Quantitative Cell-Based Bioassays for Individual and Combination Immune Checkpoint Immunotherapy Targets

Zhi-jie Jey Cheng, Jamison Grailer, Pete Stecha, Jun Wang, Jim Hartnett, Frank Fan, and Mei Cong

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

1. Introduction

The human immune system is comprised of a complex network of immune checkpoint receptors that are promising new immunotherapy targets for the treatment of a variety of diseases including cancer and autoimmune-mediated disorders.

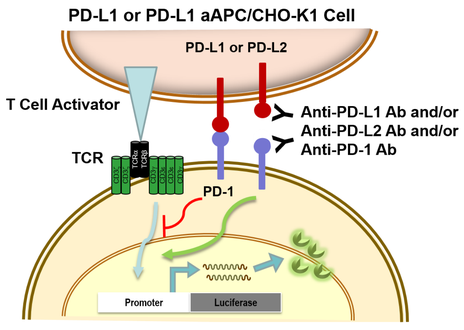
Current methods used to measure the activity of antibody and other biologics drugs designed to target immune checkpoint receptors rely on primary human cells and measurement of functional endpoints such as cell proliferation, cell surface marker expression, and cytokine production. These assays are laborious and highly variable due to their reliance on donor primary cells, complex assay protocols, and unqualified assay reagents. As a result, these assays are difficult to establish in quality-controlled drug development settings.

To overcome these challenges, we developed a suite of cell-based bioluminescent reporter bioassays for individual and combination immune checkpoint immunotherapy targets including:

- **PD-1 (PD-L1 or PD-L2), CTLA-4, LAG-3, TIGIT, PD-1+TIGIT**
- **GITR, 4-1BB, CD40, OX40**

These mechanism of action (MOA)-based bioassays are available in “thaw-and-use” format and demonstrate high specificity, sensitivity, and reproducibility. The bioassays are qualified according to ICH guidelines and demonstrate the performance required for use in antibody screening, potency testing, and stability studies.

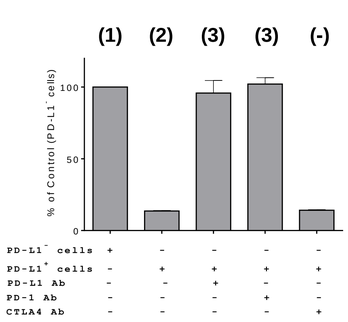
2. PD-1/PD-L1 and PD-1/PD-L2 Blockade Bioassay: Principle and Specificity



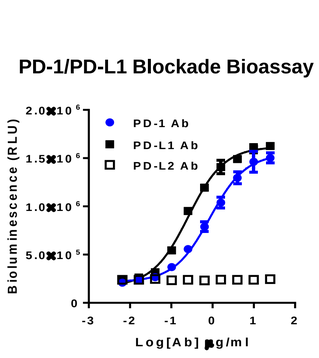
(1) TCR engagement induces luciferase activity

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

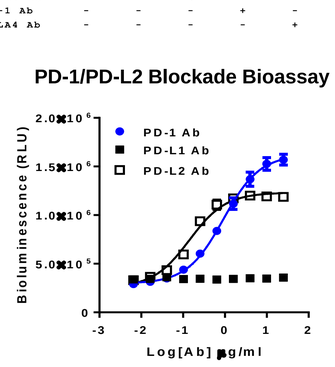
(3) Ab-mediated blockade of PD-1/PD-L1 or PD-1/PD-L2 restores luciferase activity



