



SENTI BIO

Small-molecule-regulated gene circuit for controlling cytokine expression in cell therapies

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1 Senti Biosciences, Inc.

2 Boston University

Presented by: Michelle Hung

May 14, 2021



Disclosures

- Michelle Hung is a paid employee of Senti Biosciences, Inc
- This presentation included verbal remarks by the presenter that are not included here



Executive Summary

Challenge

Safely improve efficacy of cell-based immunotherapies for solid tumors

Our Solution

Design and Build Regulator Dial – a gene circuit to regulate production of potent immune effectors using FDA-approved small molecule drugs

Test Regulator Dial in primary immune cells and **Learn** how different properties of the gene circuit can be optimized to achieve desired results

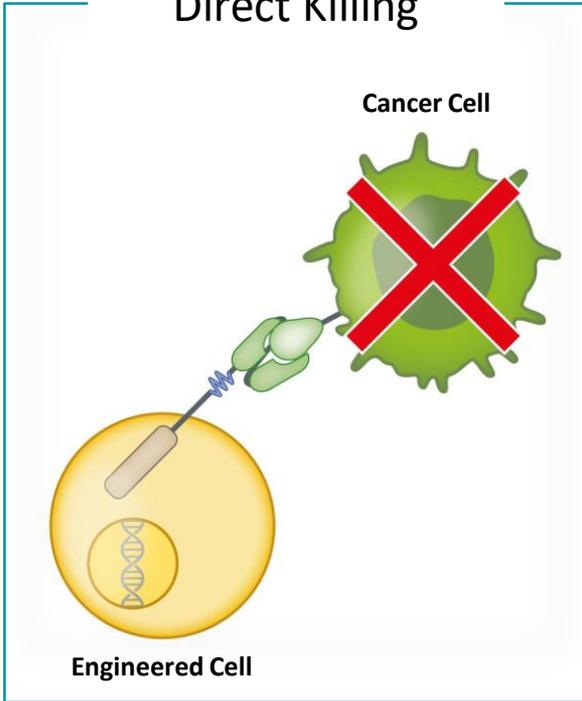


Optimized Regulator Dial gene circuit enabled Grazoprevir dose-dependent control of IL-12 production *in vivo*

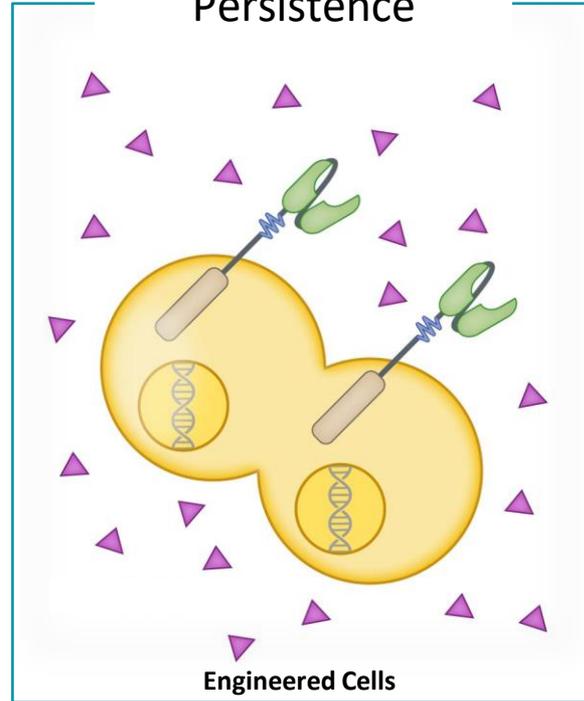


Why arm cell-based immunotherapies with cytokines?

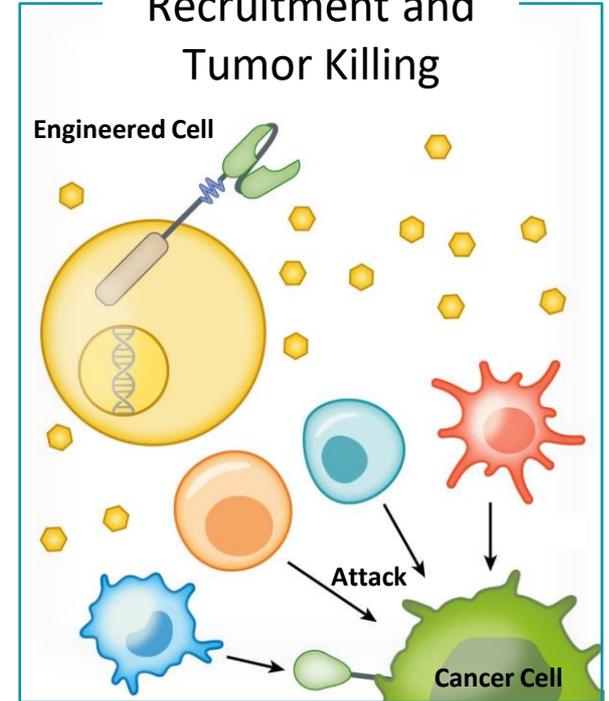
Direct Killing



Persistence



Immune Cell Recruitment and Tumor Killing

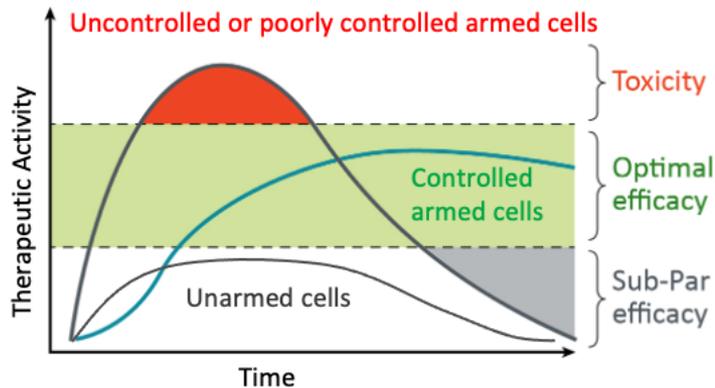




The benefit of controlling the arming of cell-based immunotherapies

Controlling IL-12 arming in cell-based immunotherapies

- **IL-12 is a highly potent immune activator** with the potential to stimulate the tumor immunity cycle
- **Overexpressing IL-12 from adoptive T cell therapies** using a poorly regulated promoter **has resulted in significant clinical toxicities** (Zhang et al., Clin. Can. Res. 2015)
- **Narrow therapeutic window** associated with IL-12 has limited success to date



OFF Dose Controlled Armed CAR-immune cells (IV)

Little/no immune effectors secreted in absence of small molecule drug

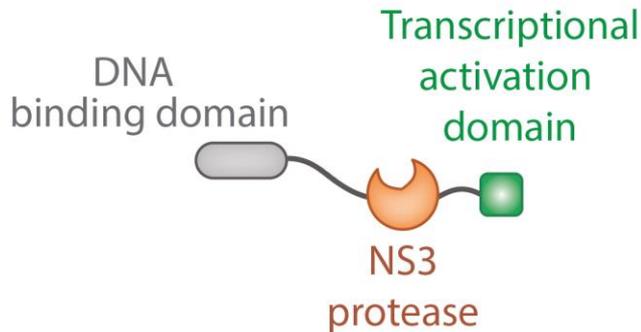
ON Dose Small Molecule Drug (Oral)

