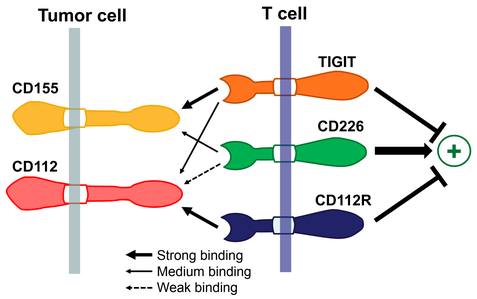
MoA-Based Potency Bioassays for Immunotherapy Programs Targeting the TIGIT/CD112R/CD226 Axis

Jamison Grailer, Denise Garvin, Jim Hartnett, Kai Hillman, Frank Fan, Mei Cong, and Zhi-Jie Jey Cheng

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

1. Introduction

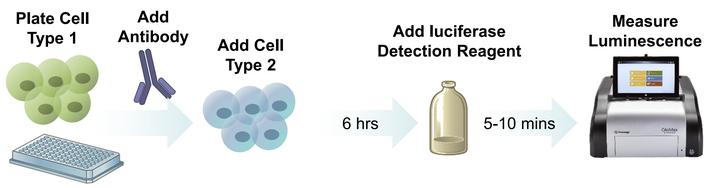
- TIGIT, CD112R and CD226 are immune checkpoints that have gained attention for cancer immunotherapy due to promising preclinical results
- We developed a portfolio of cell-based reporter bioassays to measure the potency of biologics designed to target the TIGIT/CD112R/CD226 axis



The TIGIT/CD112R/CD226 Axis

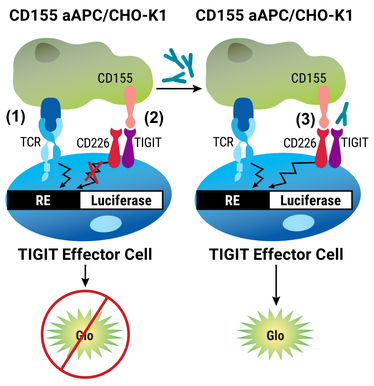
- CD226 is a co-stimulatory receptor that activates T and NK cells
- TIGIT and CD112R inhibit CD226 co-stimulation by competitive binding of their shared ligands CD155 and CD112

2. General Bioassay Workflow



The bioassays follow a simple add-mix-read format

3. The TIGIT/CD155 Blockade Bioassay Measures the Potency of TIGIT/CD155 Blocking Antibodies



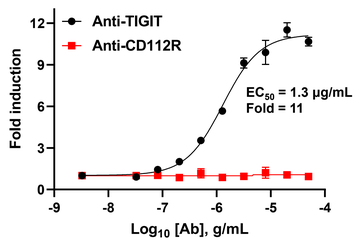
Assay Design

Luciferase activity indicating T cell activation is:

(1) induced by the T cell Receptor Activator protein that engages the TCR/CD3 complex

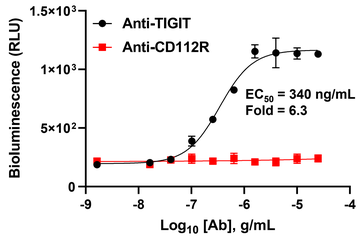
(2) inhibited by co-engagement of TIGIT with CD155

(3) restored by TIGIT blockade, co-stimulation by CD226 engagement with CD155



TCR- and CD226-mediated luciferase activity is recovered in the TIGIT/CD155 Blockade Bioassay with an anti-TIGIT blocking Ab, but not with an anti-CD112R blocking Ab.

4. The TIGIT/CD112 Blockade Bioassay Measures the Potency of TIGIT/CD112 Blocking Antibodies



Assay Design

Luciferase activity indicating T cell activation is:

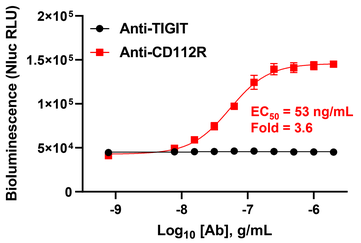
(1) induced by the T cell Receptor Activator protein that engages the TCR/CD3 complex

(2) inhibited by co-engagement of TIGIT with CD112

(3) restored by TIGIT blockade, co-stimulation by CD226 engagement with CD112

TCR- and CD226-mediated luciferase activity is recovered in the TIGIT/CD112 Blockade Bioassay with an anti-TIGIT blocking Ab, but not with an anti-CD112R blocking Ab.

