mice and small molecule inducer was dosed orally and daily at the indicated doses, starting the day before CAR-T cells were injected.
- RiboCAR-T cells' anti-tumor activity was remotely controlled by oral inducer.
- RiboCAR-T cells outperformed ConstCAR-T cells expressing constitutive CAR.

Highly dynamic inducible and reversible expression of Riboswitch CAR

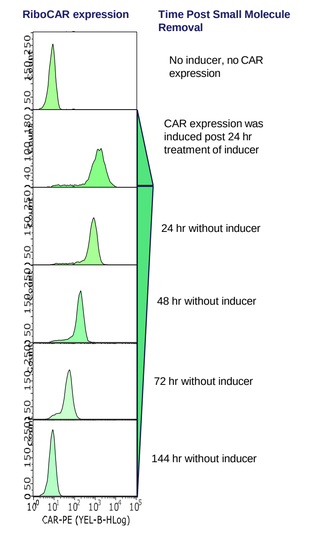
Constitutive anti-CD19 CAR (ConstCAR)

Riboswitch anti-CD19 CAR (RiboCAR)

CAR expression activated in response to small molecule dose



CAR expression switches off when small molecule removed



- RiboCAR or ConstCAR was targeted to TRAC locus by CRISPR/cas9-mediated knock-in
- Stable lines of Jurkat T cells containing RiboCAR or ConstCAR were established
- Jurkat T cells were treated with MXU-001 at different doses
- Jurkat T cells were stained with anti-FMC63 antibody 48 hours after MXU-001 treatment
- CAR expression was measured by flow cytometry
- CAR expression was induced by MXU-001 in dose-dependent manner
- CAR expression declined to undetectable levels post small molecule removal

Summary

- ✓ Our riboswitch gene regulation system has uniquely high dynamic range for regulating gene expression.
- ✓ Small molecule inducers are safe and orally bioavailable for *in vivo* use.
- ✓ Our riboswitch enables precise control of expression of therapeutic genes.
- ✓ Our gene regulation technology is applicable to CAR-T cell therapy.
- ✓ Our gene regulation technology is applicable to treatment of metabolic disease.
- ✓ Our gene regulation technology is applicable to treatment of hormone deficiency.