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

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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

Cancer Cell

Engineered Cell

Persistence

Engineered Cells

Immune Cell Recruitment and Tumor Killing

Engineered Cell

Attack

Cancer Cell
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

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**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

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<th>Regulator Dial Transcription Factor Parts</th>
<th>Controlled Payload</th>
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<td>DNA binding domain</td>
<td>minimal promoter</td>
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<td>Transcriptional activation domain</td>
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<td>cytokine payload</td>
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- 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 is a cytokine driven by a promoter that contains a binding site for the Regulator Dial DNA binding domain

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

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

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

**Regulator Dial Transcription Factor Parts**

**Controlled Payload**

- **Presence of Small Molecule Drug**
  - + NS3 inhibitor

- **NS3 protease**
  - NS3i small molecule suppresses protease activity, resulting in an intact transcription factor
  - 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

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**

- Transcriptional activation domain
- DNA binding domain
- TF binding site
- Fluorescent reporter

**Small molecule sensitivity**

Asunaprevir NS3i Concentration (uM) vs. Fluorescent reporter (AU)

Regulator Dial small molecule sensitivity graph:

- X-axis: Asunaprevir NS3i Concentration (uM)
- Y-axis: Fluorescent reporter (AU)

Graph shows an increase in fluorescent reporter activity with increasing concentrations of Asunaprevir NS3i.
Regulator Dial small molecule sensitivity

Regulator Dial

Small molecule sensitivity

Transcriptional activation domain

DNA binding domain

TF binding site

Fluorescent reporter

Fluorescent reporter (AU)

Asunaprevir NS3i Concentration (uM)

Regulator Dial small molecule sensitivity

ASV Cmax
Senti’s Design, Build, Test, Learn cycle was applied to improve Regulator Dial small molecule sensitivity.
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Senti’s Design, Build, Test, Learn cycle was applied to improve 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

Cmin to Cmax 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**

Grazoprevir dosing

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

**hIL-12 in mouse plasma**

Day 4

**Day 8**
Regulator Dial could enable us to control expression of potent immune effectors for immune cell therapies

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

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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)