Quantitative Cell-Based Reporter Gene Bioassays to Advance Individual or Combination Cancer Immunotherapy

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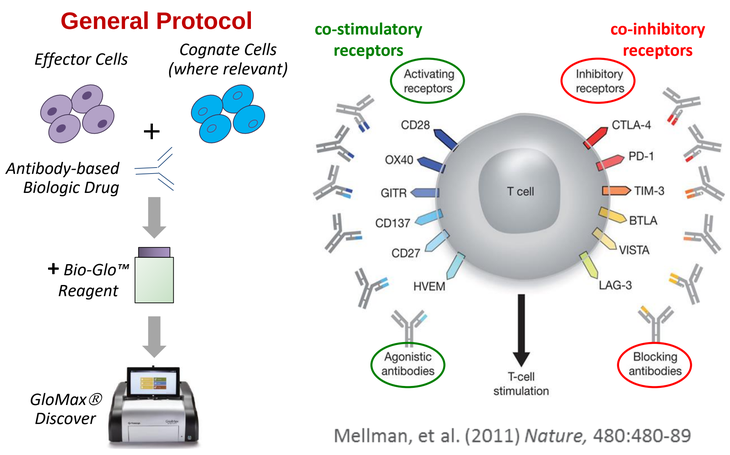
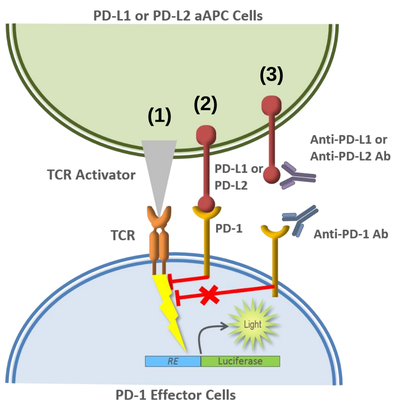
1. Introduction

A major challenge in the development of antibody-based biologics drugs is access to quantitative and reproducible functional bioassays. In contrast to the cumbersome, variable methods currently used that rely on primary cells, we have developed a portfolio of functional cell-based reporter bioassays to easily 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 drug discovery, development, potency and stability studies.



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

2. PD-1 Blockade Bioassays



Assay Design

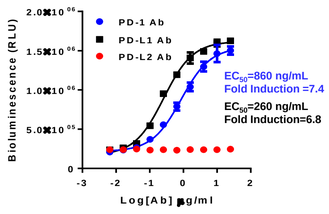
Luciferase activity indicating activation of T Cells is

(i) induced by TCR engagement

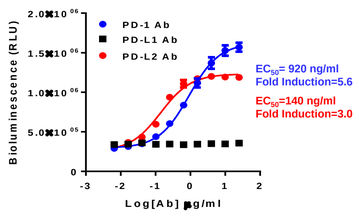
(ii) inhibited by co-engagement of PD-1 with PD-L1 or PD-L2

(iii) restored by Ab-mediated blockade of PD-1/PD-L1 or PD-1/PD-L2

PD-1/PD-L1 Blockade Bioassay



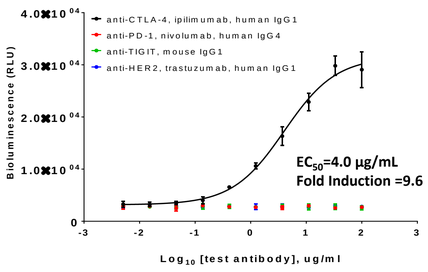
PD-1/PD-L2 Blockade Bioassay



TCR mediated luciferase activity is recovered in the PD-1 Blockade Bioassay with (**LEFT**) anti-PD-1 and PD-L1 blocking Abs and anti-PD-1 and PD-L2 blocking Abs (**RIGHT**), but not with unrelated control antibodies.

3. CTLA-4 and TIGIT Blockade Bioassays

CTLA-4 Blockade Bioassay



Assay Design

Luciferase activity indicating activation of T Cells is

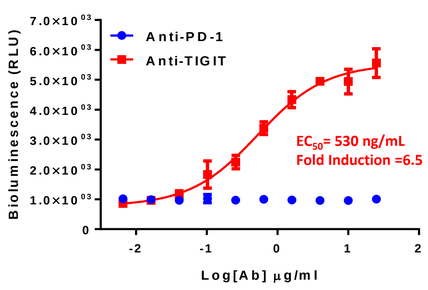
(i) induced by activation of TCR and engagement of CD28 with CD80/86

(ii) inhibited by engagement of CTLA-4 with CD80/86

(iii) restored by Ab-mediated blockade of the CTLA-4/CD80/86 interaction

TCR and CD28-mediated luciferase activity is recovered in the CTLA-4 bioassay with an anti-CTLA-4 blocking Ab (ipilimumab), but not with unrelated control antibodies.

