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Signal 1 boosters for Tmod: addressing the next obstacle in cell therapy for solid tumors

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ABSTRACT

Background: Cell therapies for solid tumors are associated with unique challenges compared with liquid tumors, such as lower access to tumor tissues that express target antigen. Antigen (signal 1) is the ignition and fuel for T cell responses and is necessary to induce chimeric antigen receptor (CAR) T-cell activation and expansion. Efforts to boost cell therapy activity have focused on improving the sensitivity of the CAR ligand-binding domains and enhancing the CAR through added intracellular domains from other signaling proteins (eg, CD28 and 4-1BB), thus boosting sensitivity and persistence while preserving antigen dependence. Little effort, other than vaccination [1], has been expended to enhance signal 1 (antigen), which may be essential to optimize responses in solid tumors. We sought to create a signal 1 booster that mimics an antigen stimulus with a small molecule that triggers signaling by the CAR or T-cell receptor (TCR).

Methods: Expression constructs were designed to control tonic signaling of T cells engineered with CARs and TCRs. These constructs contained FK506-binding protein (FKBP), ligand-binding domains that mediate protein multimerization in the presence of the small molecule rimiducid (**Figure 3**) [2].

Results: A variety of constructs, including fusions of FKBP to LAT or to CD3ε, were shown to produce the desired effects on signaling. In the absence of rimiducid, these constructs produced only small elevations of signaling in Jurkat or primary T cells. The addition of rimiducid induced dose-dependent increases in signaling 2- to 28-fold, independent of antigen (**Figure 6**). The constructs also enhanced signaling in the context of Tmod™, a dual-receptor NOT gate based on the LIR-1 inhibitory receptor (**Figure 4**) [3].

Conclusions: Signal 1 boosters that mimic an antigen stimulus with a small molecule enable the activation of CAR or TCR in the absence of antigen. The signal 1 mimetics allow tuned stimulation, which could improve the quality and performance of the T cell product in patients. Critically, they bypass the need for antigen exposure in the patient's blood, a key difference between blood and solid tumor therapy.

Figure 1: T-cell receptor signaling

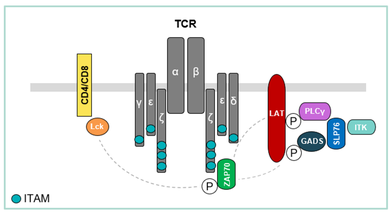


Figure 1: A schematic depicting T-cell activation and a portion of proximal signaling molecules involved in TCR signaling. Upon antigen recognition, CD4 or CD8 engage with Lck resulting in recruitment of ZAP70 to the TCR. ZAP70 phosphorylates LAT, that then recruits several signaling proteins regulating TCR responses (e.g. GADS, PLCγ, SLP76, ITK, etc.).

CAR EXPRESSION CORRELATES WITH TONIC SIGNALING

Tmod is a dual-receptor NOT gate system with an activator (accelerator) and a blocker (brake) module [3]. In the context of Tmod, higher levels of CAR (activator) expression increases tonic signaling as measured by CD25 expression (**Figure 2**) [3].

Figure 2: Increasing CAR expression increases tonic signaling in cells engineered with Tmod

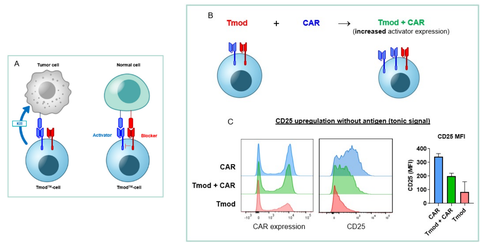


Figure 2: (A) Tmod cells target a surface antigen present on normal and tumor cells and a second surface antigen present in normal tissues that triggers the blocker, preventing cytotoxicity [3]. (B) Additional CAR constructs were transduced with Tmod constructs to increase the amount of activator expression. (C) Increased activator expression (left) correlated with antigen-independent signaling measured via CD25 MFI (center, right).

