Engineered IMiD Regulated Synthetic Transcriptional Switch for Controlled and Dose-Responsive Expression of Therapeutic Payload within FDA-Approved Drug Doses

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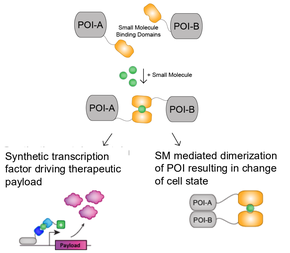
Synthetic Switches Responsive to FDA-Approved Small Molecules

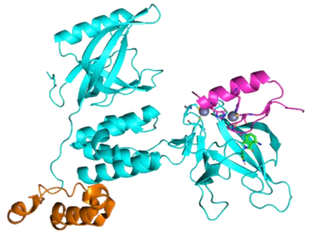


Application of small molecule (SM)-regulated switches. Many SM-sensor platforms have been developed, but few are suitable as a platform for proximity-based or degradation-based therapeutics. Here, we utilize IMiD co-binders fused to proteins of interest (POIs) to create a SM regulated proximity-based transcriptional switch and separately, a degradation-based switch for regulation at the protein level.

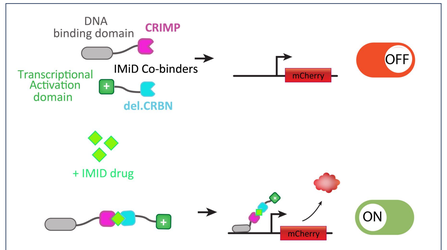
Available SM-based switches	FDA-approved & Convenient mode of delivery	Beneficial pharmacokinetics	Crosses Blood-Brain-Barrier (BBB)	
Grazoprevir	✓	✗	✗	*Tague, E, et al. Nat Methods 2018*
Rimiducid (rapamycin rapalogs)	✓	✓	✗	*Rivera VM, et al. Nat Med 1996*
Caffeine	▬	✗	✓	*Bojar, D, et al. Nat Commun 2018*
★ IMiD	✓	✓	✓	*Ebert, B, et al. Sci Trans Med 2021*

Characteristics of existing small molecule regulated switches. Few SM-sensor platforms have been developed that respond to FDA-approved SMs and have beneficial pharmacokinetics for feasible therapeutic application. IMiDs were focused on for further development because of their unique ability to cross the BBB in addition to their ability to induce degradation of IKZF3/1 class of proteins.

Design of Transcriptional Switches Regulated by IMiDs

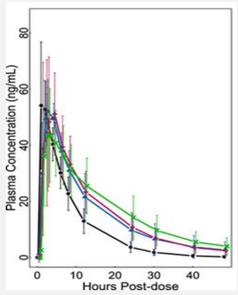


SM dimerizable parts:
CRBN in complex with CRIMP and pomalidomide. (figure generated by collaborators, Aplomex)
DDB1 subdomain (orange) is removed to prevent CRBN (teal) from complexing with E3 Ub ligase complex. The DDB1 deleted protein is subsequently referred to as del.CRBN. A ZF target of CRBN, which we call the CRIMP domain (pink), complexes with CRBN only in the presence of pomalidomide (green small molecule).



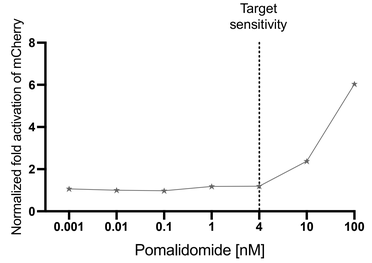
Transcriptional Switch Design. A transcriptional switch responsive to IMiD was built by fusing a modified version of CRBN (del.CRBN), with the DDB1 domain removed to prevent its association with E3 ubiquitin ligase complex, to a ZF DNA binding domain and co-expressing it with a second fusion of CRIMP, an IMiD co-binder derived from IKZF3 protein, to an activation domain. Switch performance was evaluated using an mCherry reporter assay.

