A New Class of Targeted and Conditional Therapeutics Using a Novel Dual-Binding Antibody-Based Platform

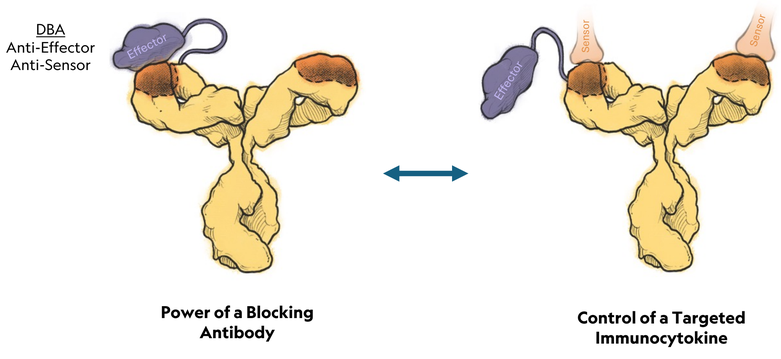
Justin R. Killebrew, Shannon L. Okada, Lynn M. Amon, David Bienvenue, Laura A. Carlucci, David W. Colby, Wendy Curtis, Kendyl Daniels, Alton Etheridge, Zane Kraft, Jamie Nguyen, Sandra Notonier, Jacqueline Pham, Megan Sprague, Kerri Thomas, Diane Hollenbaugh, and John T. Mulligan

Bonum Therapeutics, Inc., Seattle, WA

Introduction

How It Works: Dual-Binding Antibody-Based Regulation

Dual Binding Antibody (DBA): Recognizes two distinct antigens
Standard antibody and linker components
Regulation domains are portable to multiple formats

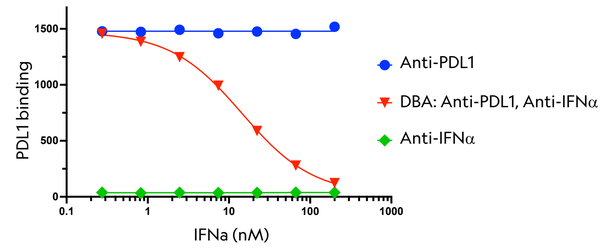


Power of a Blocking Antibody

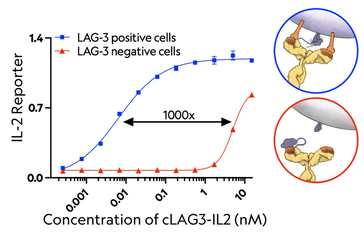
Control of a Targeted Immunocytokine

Competition between the two antigens of a DBA

DBA scFv binds to PD-L1 and IFNα, but not at the same time
Binding of the PD-L1 scFv to PD-L1 is blocked by IFNα



Tethered cytokine is "off" until bound to the sensor



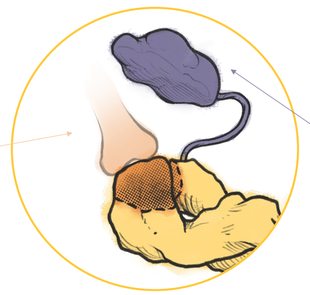
cLAG3-IL2 is active on LAG-3⁺ cells but not on cells lacking LAG-3 expression

In vitro activity of cLAG3-IL2 on LAG-3-transfected (red) or mock transfected (blue) IL2 HEK-Blue reporter cells

Where the Technology Applies

Sensor can be anything that can be bound by an antibody:

- Cell surface protein
- Soluble protein
- Small molecule
- Protein modification



Effector can be anything that can be fused to an antibody:

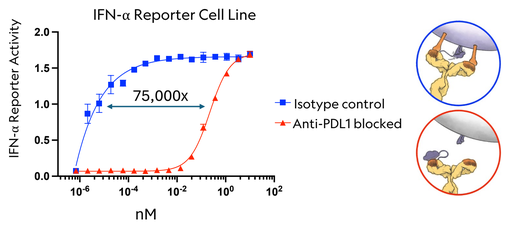
- Cytokine
- Receptor ECD
- Growth Factor
- Agonist mAb
- Blocking mAb

