# Hitting the Gas: Quantitative Cell-based Bioassays to Advance Immunotherapy Programs Targeting Co-stimulatory Immune Checkpoint Receptors

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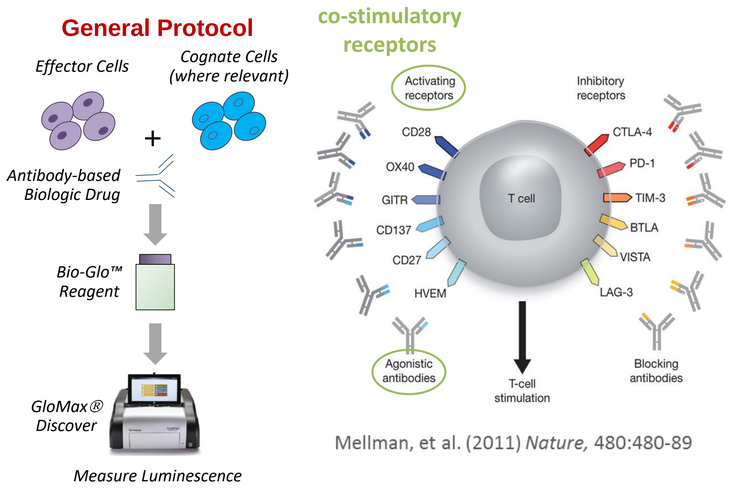
## 1. Introduction

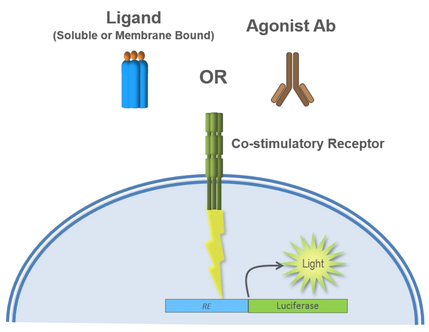
A major challenge in the development of antibody-based biologics drugs is access to quantitative and reproducible functional bioassays. Existing methods rely on primary cells and measurement of complex functional endpoints that are cumbersome, variable, and often fail to yield data quality required for drug development in a quality-controlled environment. We have developed a portfolio of functional cell-based reporter bioassays to measure the activity of biologics drugs designed to target immune checkpoint receptors including co-inhibitory (e.g. PD-1, CTLA-4, LAG-3, TIM-3) and co-stimulatory (e.g. 4-1BB, GITR, OX40, CD40, ICOS, CD28) receptors. These bioassays consist of stable cell lines that express luciferase under the precise control of receptor-mediated intracellular signals. Here we describe the application of MOA-based immune checkpoint co-stimulatory receptor bioassays for biologics drug discovery, development, potency and stability studies.




Mellman, et al. (2011) *Nature,* 480:480-89

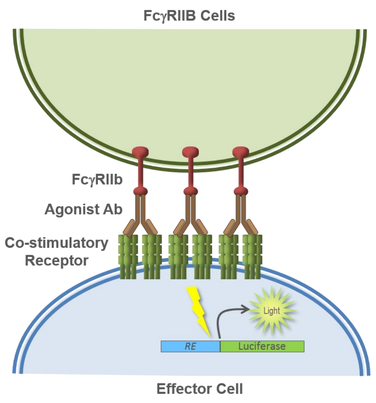
## 2. Co-stimulatory Bioassay Design

**Co-stimulatory Bioassays without FcγRIIb Crosslinking**




**Co-stimulatory receptor:**
**4-1BB (CD137)**
**GITR**
**OX40**
**CD40**
**CD28**
**ICOS**
**HVEM/LIGHT**
**CD27 (data not shown)**
**DR3 (data not shown)**

**Co-stimulatory Bioassays with FcγRIIb Crosslinking**



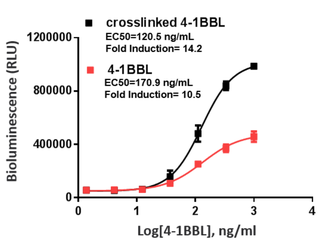

Increasing evidence suggests that crosslinking anti-TNFR agonist Abs *in vivo* via engagement of FcγR, particularly FcγRIIb, enhances receptor clustering and downstream signaling.

