Quantitative Cell-Based Bioassays for Individual or Combination Immune Checkpoint Immunotherapy

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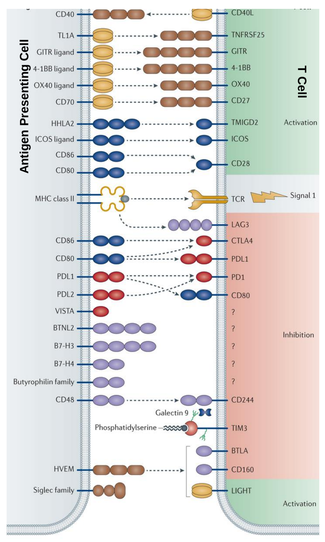
1. Introduction

Immunotherapy aims to boost a patient's own immune system to fight disease. Activation of T cells via direct stimulation of the T cell receptor or by modulating immune checkpoint pathways are two strategies being employed individually and in combination. Immune checkpoint targets include co-inhibitory (e.g. PD-1, CTLA-4, TIGIT, LAG-3) and co-stimulatory (e.g. GITR, 4-1BB, OX40, CD40) receptors.

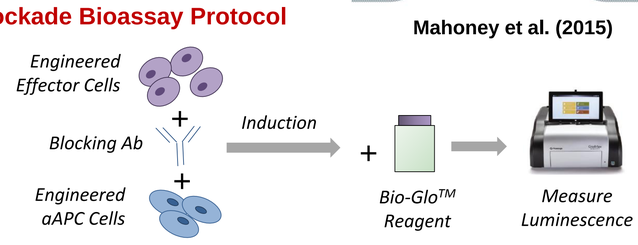
Here we describe the application of cell-based reporter bioassays for the development of therapeutic antibodies targeting co-inhibitory immune checkpoint receptors.

Immune Checkpoint Targets



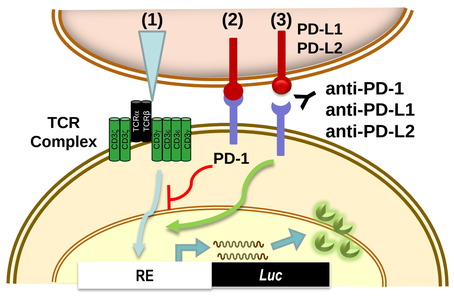
Mahoney et al. (2015)

Blockade Bioassay Protocol



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

PD-L1 or PD-L2 aAPC Cell



PD-1 Effector Cell

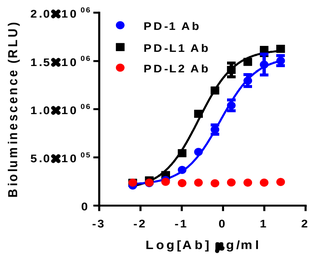
Assay Design

(1) TCR engagement induces luciferase activity

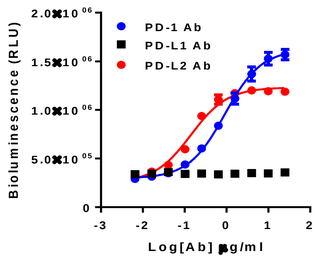
(2) Co-engagement of PD-1 with PD-L1 or PD-L2 inhibits luciferase activity

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

PD-1/PD-L1 Blockade Bioassay



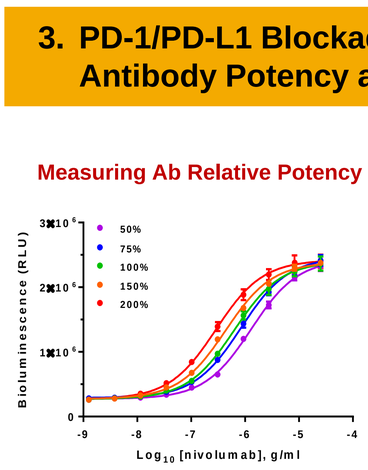
PD-1/PD-L2 Blockade Bioassay



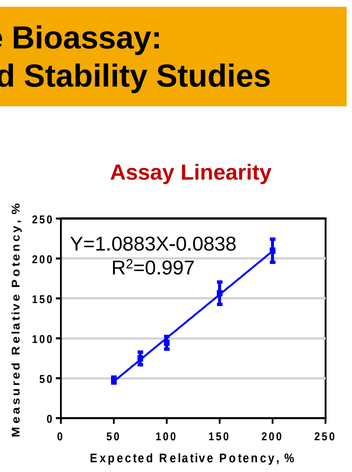
Left: TCR-mediated luciferase activity is recovered in the PD-1/PD-L1 bioassay with anti-PD-1 or anti-PD-L1 blocking Abs, but not with anti-PD-L2 blocking Ab. **Right:** TCR-mediated luciferase activity is recovered in the PD-1/PD-L2 bioassay with an anti-PD-L2 blocking Ab, but not with anti-PD-1 or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