Bonum's current pipeline is focused on cytokines and receptors in immuno-oncology

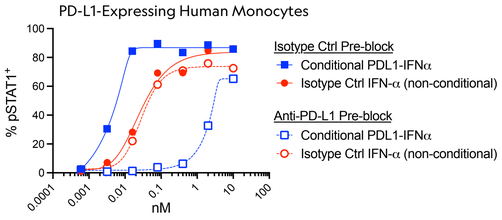
The technology is broadly applicable across potential biologic therapeutics

PDL1-IFNα – Demonstrates Efficacy and Offness

Conditional Signaling Activity in Cell-based Assays

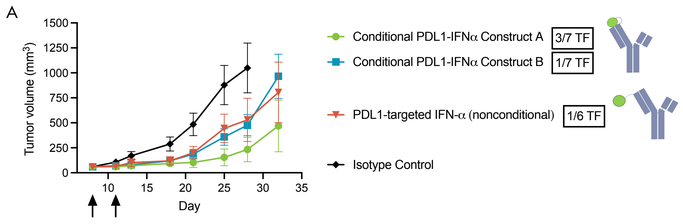


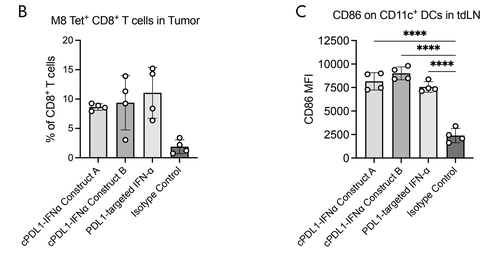
Conditional PDL1-IFNα activity on PD-L1-expressing IFN-α reporter cells pre-blocked with either anti-PD-L1 (red) or an isotype control (blue).



Human monocytes were treated with IFN-γ to induce PD-L1 expression. Cells were then pre-blocked with either anti-PD-L1 or an isotype control prior to treatment with conditional PDL1-IFNα or non-conditional isotype control IFN-α for 60 min. pSTAT1 was assessed by flow cytometry.

PDL1-IFNα Inhibits Tumor Growth and Drives Anti-Tumor Immunity

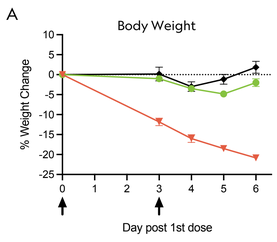




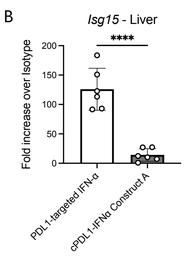
huPD-1/huPD-L1 KI mice bearing huPD-L1-expressing MC38 tumors were dosed I.V. on days 8 and 11 with conditional PDL1-IFNα, non-conditional PD-L1-targeted IFN-α, or an isotype control at 5 mg/kg. Tumor volume was measured twice weekly (A). Tissues were harvested on day 13 from a subset of mice to assess frequency of intratumoral M8 tet⁺ T cells (B) and expression of CD86 on DCs within the tumor-draining lymph node (C). *P* values were determined using one-way ANOVA with Tukey post hoc test. **** p<0.0001

PDL1-IFNα – Demonstrates Efficacy and Offness

PDL1-IFNα Shows Minimal Activity in the Absence of PD-L1 Binding

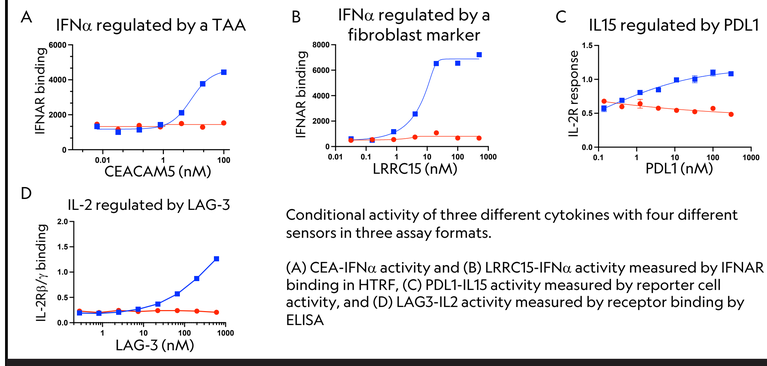


The PDL1-IFNα DBA does not bind mouse PD-L1, and therefore remains in an inactive conformation in WT mice



