Quantitative Cell-Based Bioassays for Individual or Combination
Imune 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-IBB, 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.

Blockade Bioassay Protocol


CD155 aAPC Cell

Demonstrated precision, accuracy, reproducibility, robustness

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Log [Ab] g/ml

Bio luminescence (RLU)

No human serum

1% human serum

5% human serum

10% human serum

Log[PD-1 Ab] g/ml

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

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

CTLA-4 aAPC Cell

4. TIGIT/CD155 Blockade Bioassay: Principle and Specificity

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

6. CTLA-4 Blockade Bioassay: Principle and Specificity

7. CTLA-4 Blockade Bioassay: Principle and Potency Study

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

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, CTLA-4/CD80/86 and LAG3/MHCI, 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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