Presence of small molecule triggers controlled secretion of immune effectors in the tumor



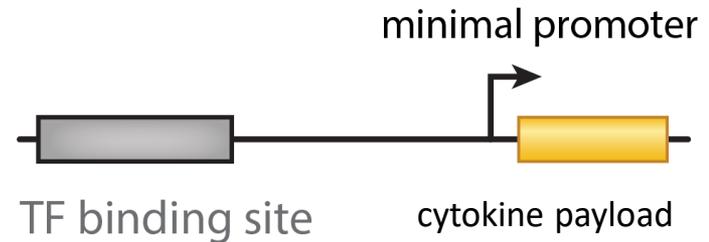
Regulator Dial is intended to enable control of gene expression via FDA approved small molecules

Regulator Dial Transcription Factor Parts



- Regulator Dial Transcription Factor uses a highly specific ZF DNA binding domain linked to an activator that drives target gene expression without impacting the rest of the transcriptome

Controlled Payload



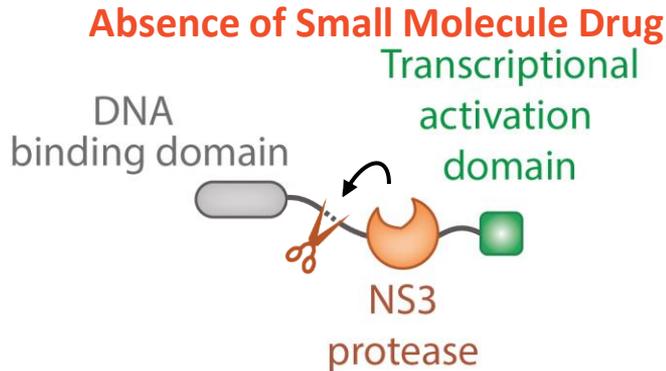
- Controlled payload is a cytokine driven by a promoter that contains a binding site for the Regulator Dial DNA binding domain

Clinically-driven design of synthetic gene regulatory programs in human cells. Divya V. Israni, Hui-Shan Li, Keith A. Gagnon, Jeffry D. Sander, Kole T. Roybal, J. Keith Joung, Wilson W. Wong, Ahmad S. Khalil. bioRxiv 2021.02.22.432371



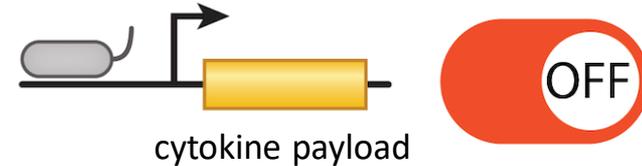
Regulator Dial is intended to enable control of gene expression via FDA approved small molecules

Regulator Dial Transcription Factor Parts



- Regulator DNA binding domain is linked to the transcriptional activation domain by a protease cleavable linker

Controlled Payload



- When the protease is active, the DNA binding domain is not linked to the transcriptional activation domain, and is unable to activate transcription
- Cytokine payload is OFF

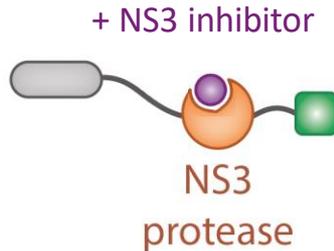
Clinically-driven design of synthetic gene regulatory programs in human cells. Divya V. Israni, Hui-Shan Li, Keith A. Gagnon, Jeffry D. Sander, Kole T. Roybal, J. Keith Joung, Wilson W. Wong, Ahmad S. Khalil. bioRxiv 2021.02.22.432371



Regulator Dial is intended to enable control of gene expression via FDA approved small molecules

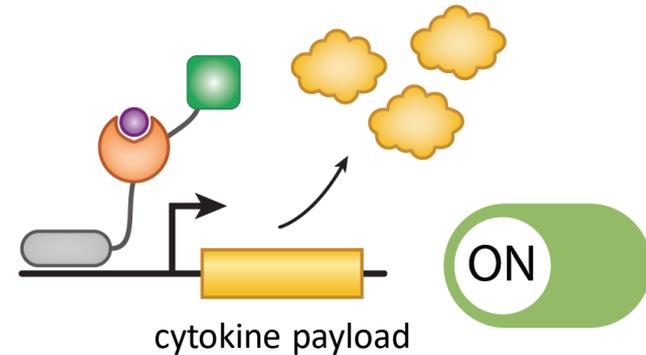
Regulator Dial Transcription Factor Parts

Presence of Small Molecule Drug



- NS3i small molecule suppresses protease activity, resulting in an intact transcription factor

Controlled Payload



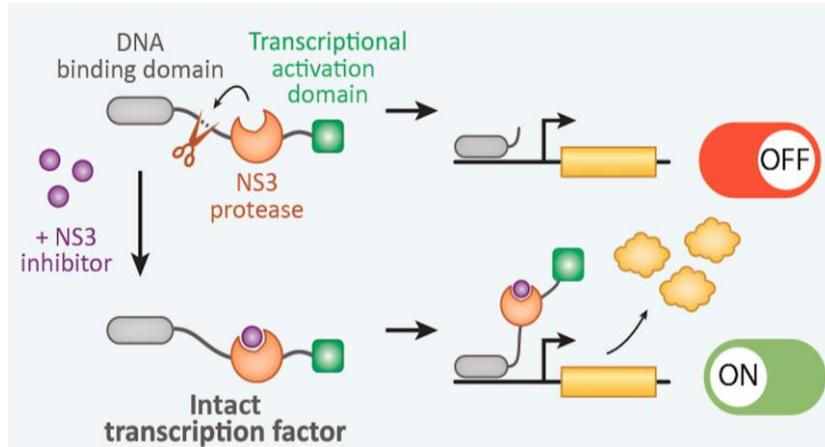
- Regulator Dial transcription factor can activate transcription
- Cytokine payload is ON

Clinically-driven design of synthetic gene regulatory programs in human cells. Divya V. Israni, Hui-Shan Li, Keith A. Gagnon, Jeffry D. Sander, Kole T. Roybal, J. Keith Joung, Wilson W. Wong, Ahmad S. Khalil. bioRxiv 2021.02.22.432371



Regulator Dial is intended to enable control of gene expression via FDA approved small molecules

