Novel Cell-based Bioassays for Monoclonal Antibody and Bispecific Molecules in PD-1 Blockade Monotherapy and Combination Therapy

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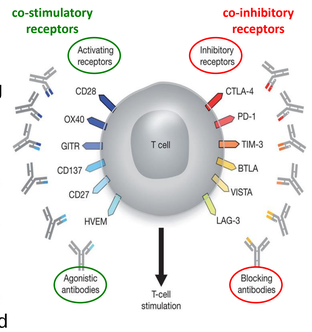
Abstract# 1875

1. Introduction

Immunotherapy targeting PD-1 has led to a paradigm shift in cancer drug discovery, due to its durable effect against a wide variety of cancers. Combining PD-1 checkpoint inhibitors with other clinically active treatments, including those targeting other immune checkpoint (IC) receptors, has also shown improved clinical results.

Here, we report the development of a suite of cell-based reporter bioassays for monoclonal antibodies targeting PD-1/PD-L1, or bispecific molecules targeting PD-1/PD-L1 and a co-stimulatory receptor (e.g., 4-1BB, OX40) or an immune inhibitory receptor (e.g., CTLA-4, LAG-3, TIGIT) and show that the combination bioassays are able to measure the synergetic effect of PD-1 blockade with a second IC inhibitor receptor blockade or a costimulatory receptor activation.



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

2. Assay Workflow of PD-1/PD-L1 Bioassay

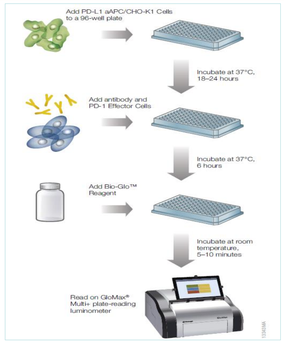
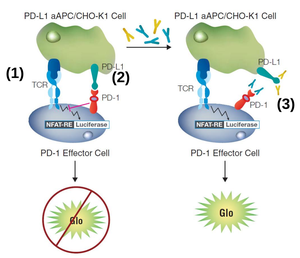
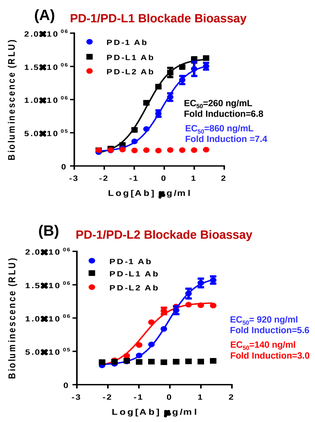


Plate PD-L1 aAPC cells
⇩
Add blocking antibody
⇩
Add PD-1 Effector cells
⇩
6-hour Induction
⇩
Add Bio-Glo™ detection reagent
⇩
Read plates

3. PD-1 Blockade Bioassays





Assay Design

Luciferase activity indicating activation of T cells is:

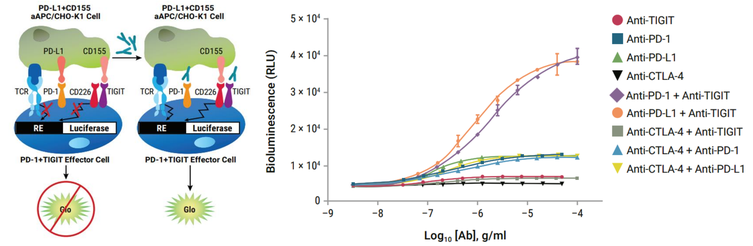
(1) Induced by TCR engagement

(2) Inhibited by engagement of PD-1/PD-L1 or PD-1/PD-L2

(3) Restored by Ab-mediated blockade of PD-1/PD-L1 or PD-1/PD-L2

TCR-mediated luciferase activity is specially activated in PD-1/PD-L1 Bioassay (**A**) with anti-PD-1 or PD-L1 blocking Abs, and in PD-1/PD-L2 Bioassay (**B**) with anti-PD-1 or PD-L2 blocking Abs.