C57BL/6 mice were dosed I.V. on days 0 and 3 with conditional PDL1-IFNα, non-conditional PD-L1-targeted IFN-α, or an isotype control at 5 mg/kg. Body weight was measured daily (A). On day 5, *Isg15* expression in the liver was assessed by qPCR (B). *P* values were determined using student's t test **** p<0.0001

Breadth Demonstrated by Multiple Pairs



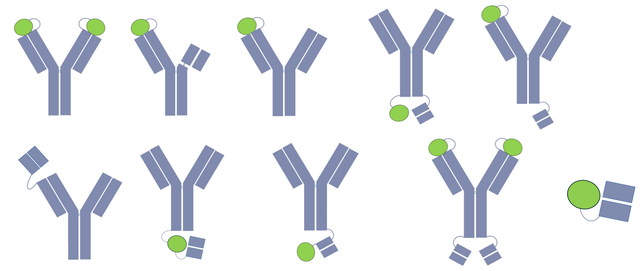
Conditional activity of three different cytokines with four different sensors in three assay formats.

(A) CEA-IFNα activity and (B) LRRC15-IFNα activity measured by IFNAR binding in HTRF, (C) PDL1-IL15 activity measured by reporter cell activity, and (D) LAG3-IL2 activity measured by receptor binding by ELISA

Multiple Formats to Address Specific Biology

Multiple formats have been employed that explore the impact of

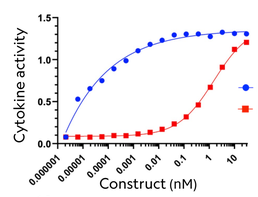
Valency for the Sensor
Valency for the Effector
Geometry
Effector Modality

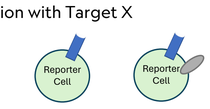


Regulation in Multiple Cell Assay Formats

Regulation in Reporter cells

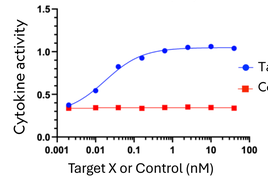
Reporter cells +/- transfection with Target X

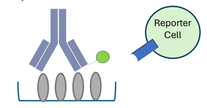




Sensor captured on a plate

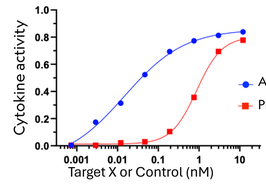
Target X is captured on a plate

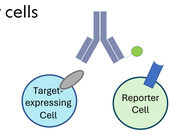




Sensor provided by a cocultured cell

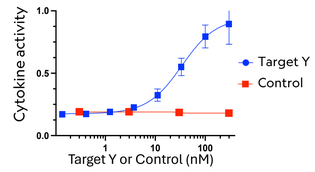
Target X⁺ cells mixed with reporter cells





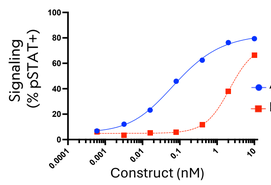
Sensor is a soluble factor

Target Y monovalent in solution with reporter cells



Regulation in Primary Cells

Primary cells that express Target Z and respond to the effector



Conclusions

- Our proprietary dual-binding antibody (DBA)-based platform allows the creation of targeted and conditionally active biologics with broad potential across therapeutic areas
- We have explored multiple sensors, effectors, and formats demonstrating the power and flexibility of the platform
- Our technology can be applied to the regulation of any functional protein moiety including agonistic and antagonistic antibody binding domains, growth factors, and receptors
- We are applying our technology to multiple sensors, including LAG-3, PD1, PDL1, ATP, and LRRC15 and to multiple effectors including IL-2, IFN-α, IL-12 and TGFβ inhibition