Therefore, functional analysis of Ab biologics in the context of FcγR crosslinking is critical in drug development.

- White, et al. (2011) *J Immunol*, 187:1754-63
- Wilson, et al. (2011) *Cancer Cell*, 19:101-13
- Li and Ravetch (2011 *Science*, 1030-34

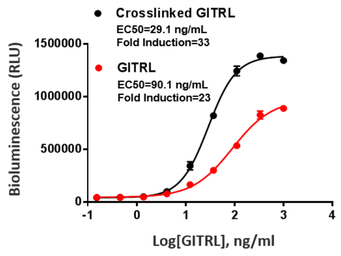
## 3. Ligand-induced Agonist Activity

**4-1BB Bioassay**



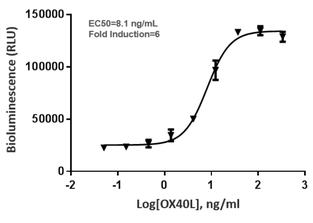

rh4-1BB Ligand (His tag) – R&D Systems (#2295-4L-025)
anti-His tag Ab – BioLegend (#652502)

**GITR Bioassay**



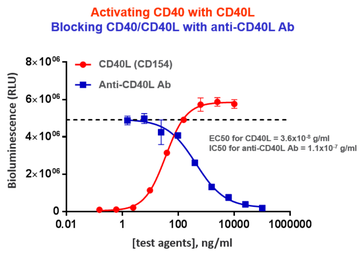

rhGITR Ligand (HA tag) – R&D Systems (#6987-GL-025)
anti-HA tag Ab – R&D Systems (#MAB060)

**OX40 Bioassay**




rhOX40 Ligand – BioLegend (#555706)

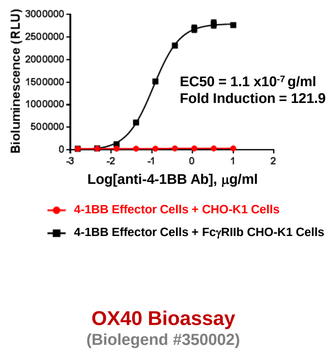
**CD40 Bioassay**



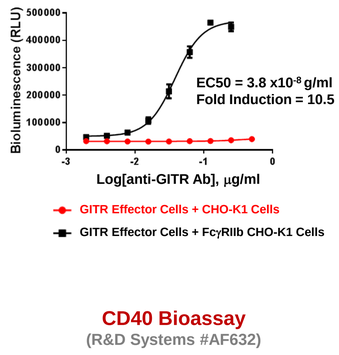

anti-hCD40L Ab – BioLegend (#310828)

## 4. FcγRIIb-dependent Agonist Activity

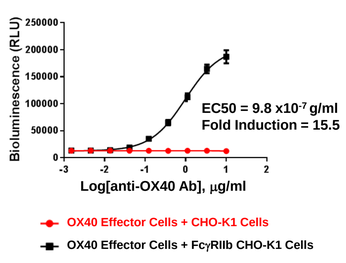
**4-1BB Bioassay**
(Biolegend #309811)