Ideal properties of Regulator Dial



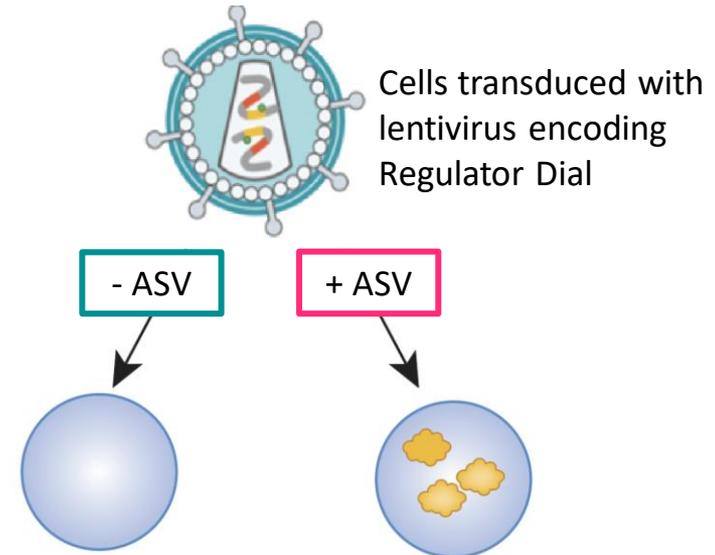
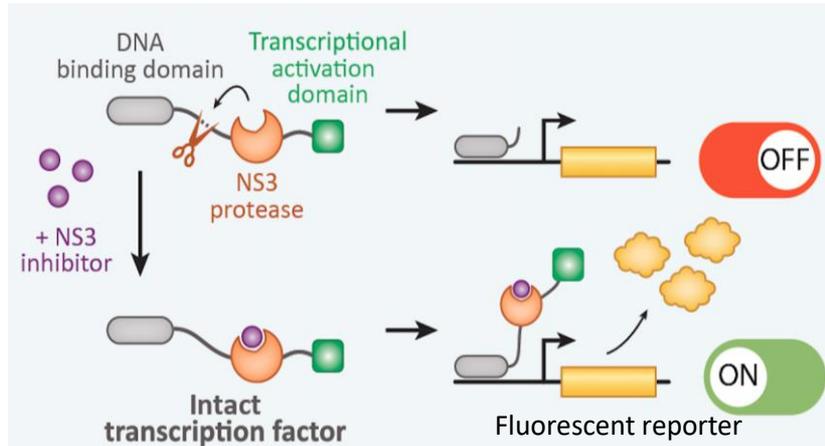
- **Versatile:** transcriptional regulation has the potential to control any payload of interest; can regulate multiple payloads
- **Safe:** small molecule is FDA approved (hepatitis drug); NS3 protease incorporates mutations to avoid immunogenicity
- **Dose Dependent:** payload level depends on small molecule dose
- **Convenient:** orally-dosed favorable PK profile

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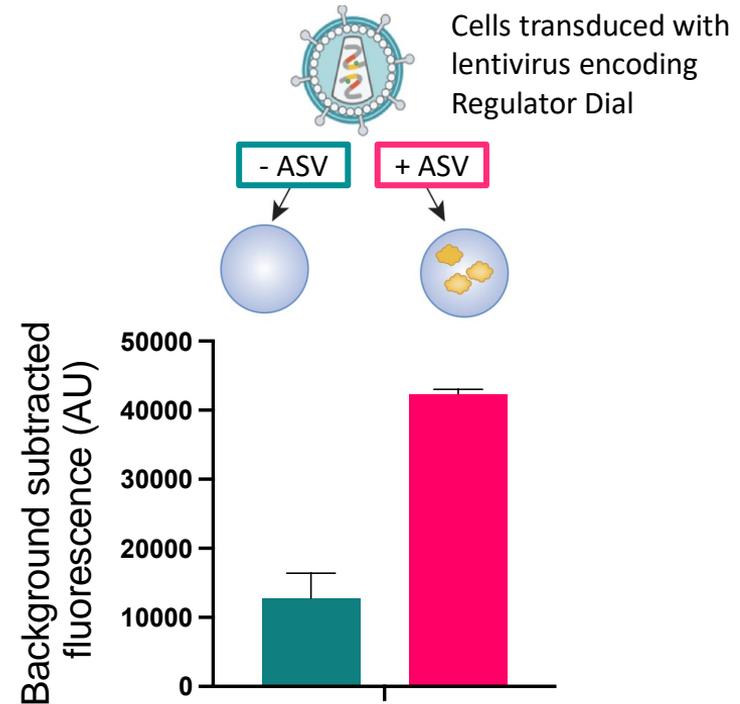
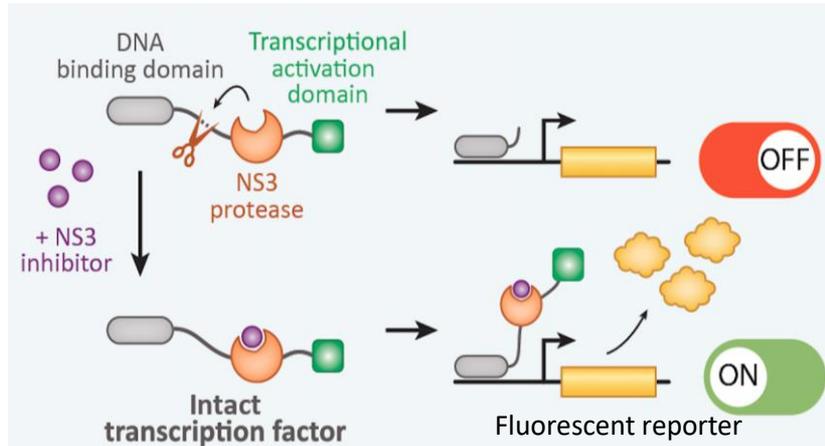
Can Regulator Dial control gene expression in primary immune cells?

- NS3 inhibitors are a family of well tolerated, orally available, FDA-approved small molecules
- Here, Asunaprevir (ASV) was the NS3 inhibitor used





Can Regulator Dial control gene expression in primary immune cells?