PD-1/PD-L1 Blockade Bioassay

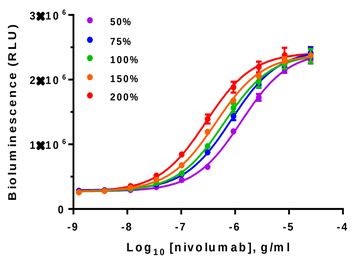


PD-1/PD-L2 Blockade Bioassay

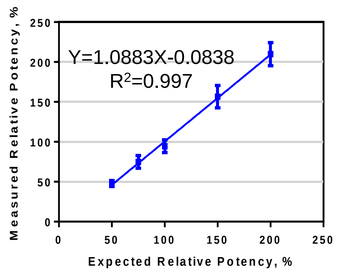


3. PD-1/PD-L1 Blockade Bioassay: Antibody Potency and Stability Studies

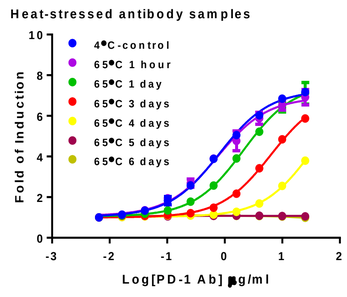
Measuring Ab Relative Potency



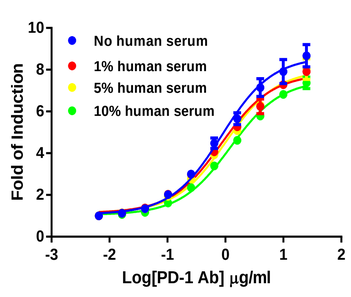
Assay Linearity



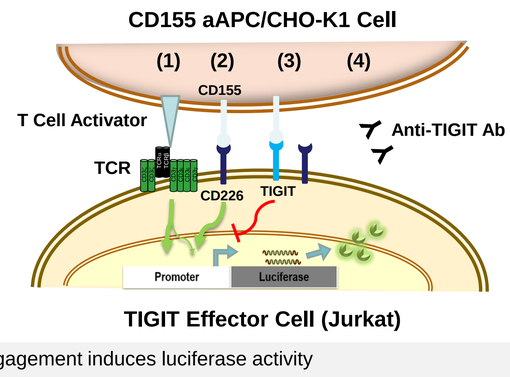
Measuring Ab Stability



Human Serum Tolerance
(potential application as a NAb assay)



4. TIGIT/CD155 Blockade Bioassay: Principle and Specificity



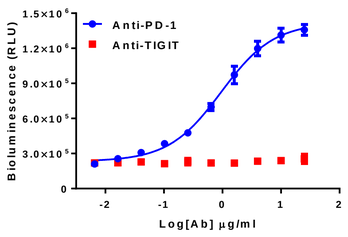
(1) TCR engagement induces luciferase activity

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

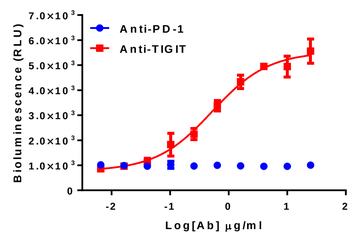
(3) When TIGIT is present, TIGIT competes with CD226 for binding to CD155 and inhibits luciferase activity

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

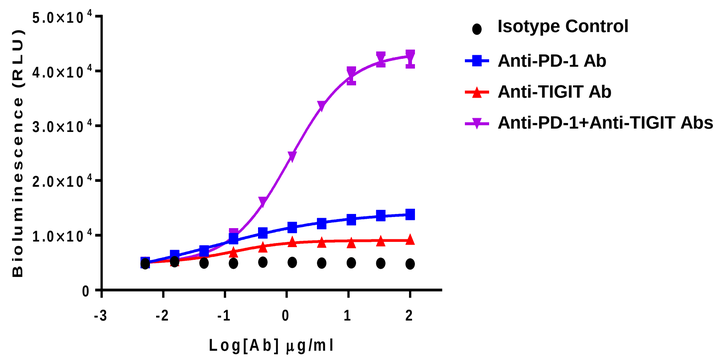
PD-1/PD-L1 Blockade Bioassay



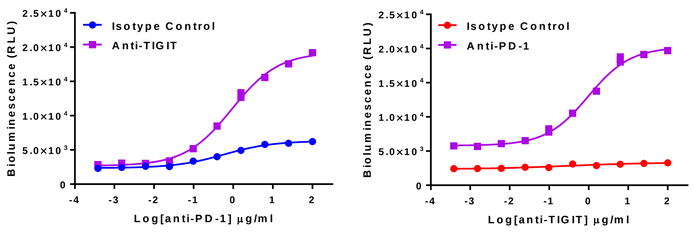
TIGIT/CD155 Blockade Bioassay



5. PD-1+TIGIT Combination Bioassay



Anti-PD-1 (BLUE) or anti-TIGIT (RED) blocking antibodies alone induced an increase in luciferase activity (2.9 and 1.8-fold, respectively). However, the combination of both blocking antibodies (PURPLE) showed a synergistic effect (8.8-fold).



Left Panel: An anti-PD-1 blocking antibody induced a robust assay response (8.4-fold) in the presence of an anti-TIGIT blocking antibody (PURPLE) but only a moderate response (2.8-fold) in the absence of anti-TIGIT antibodies (BLUE).