4. PD-1+TIGIT Combination Bioassay



Assay Design

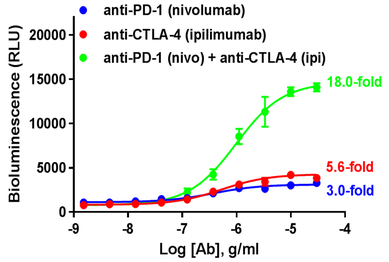
PD-L1 and CD155 are co-expressed on aAPC cells. PD-1 and TIGIT are co-expressed on effector cells. Luciferase activity indicating activation of T cells is:

(1) Induced by engagement of TCR/CD226

(2) Inhibited by engagement of PD-1/PD-L1 and TIGIT/CD155

(3) Restored by mAb-mediated blockade of PD-1/PD-L1 and/or TIGIT/CD155.

5. PD-1+CTLA-4 Combination Bioassay



Assay Design

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

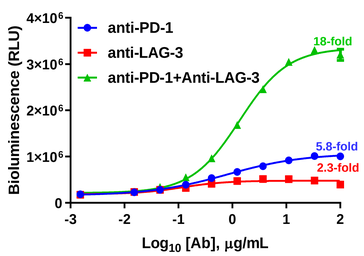
(1) Induced by engagement of TCR/CD28

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

(3) Restored by mAb-mediated blockade of PD-1/PD-L1 and/or CTLA-4/CD80

Anti-PD-1 or anti-CTLA-4 blocking Ab alone induced a 3.0- and 5.6-fold increase, while a combination of both Abs induced an 18-fold increase in luciferase activity.

6. 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:

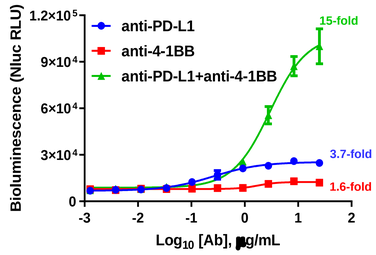
(1) Induced by engagement of TCR

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

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

Anti-PD-1 or anti-LAG-3 blocking Ab alone induced a 5.8- and 2.3-fold increase, while a combination of both Abs induced an 18-fold increase in luciferase activity.

7. PD-1+4-1BB Combination Bioassay



Assay Design

PD-L1 is expressed on aAPC cells; PD-1 and 4-1BB are co-expressed on effector cells. Luciferase activity indicating activation of T cells is:

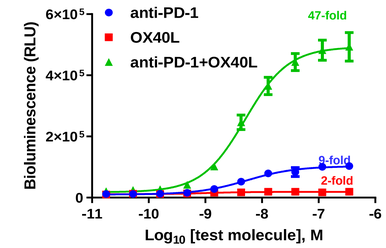
(1) Induced by activation of TCR

(2) Inhibited by engagement of PD-1/PD-L1

(3) Restored by Ab-mediated blockade of PD-1/PD-L1 and enhanced by 4-1BB agonist antibody

Anti-PD-L1 blocking Ab or anti-4-1BB agonist Ab alone induced a 3.7- and 1.6-fold increase, while a combination of both Abs induced a 15-fold increase in luciferase activity.

8. PD-1+OX40 Combination Bioassay



Assay Design

PD-L1 is expressed on aAPC cells and PD-1 and OX40 are co-expressed on effector cells. Luciferase activity indicating activation of T cells is:

(1) Induced by activation of TCR

(2) Inhibited by engagement of PD-1/PD-L1

(3) Restored by Ab-mediated blockade of PD-1/PD-L1 and enhanced by OX40 ligand

Anti-PD-1 blocking Ab or OX40L alone induced a 9- and 2-fold increase, while a combination of PD-1 Ab and OX40L induced a 47-fold increase in luciferase activity.

9. Conclusions

MOA-based reporter bioassays targeting PD-1 and a second immune checkpoint receptor overcome the limitations of primary cell-based assays and 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-mediated luciferase activity that reflects the native biology of T cell activation.
- Ability to measure the potency for immune checkpoint-targeted antibody alone or in combination.

Consistent and reliable measure of antibody activity

- Demonstrated precision, accuracy, reproducibility, robustness
- Functional performance suitable for development into potency, stability, and NAb assays

Easy-to-implement

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

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