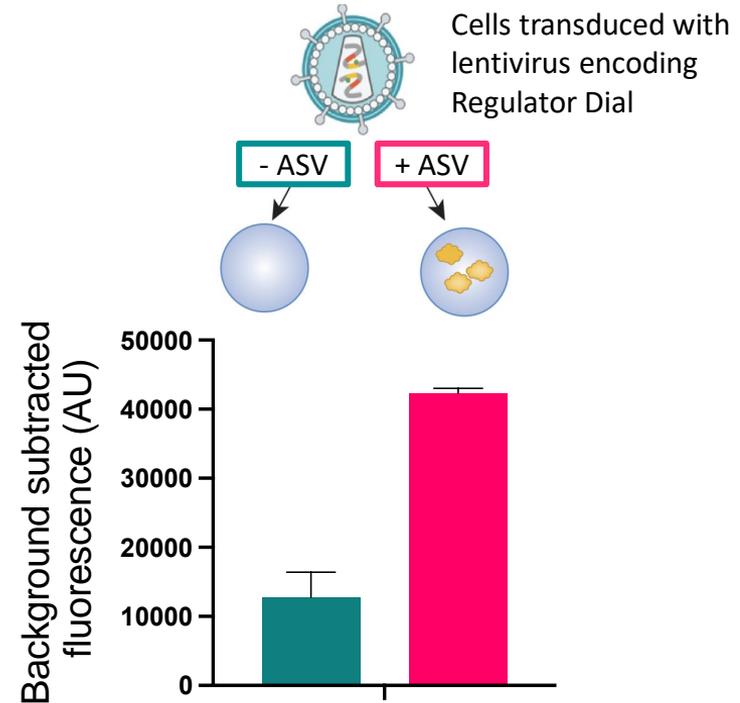
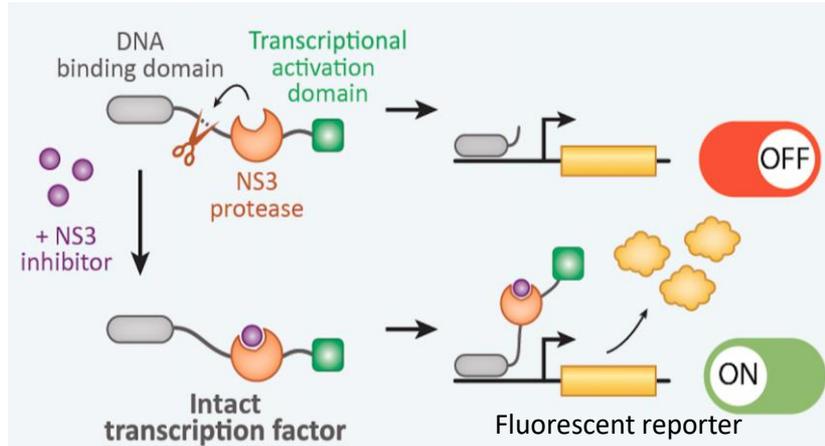


Regulator Dial induced a 3.3-fold increase in fluorescent reporter upon small molecule treatment





Can Regulator Dial control gene expression in primary immune cells?



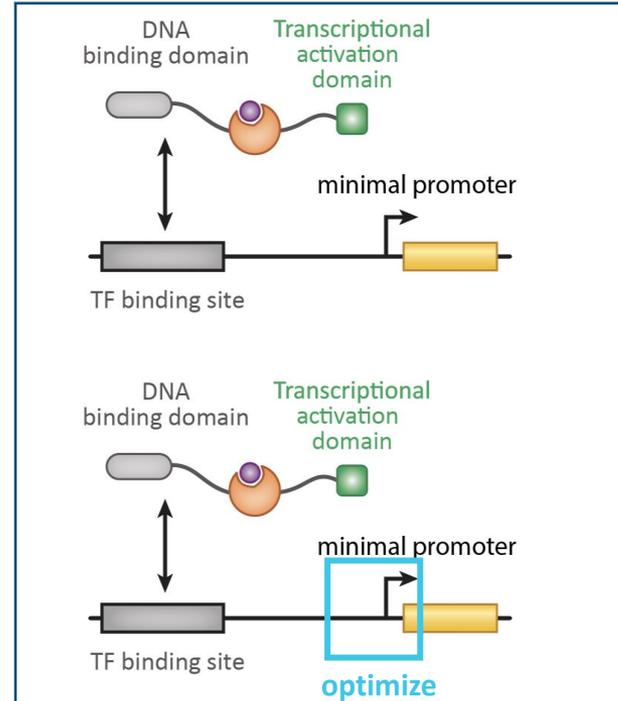
High basal activity is potentially toxic for potent immune effectors such as IL-12





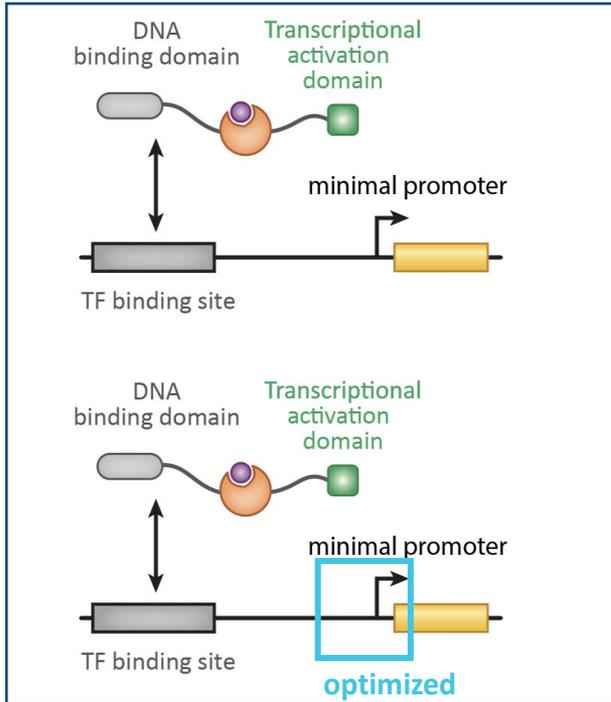
Can promoter optimization reduce basal activity of the Regulator Dial?

- Initial regulator dial promoter had high basal activity, which could be potentially toxic if a potent immune effector such as IL-12 is being controlled
- We identified alternative minimal promoters that could have lower basal activity

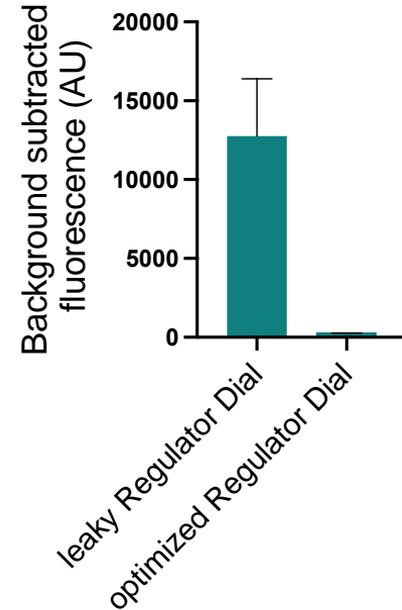
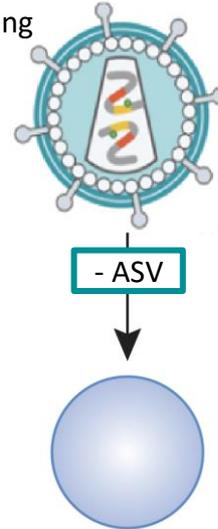




Can promoter optimization reduce basal activity of the Regulator Dial?



