Quantitative Cell-Based Bioassays for Immunotherapy Programs Targeting Immune Checkpoint Co-Stimulatory Receptors

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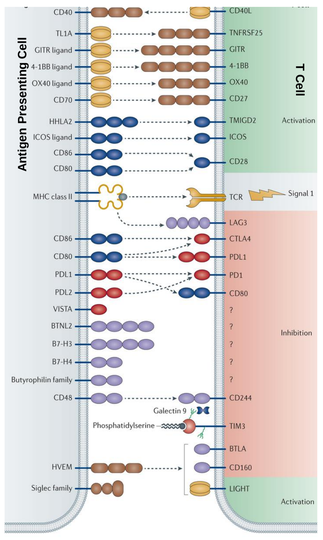
1. Introduction

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.

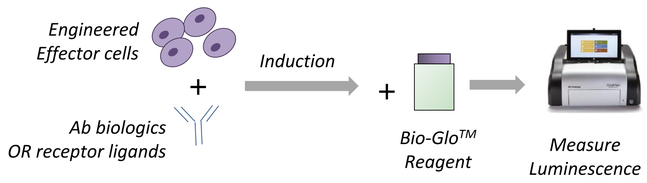
Here we describe the application of cell-based reporter bioassays for the development of therapeutic antibodies targeting co-inhibitory immune checkpoint receptors.

Immune Checkpoint Targets

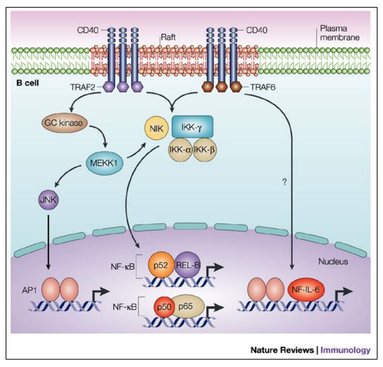


Mahoney et al. (2015)

Co-stimulatory Bioassay Protocol

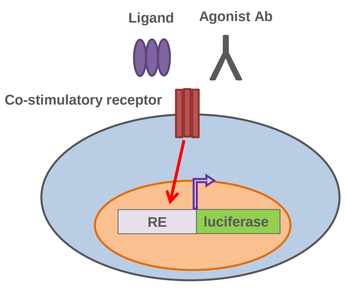


2. Immune Checkpoint Co-stimulatory Receptor Bioassays Design and Features



- Many immune checkpoint co-stimulatory receptors (e.g. GITR, 4-1BB, OX40, CD40, HVEM) belong to the TNFR superfamily
- They are expressed on T lymphocytes, NK cells and antigen-presenting cells (APCs)
- Ligand-induced activation of immune checkpoint co-stimulatory receptors is important for the development of an immune response

Assay Design



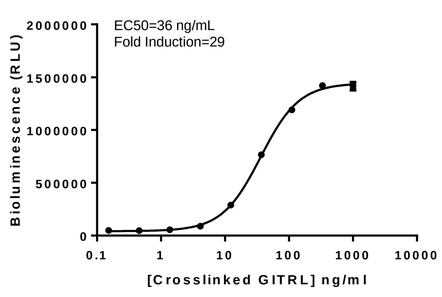
Effector Cell

Assay Features

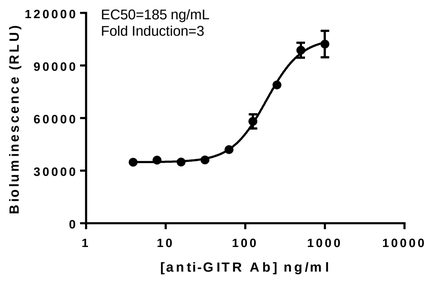
- MOA-based
- Simple and homogenous
- Specific, sensitive, precise
- Low variability
- Thaw-and-use cells
- Single day protocol

3. GITR Bioassay Principle

GITR Ligand-Induced Activation



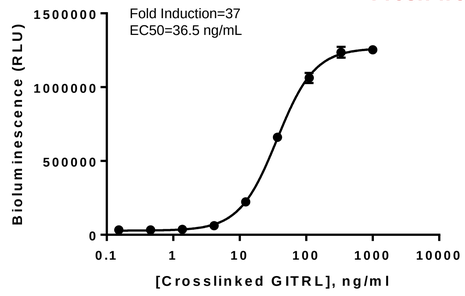
GITR Agonist Antibody-Induced Activation



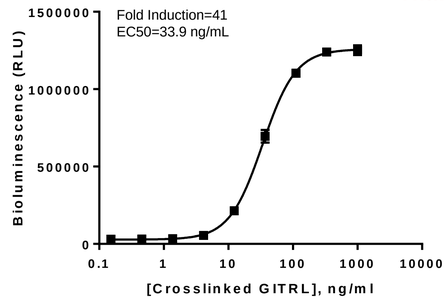
GITR effector cells were stimulated with increasing concentrations of crosslinked GITR ligand (**Top**) or a GITR agonist antibody (**Bottom**).