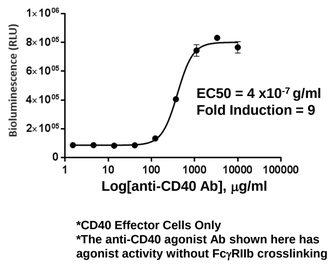

**GITR Bioassay**
(R&D Systems #MAB689)




**OX40 Bioassay**
(Biolegend #350002)

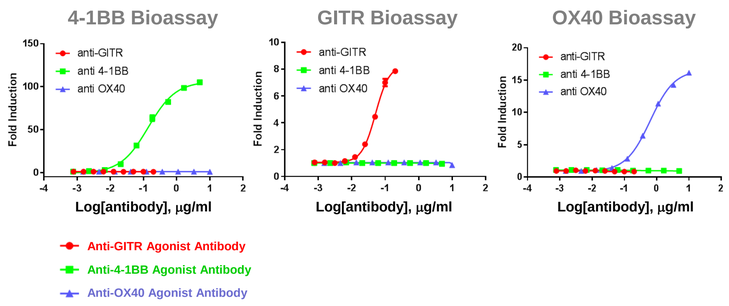



**CD40 Bioassay**
(R&D Systems #AF632)

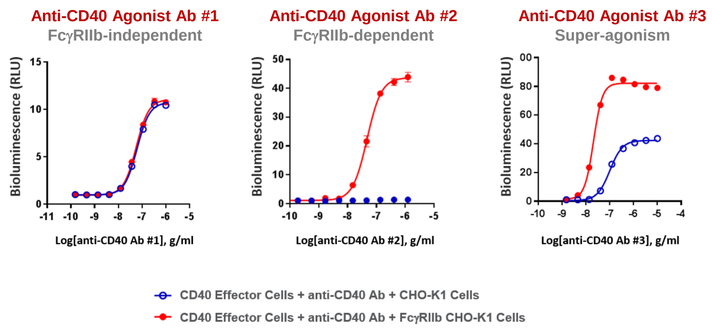



*CD40 Effector Cells Only
*The anti-CD40 agonist Ab shown here has agonist activity without FcγRIIb crosslinking

