1358-D

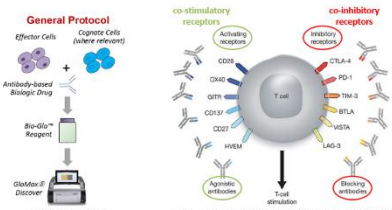
Quantitative Cell-Based Bioassays to Advance Immunotherapy Programs Targeting Immune Checkpoint Receptors

Jamison Grailer, Julia Gilden, Pete Stecha, Denise Garvin, Jun Wang, Michael Beck, Jim Hartnett, Frank Fan, Mei Cong and Zhi-jie Jey Cheng

Promega Corporation, 2800 Woods Hollow Rd, Madison, WI 53711

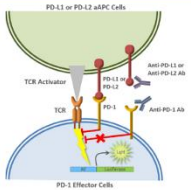
1. Abstract

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 drug discovery, development, potency and stability studies. 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) and co-stimulatory (e.g., 4-1BB, GITR, OX40) 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 these MOA-based bioassays for biologics..



Mellman, I, Coukos, G & Dranoff, G. (2011) *Nature* 480:480-489.

2. PD-1 Blockade Bioassays



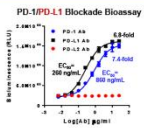
Assay Design

(1) TCR engagement induces luciferase activity

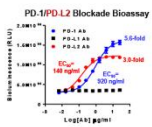
(2) Co-engagement of PD-1 with PD-L1 or PD-L2 inhibits luciferase activity

(3) Ab-mediated blockade of PD-1/PD-L1 or PD-1/PD-L2 restores luciferase activity

PD-1/PD-L1 Blockade Bioassay



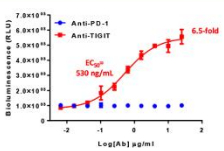
PD-1/PD-L2 Blockade Bioassay



Top: TCR-mediated luciferase activity is recovered in the PD-1/PD-L1 bioassay with anti-PD-1 or anti-PD-L1 blocking Abs, but not with anti-PD-L2 blocking Ab.

Bottom: TCR-mediated luciferase activity is recovered in the PD-1/PD-L2 bioassay with an anti-PD-L2 blocking Ab, but not with anti-PD-1 or anti-PD-L1 blocking Abs. All Abs shown here are research grade.

3. TIGIT Blockade Bioassay



Assay Design

(1) TCR engagement induces luciferase activity

(2) When TIGIT is absent, CD226/CD155 provides a co-stimulatory signal

(3) When TIGIT is present, TIGIT competes with CD226 for binding to CD155 and inhibits luciferase activity

(4) Ab-mediated blockade of TIGIT/CD155 restores luciferase activity

TCR- and CD226-mediated luciferase activity is recovered in the TIGIT/CD155 bioassay with an anti-TIGIT blocking Ab (clone MBSA-43), but not with an anti-PD-1 blocking Ab (nivolumab).

Similar results were achieved using the CTLA-4 Blockade Bioassay (data not shown).

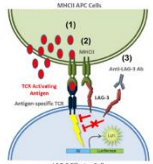
4. LAG-3 Blockade Bioassay

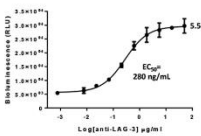
Assay Design

(1) Antigen-dependent activation of the TCR induces luciferase activity

(2) Co-engagement of LAG-3 inhibits luciferase activity

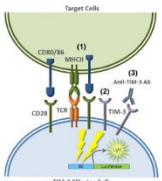
(3) Ab-mediated blockade of LAG-3 binding to its ligand restores luciferase activity

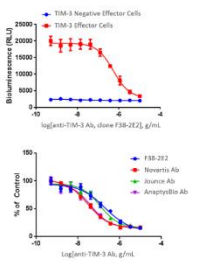




TCR-mediated luciferase activity is recovered in the LAG-3 bioassay with an anti-LAG-3 blocking Ab (clone 17B4).

5. TIM-3 Bioassay





Assay Design

(1) TCR activation and CD28 engagement with CD80/86 induce luciferase activity

(2) Expression of TIM-3 enhances TCR/CD28-mediated activation and luciferase activity

(3) Blockade of TIM-3 inhibits luciferase activity

Top: TCR/CD28-mediated luciferase activity is enhanced with expression of TIM-3 in Effector Cells. TIM-3-mediated luciferase activity is inhibited in a dose-dependent manner with the anti-TIM-3 blocking Ab, clone F38-2E2.