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

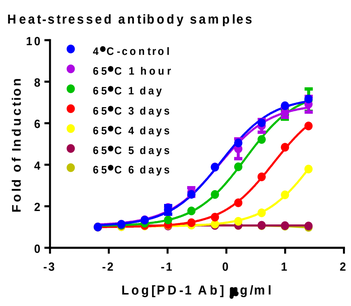
Measuring Ab Relative Potency



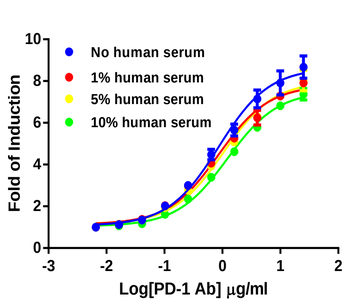
Assay Linearity



Measuring Ab Stability

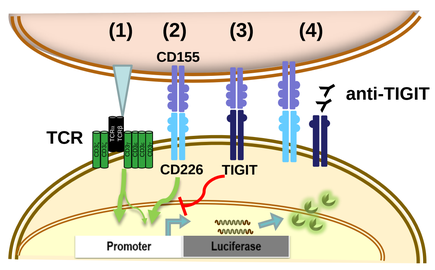


Human Serum Tolerance
(potential application as a NAb assay)



4. TIGIT/CD155 Blockade Bioassay: Principle and Specificity

CD155 aAPC Cell



TIGIT Effector Cell

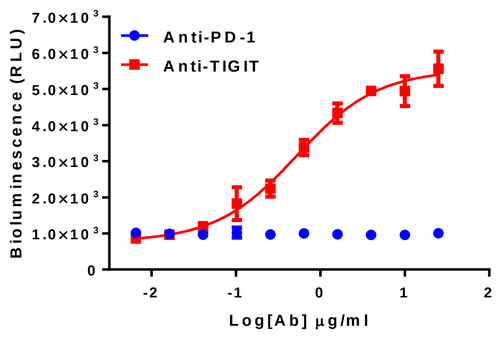
Assay Design

(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-mediated luciferase activity is recovered in the TIGIT/CD155 bioassay with an anti-TIGIT blocking Ab, but not with an anti-PD-1 blocking Ab.

5. PD-1+TIGIT Combination Bioassay: Synergy of anti-PD-1 & anti-TIGIT Blocking Abs

Assay Design

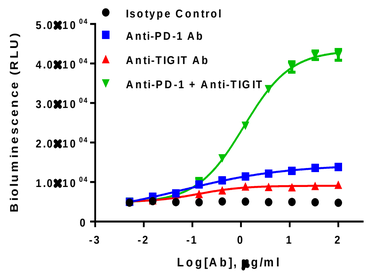
(1) PD-L1 and CD155 are expressed on aAPC cells

(2) PD-1, TIGIT, and CD226 are co-expressed on effector cells

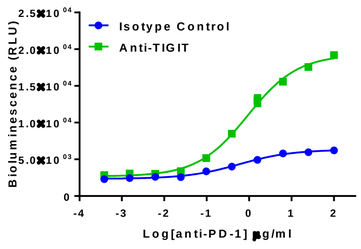
(3) TCR activation and CD226/CD155 engagement induce luciferase activity

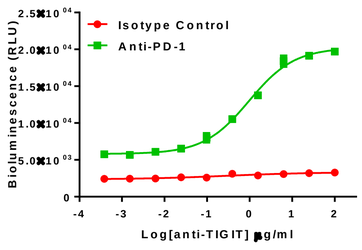
(4) Engagement of PD-1/PD-L1 and TIGIT/CD155 inhibits luciferase activity

(5) Blockade of PD-1/PD-L1 and/or TIGIT/CD155 restores luciferase activity



Anti-PD-1 or anti-TIGIT blocking Abs induce a 2.9 and 1.8-fold increase in luciferase activity, respectively. A combination of both Abs induces an 8.8-fold increase in activity.