Cells transduced with
lentivirus encoding
Regulator Dial

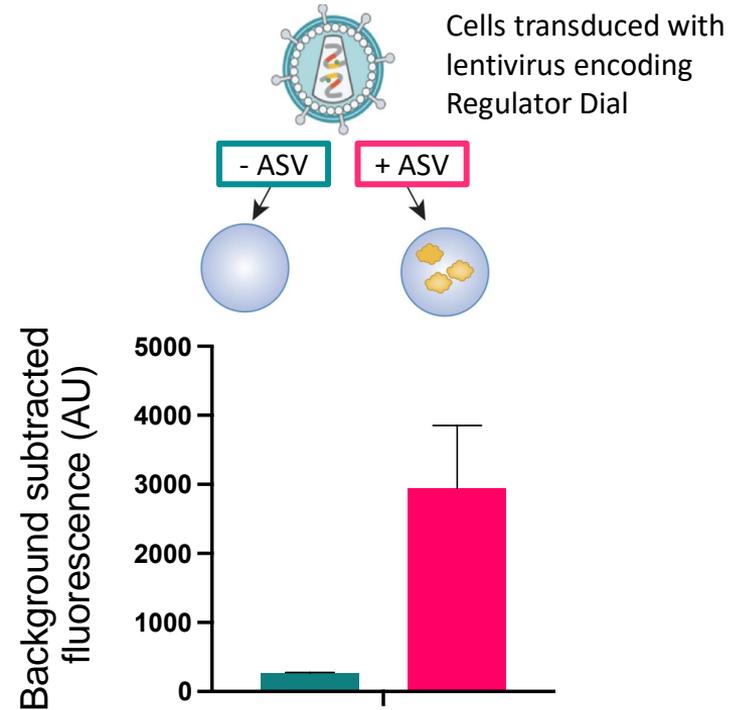
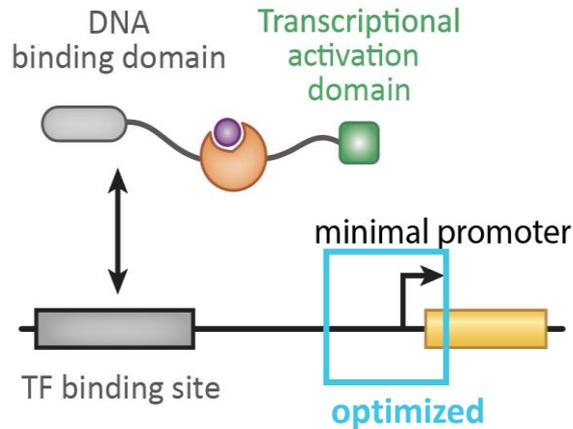


Basal expression was reduced by 20-fold upon promoter optimization





How well does the optimized Regulator Dial induce gene expression?

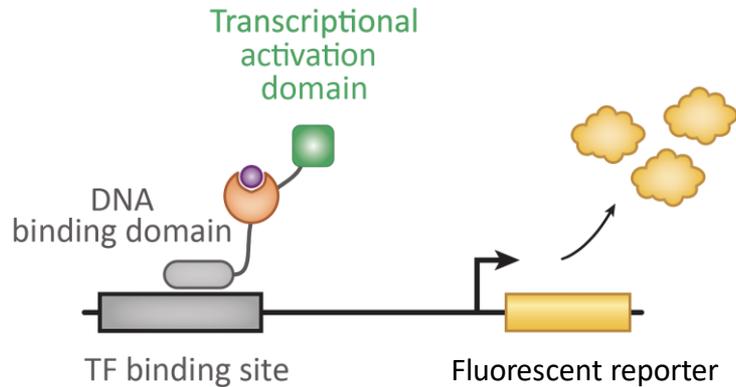


Optimized Regulator Dial induced an 11-fold increase in fluorescent reporter upon small molecule treatment