Right Panel: An anti-TIGIT blocking antibody induced a robust assay response (8.3-fold) in the presence of an anti-PD-1 blocking antibody (PURPLE) but only a moderate response (1.4-fold) in the absence of anti-TIGIT antibodies (BLUE).

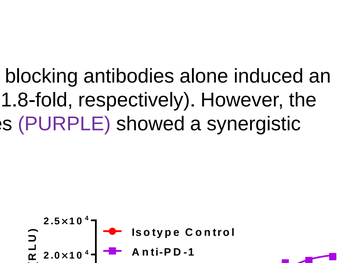
6. CTLA-4 Blockade Bioassay: Specificity and Antibody Potency Study

(1) TCR engagement induces luciferase activity

(2) Co-engagement of CTLA-4 with CD80:CD86 inhibits luciferase activity

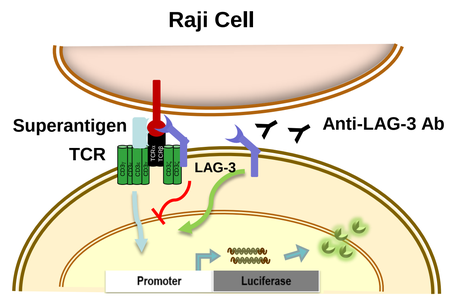
(3) Ab-mediated blockade of the CTLA-4 interaction restores luciferase activity

(4) An anti-PD-1 blocking antibody has no effect on luciferase activity



Potency Measurement of Ipilimumab (trade name Yervoy)

7. LAG-3 Blockade Bioassay: Principle and Potency Study

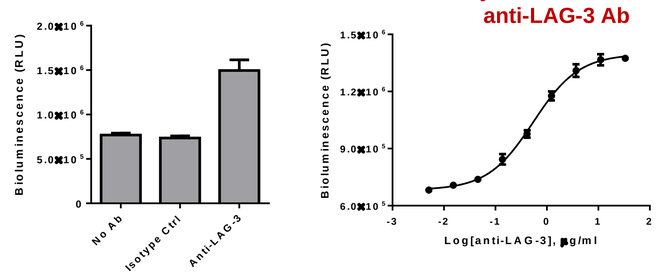


(1) Antigen-independent activation of the TCR using Superantigen induces luciferase activity

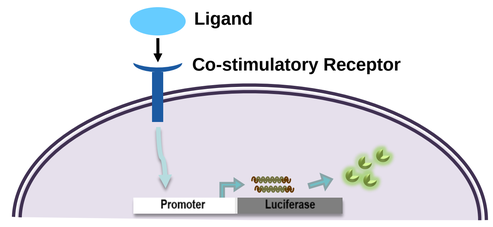
(2) Co-engagement of LAG-3 inhibits luciferase activity

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

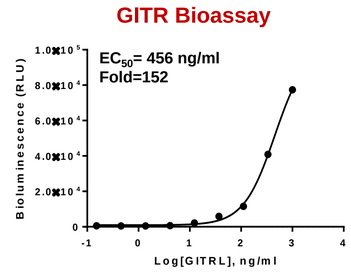
Potency Measurement of anti-LAG-3 Ab



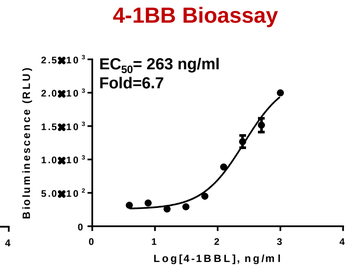
8. GITR, 4-1BB, OX40, CD40 Bioassays



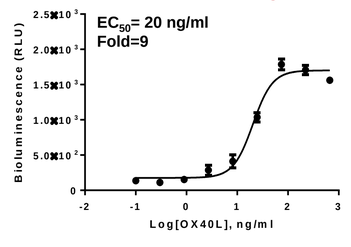
GITR Bioassay



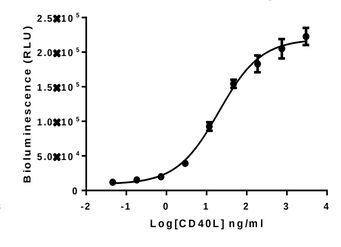
4-1BB Bioassay



OX40 Bioassay



CD40 Bioassay



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 immune checkpoint bioassays 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 native biology.
- Demonstrated ability to measure the activity of immune checkpoint-targeted antibodies

Consistent and reliable measure of antibody activity

- Demonstrated precision, accuracy, reproducibility, robustness
- “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

For Research Use Only