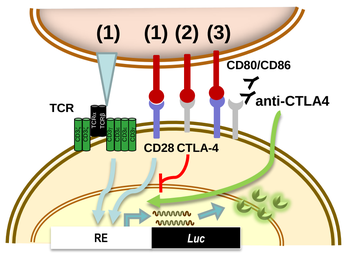




Left: An anti-PD-1 blocking Ab induces a robust assay response (8.4-fold) in the presence of an anti-TIGIT blocking Ab, but only a moderate response (2.8-fold) in the presence of an isotype control Ab. **Right:** An anti-TIGIT blocking Ab induces a robust assay response (8.3-fold) in the presence of an anti-PD-1 blocking Ab, but only a moderate response (1.4-fold) in the presence of an isotype control Ab.

6. CTLA-4 Blockade Bioassay: Principle and Specificity

aAPC



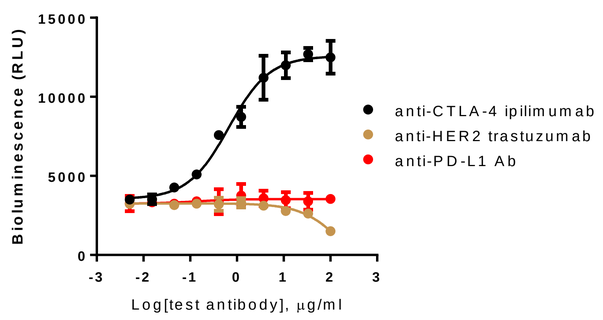
CTLA-4 Effector Cell

Assay Design

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

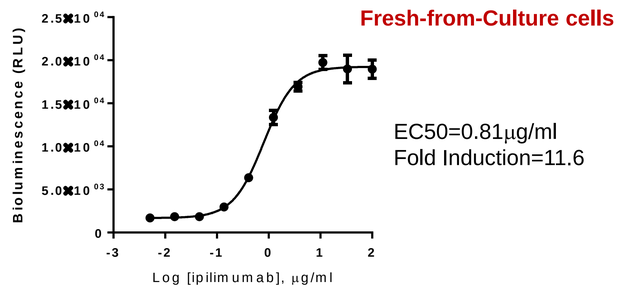
(2) Engagement of CTLA-4 with CD80/CD86 inhibits luciferase activity

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



TCR and CD28-mediated luciferase activity is recovered in the CTLA-4 bioassay with an anti-CTLA-4 blocking Ab (ipilimumab), but not with anti-HER2 (trastuzumab) or anti-PD-L1 blocking Abs.

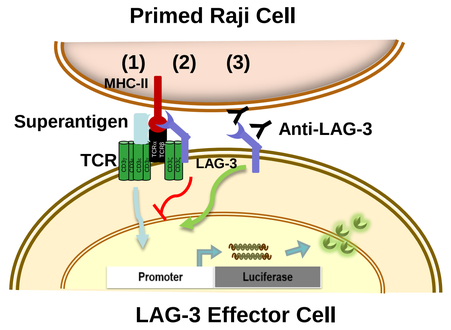
7. CTLA-4 Blockade Bioassay: Thaw-and-Use Cells Perform Equivalently to Fresh Cells



An anti-CTLA-4 blocking Ab (ipilimumab) induces an equivalent dose-dependent increase in luciferase activity in the CTLA-4 bioassay in fresh-from-culture (EC50=0.81µg/ml) and thaw-and-use (EC50=1.2µg/ml) cell format.

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

Primed Raji Cell



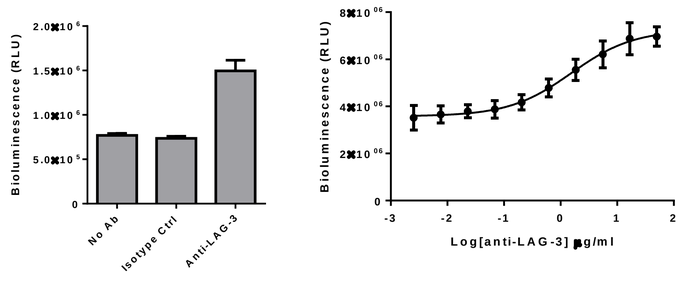
LAG-3 Effector Cell

Assay Design

(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 binding to its ligand restores luciferase activity



Left: TCR-mediated luciferase activity is recovered in the LAG-3 bioassay with anti-LAG-3 blocking Ab, but not with a non-specific isotype control Ab.

Right: An anti-LAG-3 blocking antibody induces a dose-dependent increase in luciferase activity in the LAG-3 bioassays.

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 inhibitory checkpoint bioassays targeting PD-1/PD-L1, TIGIT/CD155, CTLA4/CD80/86 and LAG3/MHCII, 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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