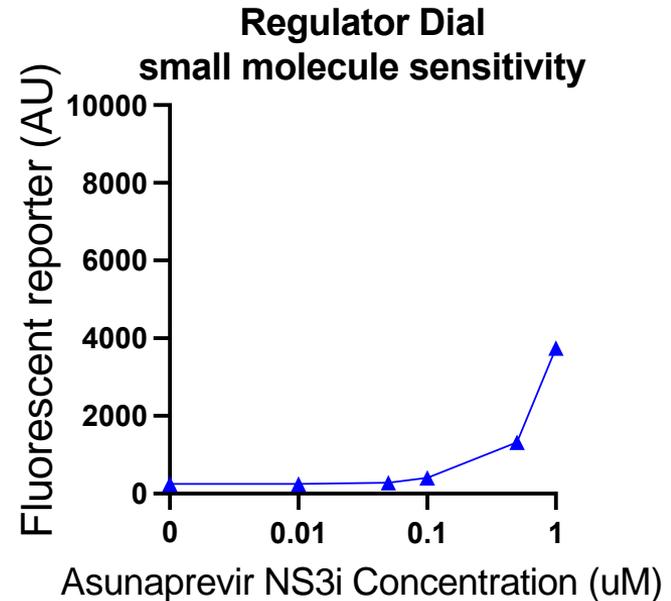


Regulator Dial small molecule sensitivity

Regulator Dial



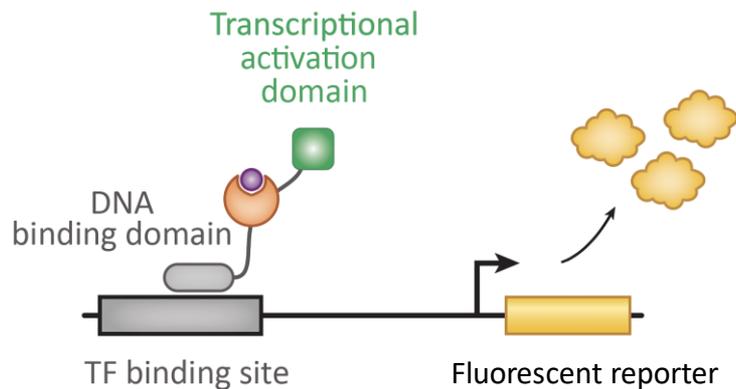
Small molecule sensitivity



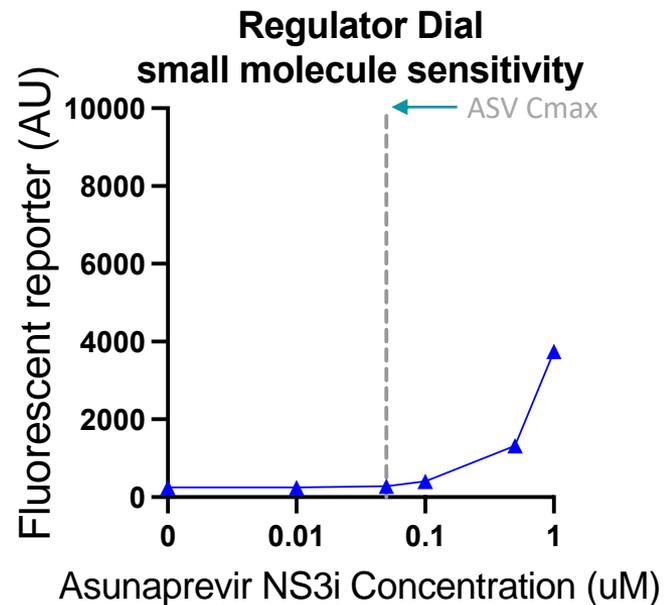


Regulator Dial small molecule sensitivity

Regulator Dial



Small molecule sensitivity

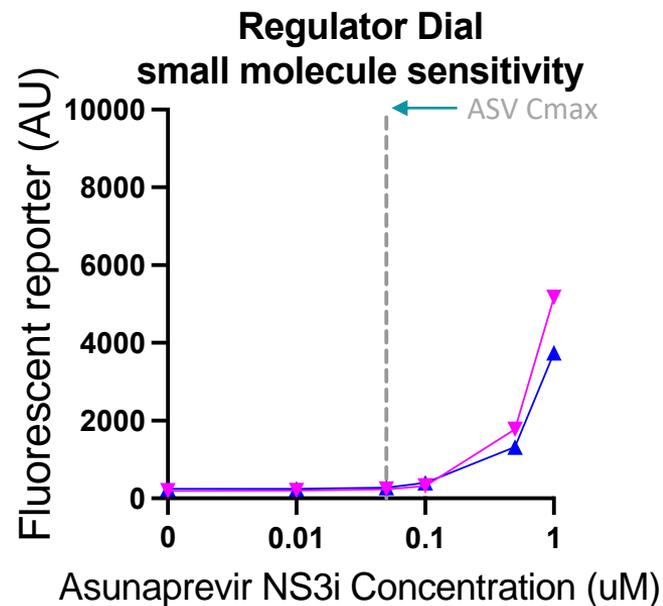
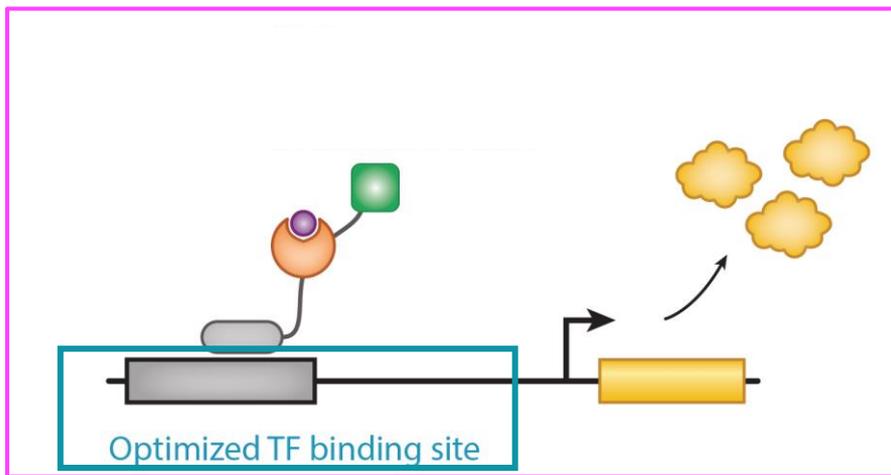
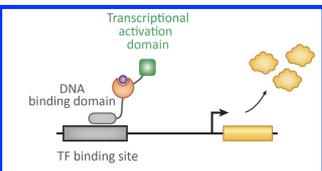




Senti's Design, Build, Test, Learn cycle was applied to improve Regulator Dial small molecule sensitivity

Regulator Dial

Small molecule sensitivity

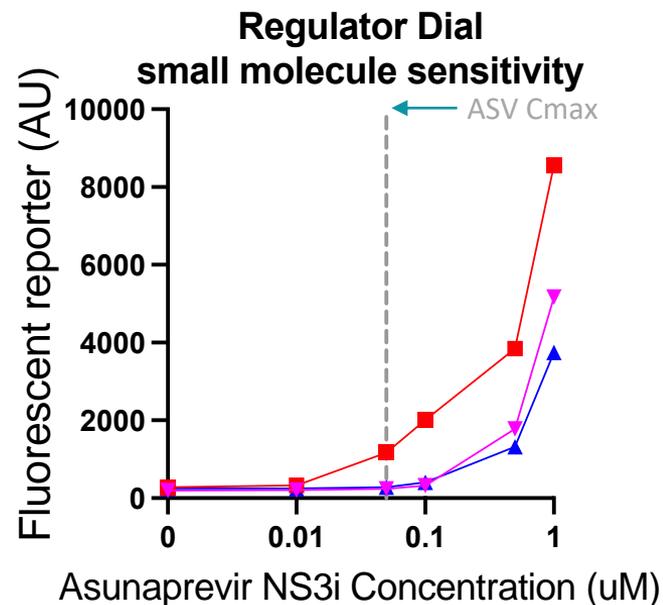
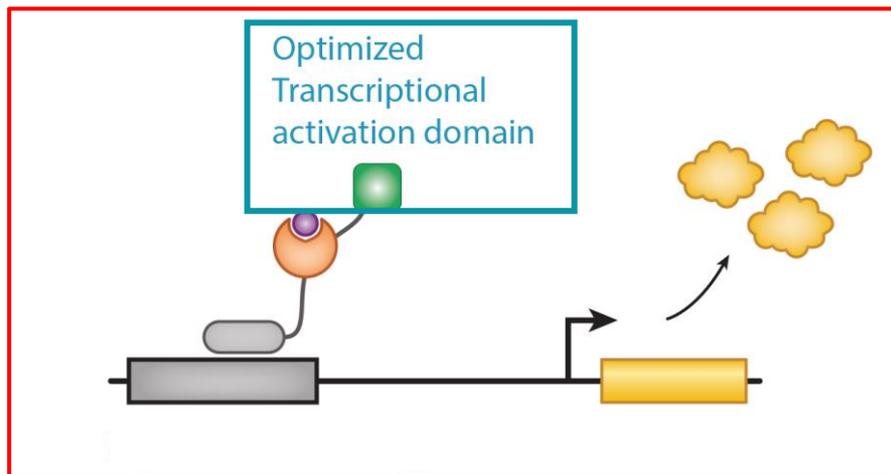
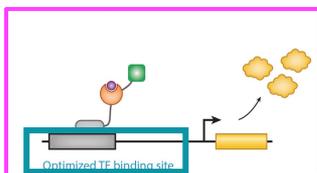
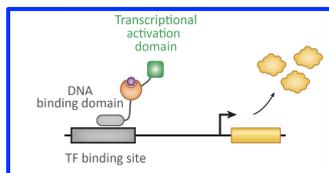