TIGIT/CD155 Blockade Bioassay



Assay Design

Luciferase activity indicating activation of T Cells is

(i) induced by activation of TCR and engagement of CD226 with CD155

(ii) inhibited by engagement of TIGIT with CD155

(iii) restored by Ab-mediated blockade of the TIGIT/CD155 interaction

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).

4. LAG-3/MHCII Blockade Bioassay

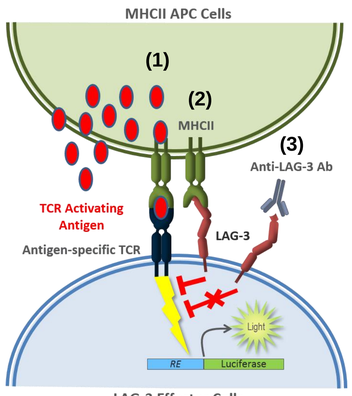
Assay Design

Luciferase activity indicating activation of T Cell is

(i) Induced by TCR engagement

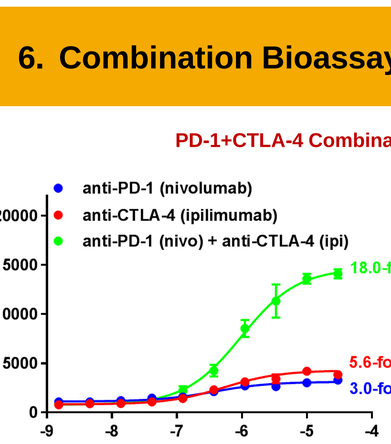
(ii) Inhibited by co-engagement of LAG-3

(iii) restored by Ab-mediated blockade of LAG-3 binding to its ligand



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

5. TIM-3 Bioassay



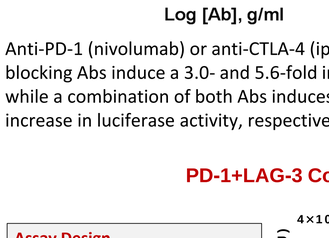
Assay Design

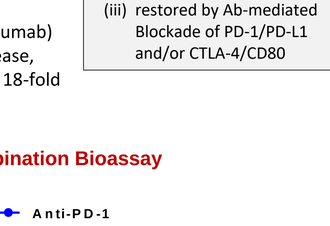
Luciferase activity indicating activation of T Cells is

(i) Induced by TCR activation and CD28 co-stimulation with CD80/86

(ii) enhanced by expression of TIM-3

(iii) Inhibited by blockade of TIM-3

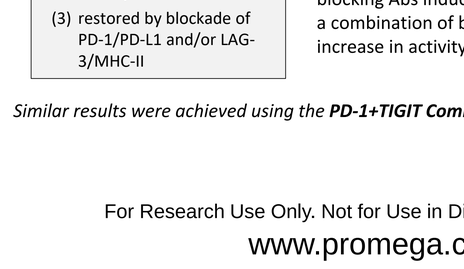




TCR/CD28-mediated luciferase activity is enhanced with expression of (**LEFT**) TIM-3 in effector cells, which is inhibited in a dose-dependent manner with the anti-TIM-3 blocking Ab. TIM-3-mediated luciferase activity is inhibited (**RIGHT**) in a dose-dependent manner with clone F38-2E2 and TIM-3 blocking antibodies from Novartis, Jounce, and AnaptysBio.

6. Combination Bioassays

PD-1+CTLA-4 Combination Bioassay



Anti-PD-1 (nivolumab) or anti-CTLA-4 (ipilimumab) blocking Abs induce a 3.0- and 5.6-fold increase, while a combination of both Abs induces an 18-fold increase in luciferase activity, respectively.

Assay Design

PD-L1 and CD80 are expressed on aAPC cells and PD-1, CTLA-4 and CD28 are co-expressed on effector cells. Luciferase activity indicating activation of T Cell is

(i) Induced by activation of TCR and engagement of CD28 with CD80/86

(ii) Inhibited by engagement of PD-1/PD-L1 and CTLA-4/CD80

(iii) restored by Ab-mediated Blockade of PD-1/PD-L1 and/or CTLA-4/CD80

PD-1+LAG-3 Combination Bioassay

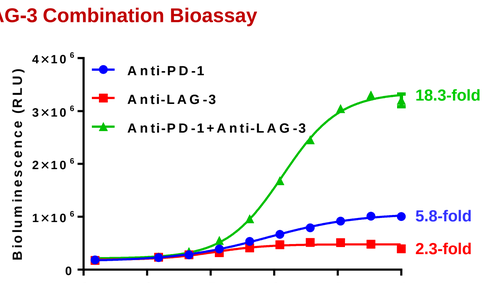
Assay Design

PD-L1 and MHC-II are co-expressed on aAPC cells. PD-1 and LAG-3 are co-expressed on effector cells. Luciferase activity indicating activation of T Cells is

(1) Induced by engagement of TCR

(2) Inhibited by engagement of PD-1/PD-L1 and LAG-3/MHC-II inhibits luciferase activity