### Co-stimulatory Bioassay Specificity

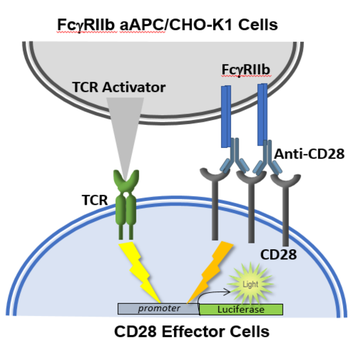



### Panel of anti-CD40 Agonist Antibodies

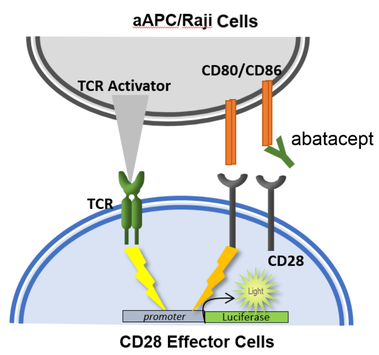



## 5. CD28 Bioassays for Agonist & Blocking Abs

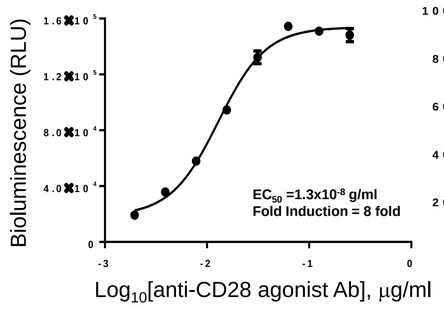
**CD28 Agonist Bioassay**



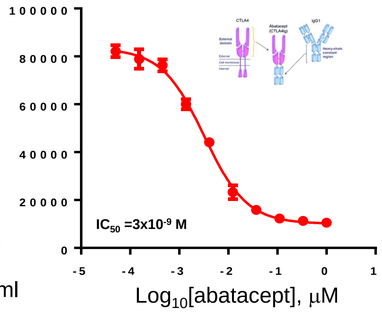

**CD28 Blocking Bioassay**




**CD28 Agonist Bioassay**

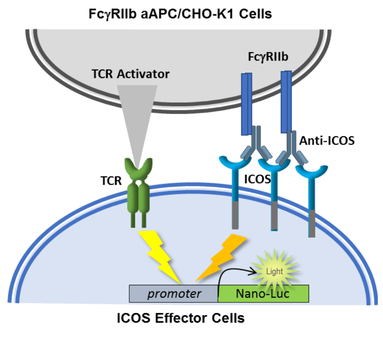



**CD28 Blocking Bioassay**

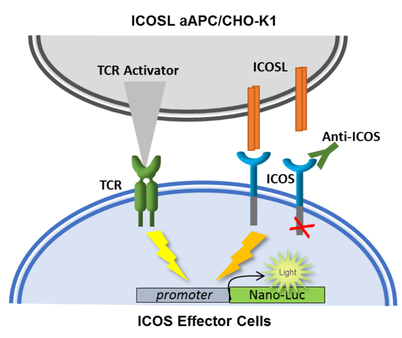



## 6. ICOS Bioassays for Agonist & Blocking Abs

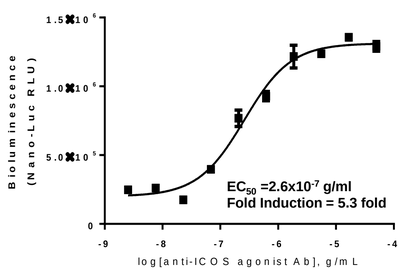
**ICOS Agonist Bioassay**



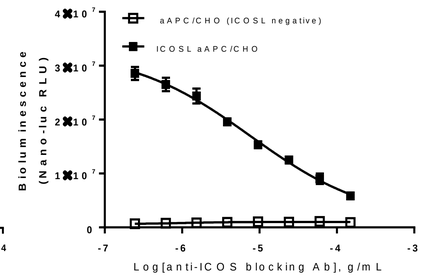

**ICOS Blocking Bioassay**



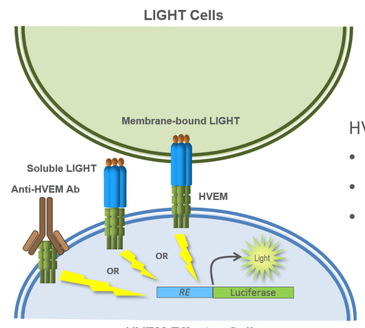

**ICOS Agonist Bioassay**




**ICOS Blocking Bioassay**



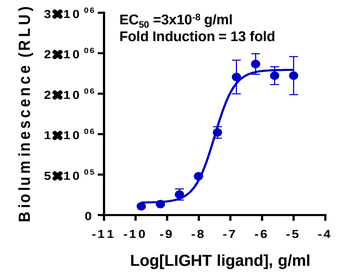

## 7. HVEM/LIGHT Bioassay



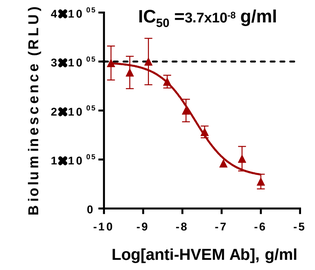

HVEM Effector Cells can be activated by:

- Agonist Ab
- Soluble LIGHT Ligand
- Membrane-bound LIGHT Ligand

**Activation with LIGHT Ligand**




**Inhibition of LIGHT signaling with anti-HVEM blocking Ab**




## 8. Conclusions

Cell-based reporter bioassays overcome the limitations of primary cell-based assays for functional characterization of antibody and other biologics drugs targeting individual or combination immune checkpoint receptors. Here we show a portfolio of MOA-based bioassays for co-stimulatory immune checkpoint receptors that can be used for antibody screening, characterization, potency and stability studies. These bioassays provide the following:

**Biologically relevant measurement of antibody MOA**

- Specific immune checkpoint regulated expression of luciferase that reflects the native biology of immune cells.
- Demonstrated critical role of FcγRIIb in modulating antibody activity, consistent with published data using primary cells.

**Consistent and reliable, easy to implement**

- All assays can be used as “Thaw-and-use” cell format, no cell culture required
- Rapid and convenient workflow
- Amenable to standard 96-well and 384-well plate formats





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