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Regulator Dial

Small molecule sensitivity

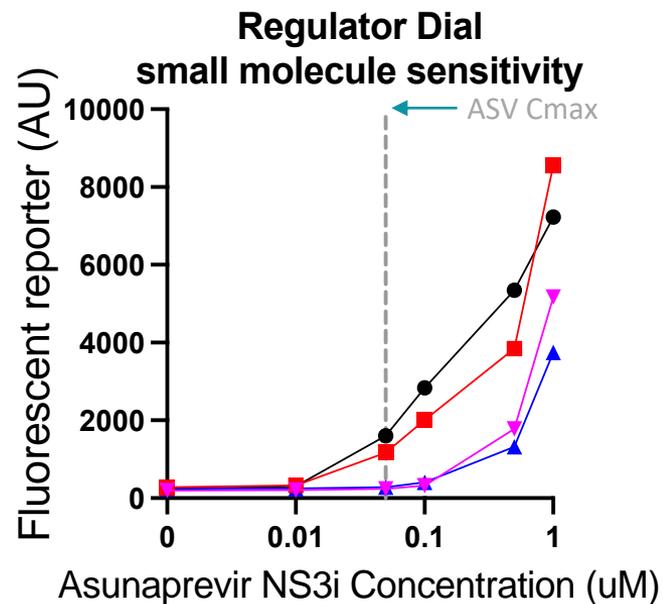
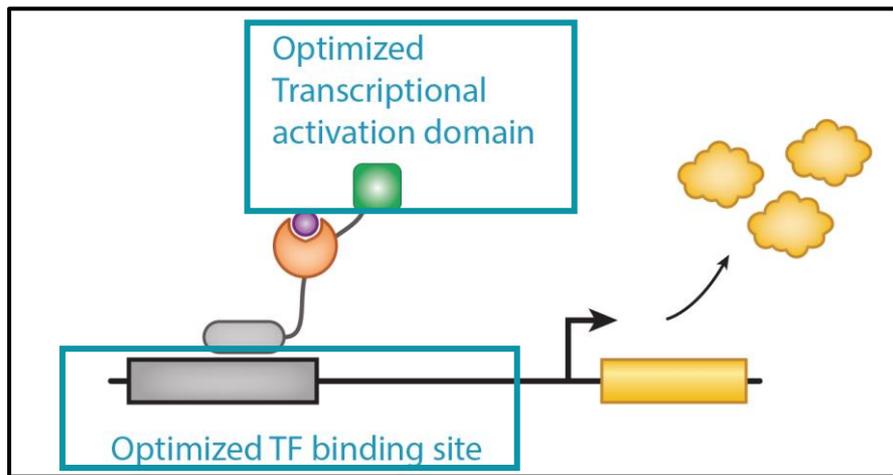
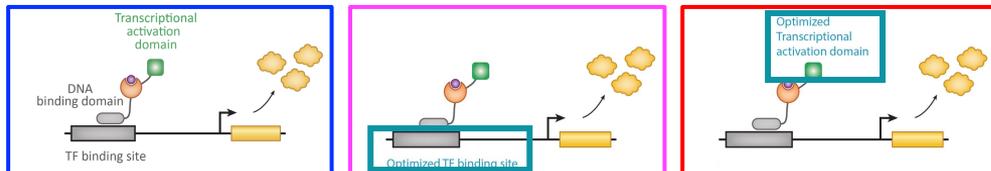




Senti's Design, Build, Test, Learn cycle was applied to improve Regulator Dial small molecule sensitivity

Regulator Dial

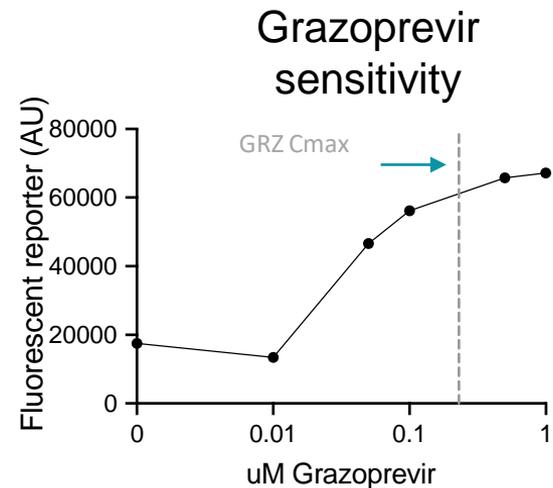
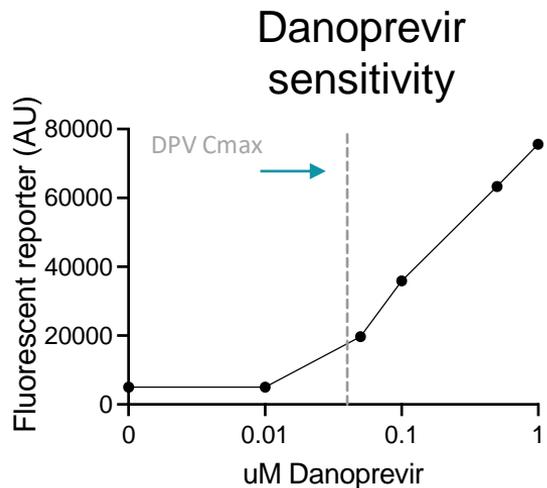
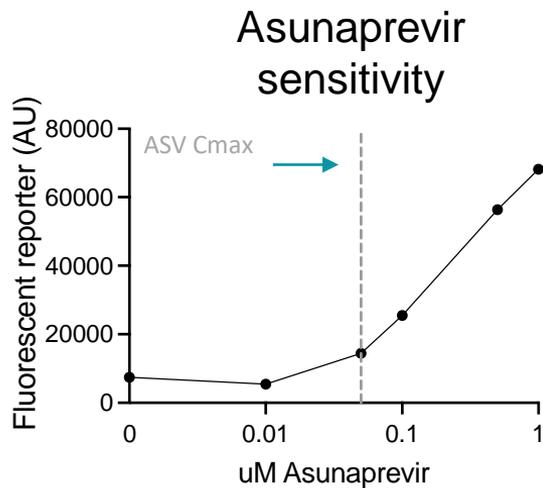
Small molecule sensitivity





Regulator Dial sensitivity to alternative NS3 inhibitors

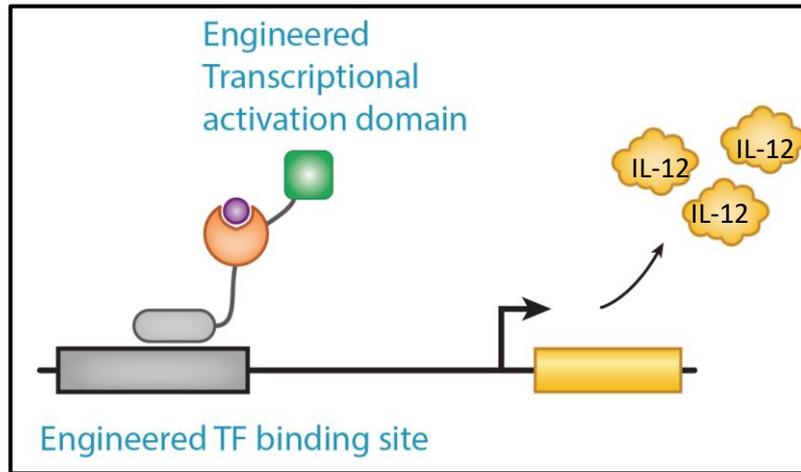
Multiple NS3i small molecules were chosen based on IC50s against NS3 and human PK data



Regulator Dial showed high sensitivity to Grazoprevir at clinically relevant levels