5. The CD112R/CD112 Blockade Bioassay Measures the Potency of CD112R Blocking Antibodies



Assay Design

Luciferase activity indicating T cell activation is:

(1) induced by the T cell Receptor Activator protein that engages the TCR/CD3 complex

(2) inhibited by co-engagement of CD112R with CD112

(3) restored by CD112R blockade, co-stimulation by CD226 engagement with CD155

TCR- and CD226-mediated luciferase activity is recovered in the CD112R/CD112 Blockade Bioassay with an anti-CD112R blocking Ab, but not with an anti-TIGIT blocking Ab.

6. The PD-1+TIGIT Combination Bioassay Measures Synergetic Effects of Blocking Antibodies for PD-1 and TIGIT

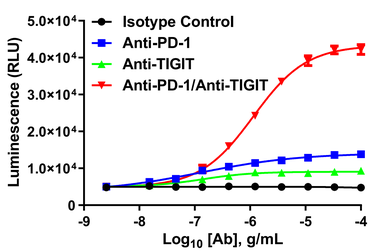
Assay Design

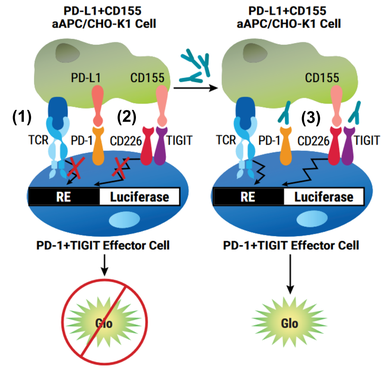
Luciferase activity indicating T cell activation is:

(1) induced by the T cell Receptor Activator protein that engages the TCR/CD3 complex

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

(3) Partially restored by TIGIT or PD-1 blockade, fully restored by TIGIT/PD-1 co-blockade



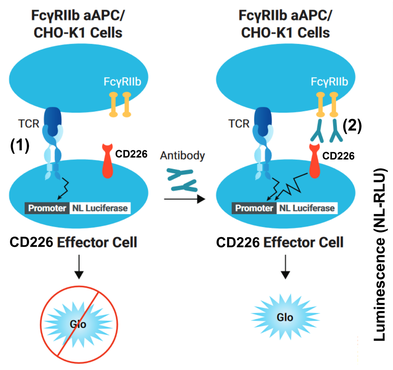


Antibody	EC_{50}	Fold
Anti-PD-1	130 ng/ml	2.8
Anti-TIGIT	140 ng/ml	1.8
Anti-PD-1/Anti-TIGIT	1.2 µg/ml	8.8

TCR- and CD226-mediated luciferase activity is recovered in the PD-1+TIGIT Combination Bioassay with an anti-TIGIT blocking Ab and an anti-PD-1 blocking Ab.

7. The CD226 Bioassays Measure the Potency of CD226 Agonist or Antagonist Antibodies

CD226 Bioassay

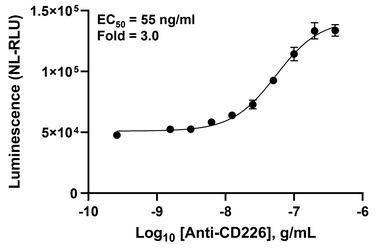


Assay Design

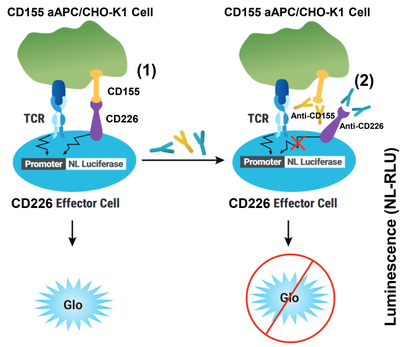
Luciferase activity indicating T cell activation is:

(1) induced by the T cell Receptor Activator protein that engages the TCR/CD3 complex

(2) Enhanced by FcγRIIb-mediated crosslinking of anti-CD226 agonist Ab



CD226/CD155 Blockade Bioassay

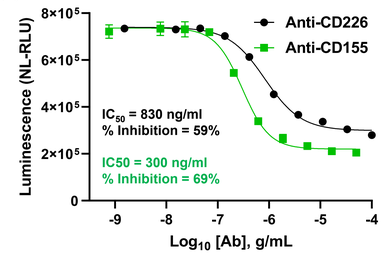


Assay Design

Luciferase activity indicating T cell activation is:

(1) induced by the T cell Receptor Activator protein that engages the TCR/CD3 complex and CD226/CD155 co-engagement

(2) inhibited by CD226 or CD155 blockade



8. Conclusions

Here we show a portfolio of MOA-based bioassays for the TIGIT/CD112R/CD226 axis 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 and stability assays

Easy-to-implement

- Rapid and convenient workflow, amenable to 96- and 384-well plates

Corresponding author: jamison.grailer@promega.com