Bottom: TIM-3-mediated luciferase activity is inhibited in a dose-dependent manner with clone F38-2E2 and blocking antibodies from Novartis, Jounce and AnaptysBio.

6. Combination Bioassays

PD-1+LAG-3 Combination Bioassay

Assay Design

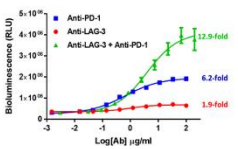
(1) PD-L1 and MHC-II are expressed on aAPC cells

(2) PD-1 and LAG-3 are co-expressed on effector cells

(3) TCR activation induces luciferase activity

(4) Engagement of PD-1/PD-L1 and LAG-3/MHC-II inhibits luciferase activity

(5) Blockade of PD-1/PD-L1 and/or LAG-3/MHC-II restores luciferase activity

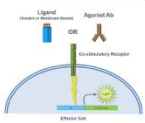


Anti-PD-1 (clone EH12.2H7) or anti-LAG-3 (clone 17B4) blocking Abs induce a 6.2 and 1.9-fold increase in luciferase activity, respectively. A combination of both Abs induces an 12.9-fold increase in activity.

Similar results were achieved using the PD-1+TIGIT and PD-1 + CTLA-4 Combination Bioassays (data not shown).

7. Co-Stimulatory Bioassays

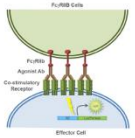
Co-Stimulatory Bioassays without FcγRIIb Crosslinking



Co-Stimulatory receptor:

- 4-1BB (CD137)
- GITR
- OX40
- CD40
- HVEM/LIGHT *(data not shown)*
- CD27 *(data not shown)*
- DR3 *(data not shown)*

Co-Stimulatory Bioassays with FcγRIIb Crosslinking

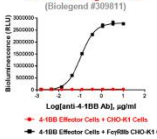


Increasing evidence suggests that crosslinking anti-TNFR agonist Abs in vivo via engagement of FcγR, particularly FcγRIIb, enhances receptor clustering and downstream signaling.

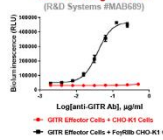
Therefore, functional analysis of Ab biologics in the context of FcγR crosslinking is critical in drug development.

- White, *et al.* (2011) *J Immunol*, 187:1754-63
- Wilson, *et al.* (2011) *Cancer Cell*, 19:101-13
- Li and Ravetch (2011) *Science*, 333:1030-34

Anti-4-1BB Agonist Ab (Biolegend #309811)



Anti-GITR Agonist Ab (R&D Systems #MAB689)

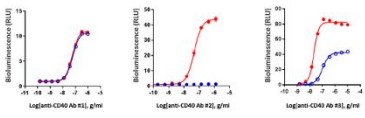


Similar agonist activity is observed using 4-1BB, GITR, OX40 and CD40 ligands (data not shown).

CD40 Agonist Ab #1 FcγRIIb-independent

CD40 Agonist Ab #2 FcγRIIb-dependent

CD40 Agonist Ab #3 Super-agonism



Three anti-CD40 agonist antibodies were analyzed using the CD40 Effector Cells and CHO-K1 Cells either with (RED) or without (BLUE) FcγRIIb crosslinking, as indicated. Similar results were achieved using anti-4-1BB, anti-GITR and anti-OX40 agonist antibodies using the relevant bioassay (data not shown).

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-inhibitory and co-stimulatory immune checkpoint receptors that can be used for antibody screening, characterization, potency and stability studies.

Biologically relevant measurement of antibody MOA

- Specific immune checkpoint regulated expression of luciferase that reflects the native biology of T cell activation.
- Demonstrated ability to measure the potencies of immune checkpoint-targeted antibodies

Consistent and reliable measure of antibody activity

- Demonstrated precision, accuracy, reproducibility, robustness
- All assays can be used as "Thaw-and-use" cell format, no cell culture required
- Functional performance suitable for development into potency, stability, and NAb assays

Easy-to-implement

- Rapid and convenient workflow
- Amenable to standard 96-well and 384-well plate formats

Corresponding author: Jey.Cheng@promega.com