Improving Sensitivity of the Small Molecule Binding Domains



Determination of target pomalidomide sensitivity of switch from pomalidomide PK. (healthy liver (black) concentrations peak within 6 hours of oral dosing at ~220 nM and then quickly decline to ~12 nM within 24 hours of initial dose (PMID: 29746728). In rats, it was observed that 39% of pomalidomide crosses the BBB (PMID: 23940785). Based on these two studies, we calculated our target concentration to be **4 nM Pomalidomide.**

Performance of existing SM-regulated transcriptional switch

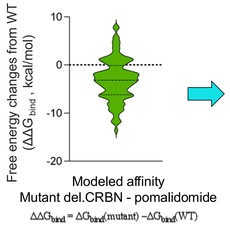


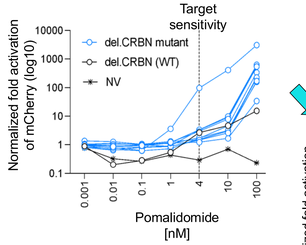
*Switch is responsive to 100 nM of pomalidomide. Platform **requires improvement** to unlock clinical applications.*

Parallel optimization of both binding domains

del.CRBN domain

Modeled binding affinity of del.CRBN mutants to pomalidomide in collaboration with Aplomex and selected mutants for *in vitro* screening (right)





CRIMP domain

Flow-seq performed to identify candidate CRIMP mutations with improved sensitivity to pomalidomide

Library of single CRIMP mutations

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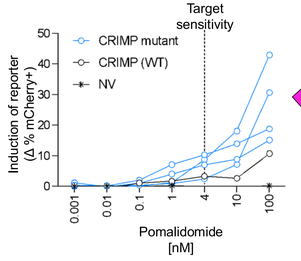
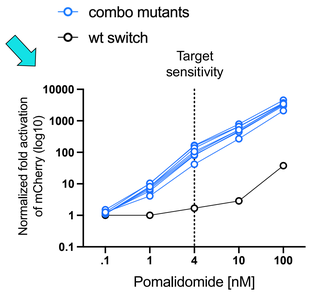
Sequential sorting for library members responsive to decreasing concentrations of pomalidomide



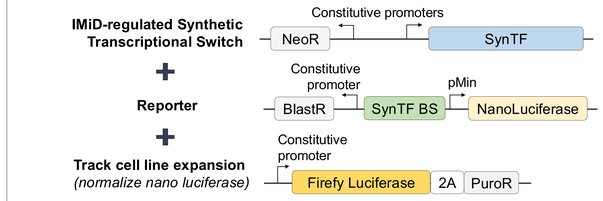
Full switch with improved del.CRBN & CRIMP domains



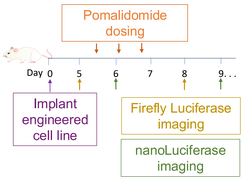
Lead del.CRBN mutants and CRIMP mutants demonstrated activity at and below target sensitivity of 4 nM pomalidomide. *In vitro* validation of selected candidates from the CRIMP and del.CRBN library identified several variants with improved sensitivity to pomalidomide compared to their wildtype counterparts.

In vivo Application of the IMiD Responsive Transcriptional Switch

Cell line created for *in vivo* evaluation



Study Design



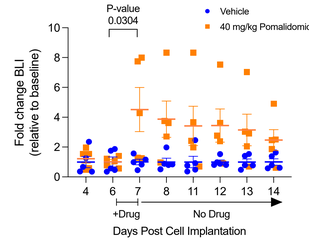
1. Normalization of Nanoluc across tumor burden:

$$Normalized\ NanoLuc = \frac{NanoLuc}{Firefly\ Luc}$$

2. Fold change of Nanoluc reporter compared to vehicle:

$$Fold\ activation = \frac{Normalized\ NanoLuc_{Drug\ treated\ mice}}{Normalized\ NanoLuc_{Vehicle\ treated\ mice}}$$

Induction of nanoLuciferase *in vivo*



IMiD transcriptional switch functions *in vivo*. 4-fold induction of reporter was detected in mice treated with max tolerated dose of Pomalidomide (40mg/kg) by oral gavage (orange).

Conclusions and Next Steps

Conclusions

- Optimization of IMiD-responsive binding domains by computational design and high throughput screening yielded transcriptional switches that induce payload expression at physiologically relevant concentrations of pomalidomide *in vitro*.
- Engineered IMiD binding domains are sensitive to pomalidomide at concentrations expected in the serum and brain of patients following an FDA-approved dosing regimen, enabling the applications in the clinic.
- We have demonstrated function of an IMiD responsive transcriptional switch *in vivo* resulting in robust activation of reporter payload.

Next Steps

- Evaluate performance of IMiD transcriptional switch in primary cells regulating effectors known to improve efficacy of cell therapies.

Acknowledgments:
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