4. GITR Bioassay: Thaw-and-Use Cells Perform Equivalently to Fresh Cells

Fresh-from-Culture Cells



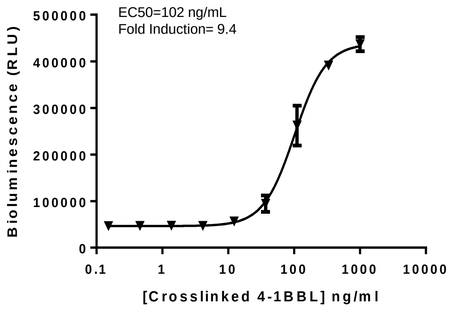
Thaw-and-Use Cells



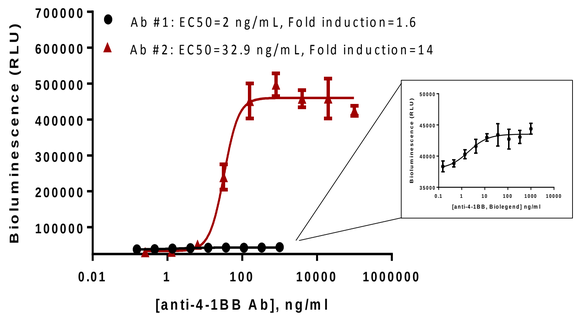
GITR effector cells in either fresh-from-culture (**Top**) or thaw-and-use (**Bottom**) cell format were stimulated with increasing concentrations of GITR ligand.

5. 4-1BB Bioassay Principle

4-1BB Ligand-Induced Activation



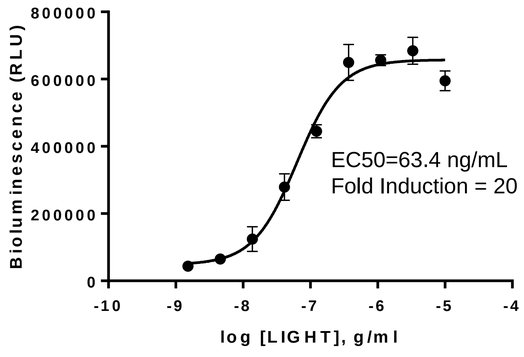
4-1BB Agonist Antibody-Induced Activation



4-1BB effector cells were stimulated with increasing concentrations of crosslinked 4-1BB ligand (**Top**) or a 4-1BB agonist antibody (**Bottom**).

6. HVEM Bioassay Principle

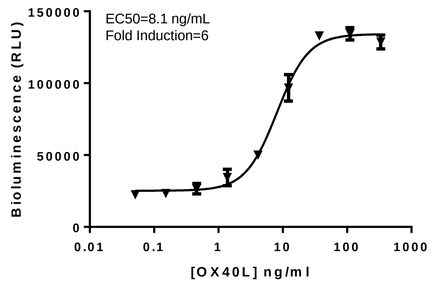
HVEM Bioassay Activation by LIGHT Ligand



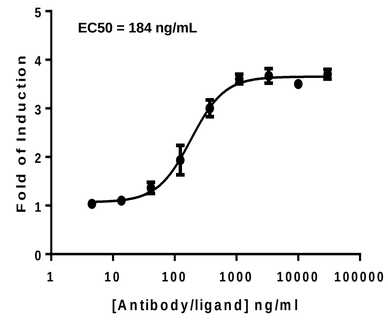
HVEM effector cells were stimulated with increasing concentrations of LIGHT ligand (without crosslinking). Data are represented in RLU (**Left**) and Fold Induction (**Right**).

7. OX40 Bioassay Principle

OX40 Ligand-Induced Activation



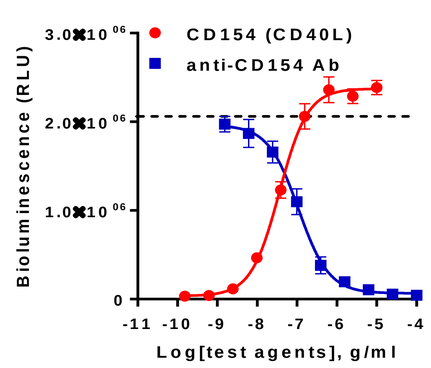
OX40 Agonist Antibody-Induced Activation



OX40 effector cells were stimulated with increasing concentrations of OX40 ligand (**Top**) or an OX40 agonist antibody (**Bottom**).

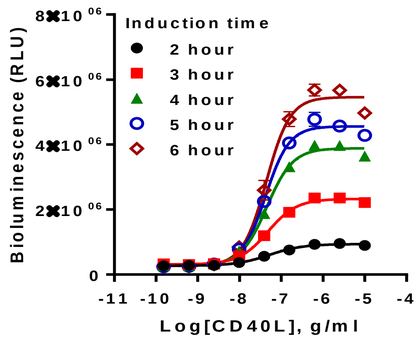
8. CD40 Bioassay Principle and Optimization

Assay Response to CD40 Ligand or a CD40L blocking antibody



CD40 effector cells were treated with a serial titration of CD40L (**Red**), or a serial titration of anti-CD154 blocking antibody (Biolegend #310828) in the presence of 0.1 ug/ml of CD40L (**Blue**).

EC50 for CD40L=3.6×10^{-8} g/ml
IC50 for anti-CD154=1.1×10^{-7} g/ml



CD40 effector cells were treated with a serial titration of CD40L for the indicated times.

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.
- Can be used in "Thaw-and-use" cell format, no cell culture required
- Demonstrated ability to measure the potencies of immune checkpoint-targeted antibodies

Easy-to-implement

- Rapid and convenient workflow
- Amenable to standard 96-well and can be further developed into 384-well plate formats

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