SIGNAL 1 BOOSTERS ARE INDUCIBLE WITH FKBP FUSIONS

Some efforts to boost performance of CAR-Ts have centered on downstream elements of TCR signaling (e.g., LAT, SLP76, Lck, etc. [4,5]. These have in some cases produced new designs for CARs that extend beyond the classical ITAMs of TCRs. LAT is an adaptor molecule phosphorylated after TCR activation that serves as a scaffolding complex for the recruitment of enzymes and signaling molecules involved in downstream events of T-cell activation (**Figure 1**). A LAT-FKBP fusion protein can be brought to CAR-FKBP via dimerization of FKBP domains with the small molecule rimiducid, triggering signaling in the absence of antigen (**Figure 3A**). The addition of rimiducid induced a dose-dependent increase in signaling 3-fold (**Figure 3B**).

Figure 3: Addition of rimiducid to Jurkat cells harboring FKBP fusions triggers tonic signaling

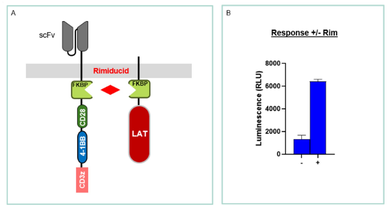


Figure 3: (A) Platform to induce activation of T-cell signal 1, independent of antigen engagement. TCR signaling molecules, such as LAT, are induced to dimerize with the CAR via FKBP domains and a small molecule (rimiducid), triggering CAR signaling. (B) Tonic signaling in Jurkat cells expressing the signal 1 booster construct after 6 hours of treatment with or without 50 nM rimiducid.

SIGNAL 1 BOOSTERS WORK WITH TMOD

The LAT-FKBP fusion protein also enhanced signaling in the context of Tmod (**Figure 4**). LAT, without an FKBP domain in combination with Tmod, increases baseline signaling, but a dose-dependent signal of rimiducid-induced oligomerization is achieved with Tmod and LAT containing FKBP (**Figure 4**).

Figure 4: Signal 1 booster in the context of Tmod

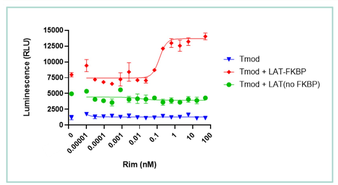


Figure 4: Signaling in Jurkat cells expressing Tmod and the LAT-FKBP construct after 6 hours of treatment with titrated rimiducid.

A RANGE OF SIGNAL 1 BOOSTERS HAVE BEEN IDENTIFIED

FKBP fusions can function as rimiducid-dependent signal 1 boosters in many contexts. Similar to activation of CARs, an FKBP domain can be added to CD3ε to oligomerize the endogenous TCR and other proximal signaling proteins involved in T-cell activation (**Figure 5**).

Figure 5: Signal 1 molecules can be recruited to the CAR/TCR/LAT

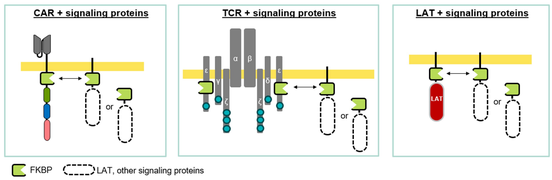
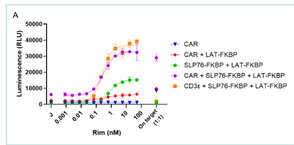
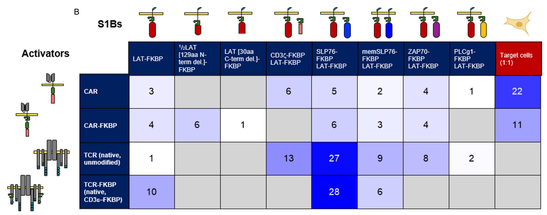


Figure 5: A schematic demonstrating how signal 1 fusions to FKBP can be applied across several activation strategies. LAT or other signaling proteins can be oligomerized with the CAR (left), the TCR (middle), or LAT (right).

Figure 6: A range of signal 1 boosters have been identified

SLP76 is a component of the ITAM signaling pathway and is recruited to the LAT complex after T-cell activation (**Figure 1**). Inclusion of SLP76 with an FKBP domain can enhance activation and is one of many examples of a signaling protein that can be oligomerized with the CAR, TCR or LAT (**Figure 6**).