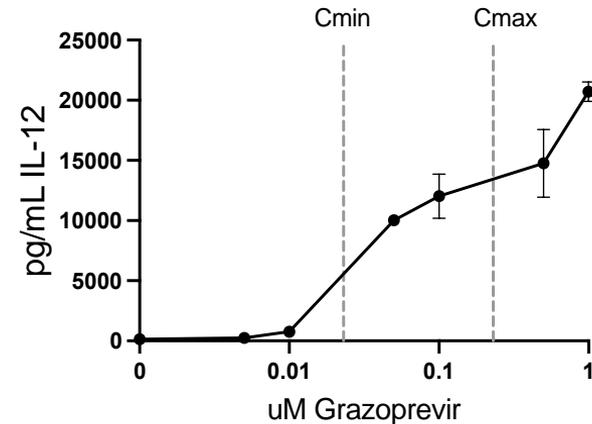


Regulator Dial enables control of IL-12 production in primary immune cells



C_{min} to C_{max} are the average minimal and maximal serum concentrations of GRZ in patients treated daily with the clinical dose (ZEPATIER[®])

Regulator Dial control of IL-12



Regulator Dial dose-dependently controlled IL-12 production in primary immune cells at clinically relevant concentrations of Grazoprevir

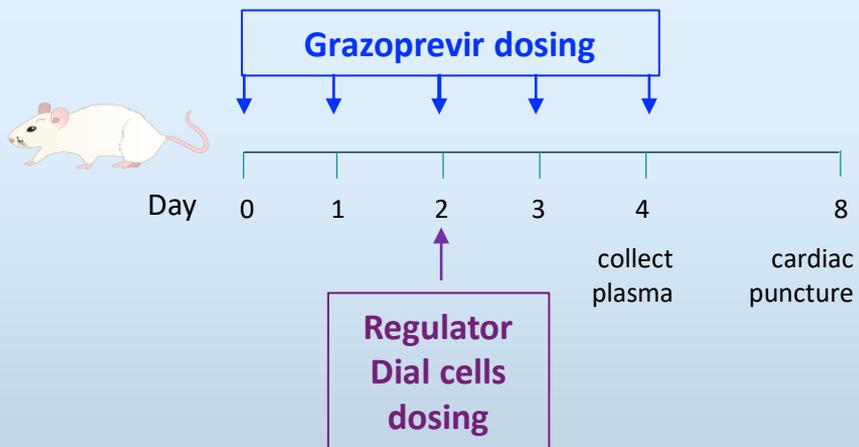




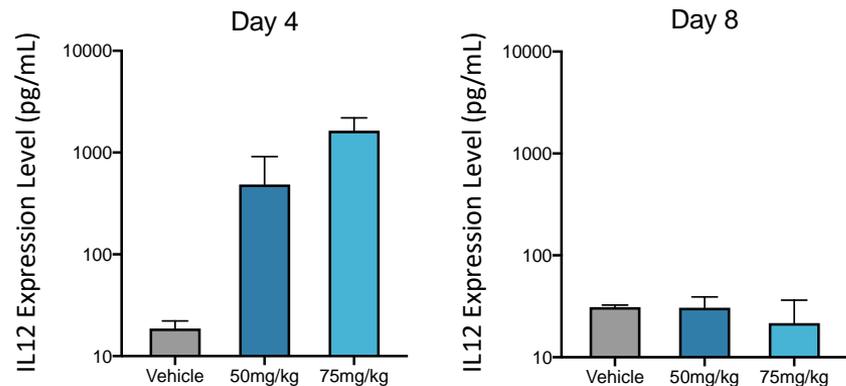
In Vivo Regulator Dial control of hIL-12 production

Study Design

In vivo



hIL-12 in mouse plasma



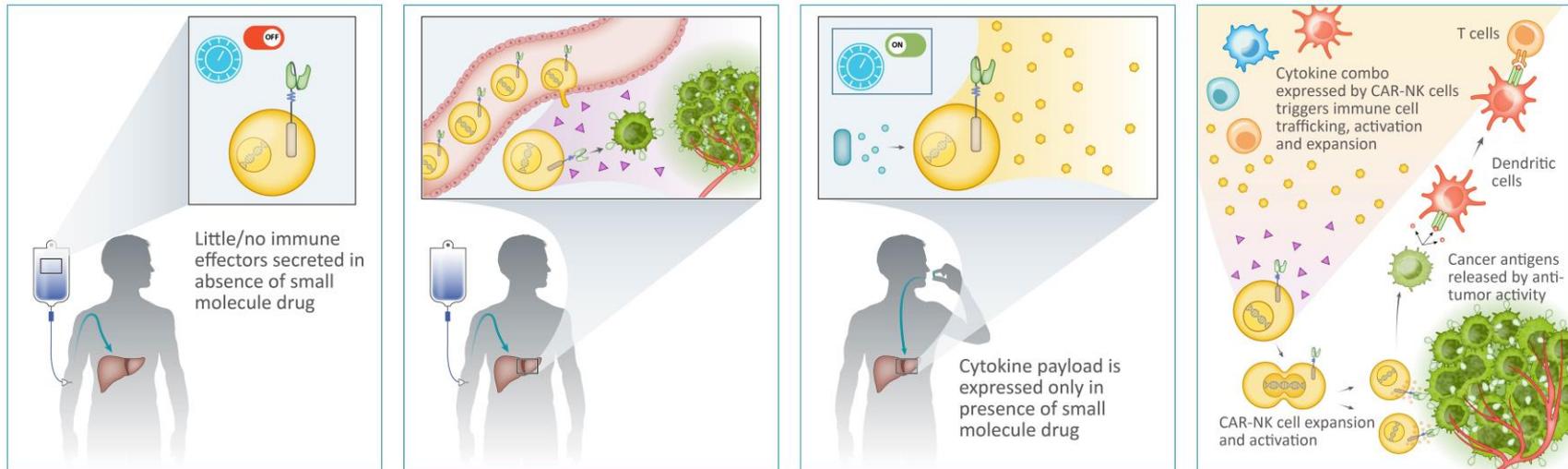
Regulator Dial enabled dose dependent and reversible IL-12 production *in vivo*





Regulator Dial could enable us to control expression of potent immune effectors for immune cell therapies

Armed, Controlled Therapy enabled by Regulator Dial



Regulator Dial has the potential to enable the following benefits:

- **Optimized** for **safe**, low expression of potent cytokines in the absence of small molecule drug and **strong, dose dependent** induction of cytokine production in the presence of small molecule drug
- **Versatile** to regulate potentially any payload of interest
- **Convenient** regulation by **orally dosed, FDA-approved small molecule**



Acknowledgements

Thank you to the fantastic team at Senti Biosciences



and our collaborators in the Khalil lab
at Boston University!



See our other Senti Abstracts:

Title: Precise Targeting of AML with First-in-Class OR / NOT Logic-Gated Gene Circuits in CAR-NK Cells

Garrison et al. (abstract 77)

Title: Precise Tumor Targeting with NOT Logic-Gated Chimeric Antigen Receptor Gene Circuits

Frankel et al. (abstract 960)