(3) restored by blockade of PD-1/PD-L1 and/or LAG-3/MHC-II



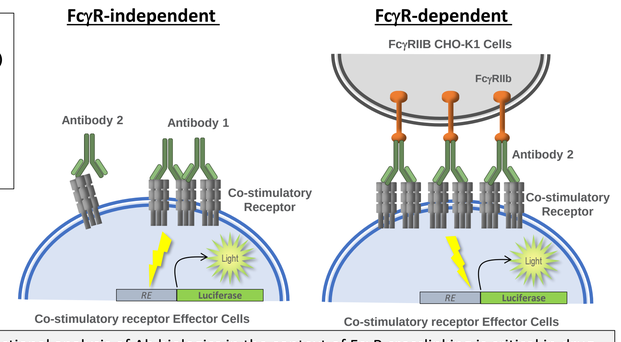
Anti-PD-1 (clone EH12.2H7) or anti-LAG-3 (clone 17B4) blocking Abs induce a 5.8- and 2.3-fold increase, while a combination of both Abs induces an 18.3-fold increase in activity, respectively.

*Similar results were achieved using the **PD-1+TIGIT Combination Bioassay** (data not shown).*

7. FcγR-independent and –dependent Co-stimulatory Bioassays

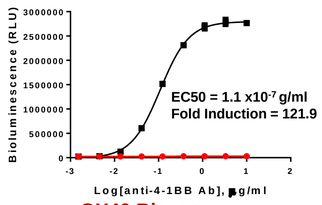
Co-stimulatory receptors:
- 4-1BB (CD137)
- GITR
- OX40
- CD40

Data not shown:
- HVEM/LIGHT
- CD27
- DR3

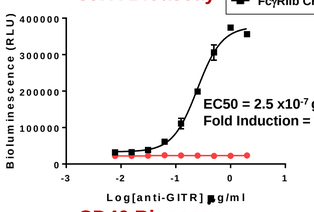


Assay Design: Functional analysis of Ab biologics in the context of FcγR crosslinking is critical in drug development since there is iIncreasing evidence that crosslinking anti-TNFSFR agonist Abs with FcγRIIb enhances receptor clustering and downstream signaling (**RIGHT**) whereas such cross-linking may not be always necessary (**LEFT**) to induce luciferase activity indicating activation of T Cells.

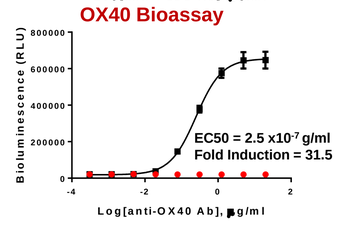
4-1BB Bioassay



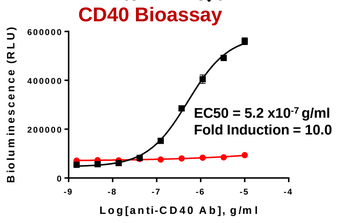
GITR Bioassay



OX40 Bioassay



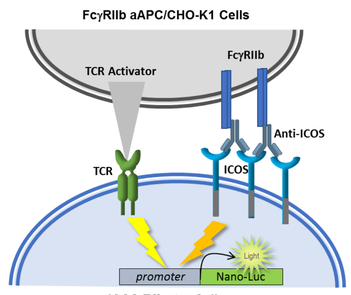
CD40 Bioassay

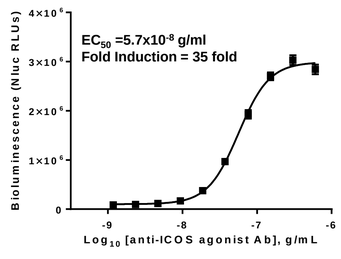


*Similar agonist activity is observed using **4-1BB, GITR, OX40 and CD40 ligands** (data not shown).*

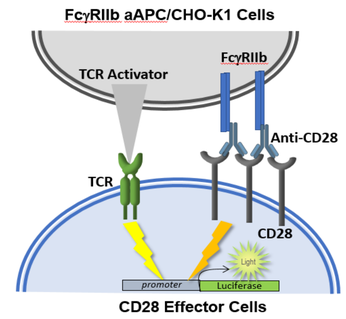
8. ICOS and CD28 Bioassays

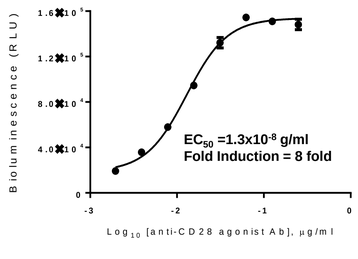
ICOS Agonist Bioassay





CD28 Agonist Bioassay





TCR mediated luciferase activity is enhanced by crosslinking of anti-ICOS agonist Ab by FcγRIIb (**UPPER**) and anti-CD28 agonist Ab crosslinked by FcγRIIb (**LOWER**)

***ICOS and CD28 Bioassays** for blocking antibodies are also available (data not shown).*

9. 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. These bioassays provide the following:

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

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