	LAT-FKBP	½LAT (129aa N-term del.)-FKBP	LAT (30aa C-term del.)-FKBP	CD3ζ-FKBP LAT-FKBP	SLP76-FKBP LAT-FKBP	memSLP76-FKBP LAT-FKBP	ZAP70-FKBP LAT-FKBP	PLCg1-FKBP LAT-FKBP	Target cells (1:1)
CAR	3			6	5	2	4	1	22
CAR-FKBP	4	6	1		6	3	4		11
TCR (native, unmodified)	1			13	27	9	8	2	
TCR-FKBP (native, CD3ε-FKBP)	10				28	6			

Figure 6: (A) Signaling in Jurkat cells expressing CAR or CD3ε and the LAT-FKBP construct or that of another proximal signaling molecule with an FKBP fusion. SLP76-FKBP, after 6 hours of treatment with titrated rimiducid. (B) A table summarizing different induction ratios of signal 1 boosters in Jurkat cells (+/- rimiducid; mem: membrane bound).

Visit poster #292 to hear about a high-throughput screen to identify and optimize signal 1 boosters.

THREE INDUCIBLE SYSTEMS WORK WITH SIGNAL 1 BOOSTERS

Figure 7: At least three different clinically-approved molecules work as S1B inducers

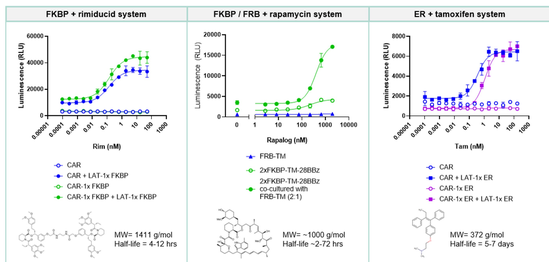


Figure 7: Signaling in Jurkat cells expressing a CAR and the signal 1 booster LAT construct with titrated small molecule rimiducid (left), rapamycin analog (rapalog, center) (FRB: FKBP-rapamycin-binding), or tamoxifen (right) (ER: estrogen receptor).

A SIGNAL 1 BOOSTER INDUCES IFN-γ SECRETION IN PRIMARY T CELLS

LAT-FKBP also induced interferon-gamma (IFN-γ) secretion in primary T cells. IFN-γ is a cytokine that plays a role in many functions of T cells including activation, proliferation and cytolytic activity [4]. Donor cells co-transduced with CAR and LAT-FKBP fusions secrete IFN-γ in a dose dependent manner when incubated with titrated rimiducid (**Figure 8**).

Figure 8: Addition of rimiducid to primary T cells harboring FKBP fusions triggers signaling

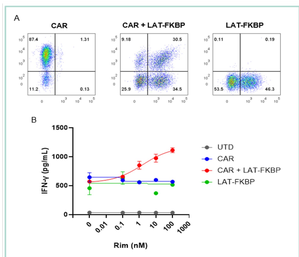


Figure 8: (A) Co-transduction and expression of CAR and LAT-FKBP lentivirus in primary T donor cells measured by flow cytometry. CAR expression is shown on the y-axis and LAT-FKBP expression is shown on the x-axis (B) Tonic signaling in donor cells after 24 hours of treatment with titrated rimiducid as measured by secreted IFN-γ.

CONCLUSIONS and NEXT STEPS:

- Access to tumor tissues that express target antigen is severely restricted by the blood vessel walls.
- With limiting antigen, it is unclear how antigen-dependent boosters can be brought into action.
- We address this problem by mimicking antigen with a small molecule that triggers signaling by the CAR or TCR.
- The signal 1 booster allows tuned stimulation to potentially improve the quality and performance of the T cell product in patients.
- The signal 1 booster is complementary to signal 3 boosters.

TUNED SIGNAL 1 AND SIGNAL 3 IN IMMUNE CELLS

With the selective power of Tmod, an inducible system can be utilized to combine several boosters to enhance potency. An NFAT-regulated signal 3 cytokine booster can be combined to amplify activation by the signal 1 booster (**Figure 9**).

Figure 9: Development of a cell therapy that has tumor selectivity with tunable activity

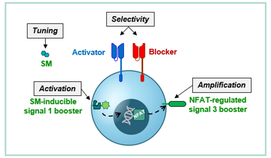


Figure 9: Tmod constructs engineered with selectivity via activator and blocker receptor, a signal 1 booster under the control of a small molecule (SM) which in turn controls a downstream NFAT-regulated signal 3 booster.

Visit poster #341 to hear about signal 3 boosters and Tmod.

References

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