NanoBiotec
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IMMUNE CHECKPOINT BLOCKADE ASSAYS

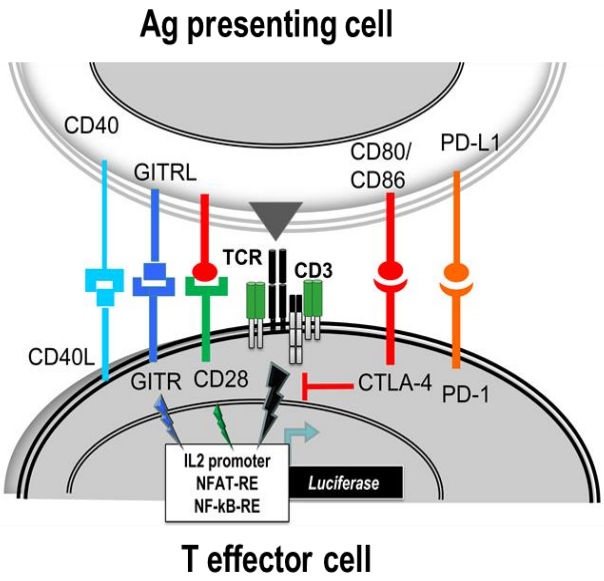
INTRODUCTION

The human immune system is modulated by a complex network of co-inhibitory and co-stimulatory pathways that facilitate the elimination of cells expressing foreign antigens while maintaining tolerance to self-antigen. Immune checkpoint pathways mediated by PD-1, CTLA-4, GITR, 4-IBB, OX4-O and TIGIT are promising immunotherapy targets for the treatment of cancer and autoimmunity. Our immune checkpoint bioassays provide simple, consistent and reliable cell-based assays for screening antibody and small molecule activities as immunotherapeutics.

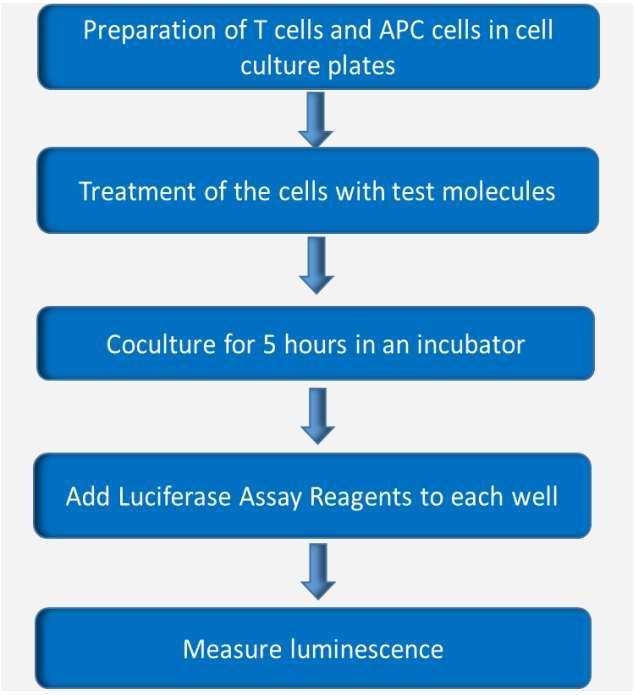
CELL-BASED REPORTER ASSAYS

These assays consist of two genetically engineered cell lines: T effector cells (cells expressing PD-1, TIGIT or PD-1 + TIGIT on surface and NFAT-Luc as reporter) and artificial APC cells (cells expressing PD-L1, CD155 or PD-L1 + CD155 and a cell surface protein designed to activate cognate TCRs in an antigen-independent manner). Co-culture of T effector cells with their corresponding APC cells leads to inhibition of TCR-mediated proliferation, transcriptional activation and cytokine production. Addition of anti-PD-1, PD-L1, or CTLA-4 antibodies or small molecule mimetics block the checkpoint inhibition to restore T effector cell activation and result in luciferase activity. The assay can be utilized to measure the potency and stability of large or small molecules designed to block the checkpoint interaction.

Assay Mechanism:



Assay Procedure:



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T-CELL ACTIVATION ASSAY: OVERCOMING CHECKPOINT MOLECULE–MEDIATED INHIBITION

Primary human T cells are stimulated with anti-CD3 and anti–CD28-coated beads to induce proliferation and IL-2/IFN-γ release. Both proliferation and cytokine release is reduced when recombinant PD-L1 is added. T-cell proliferation measured by BrdU incorporation, and cytokine release measured by multiplex immunoassay, are used as the assay endpoints. Using these assays, we screen large and small molecules which have potentials to overcome the immune checkpoint caused T cell inhibition.

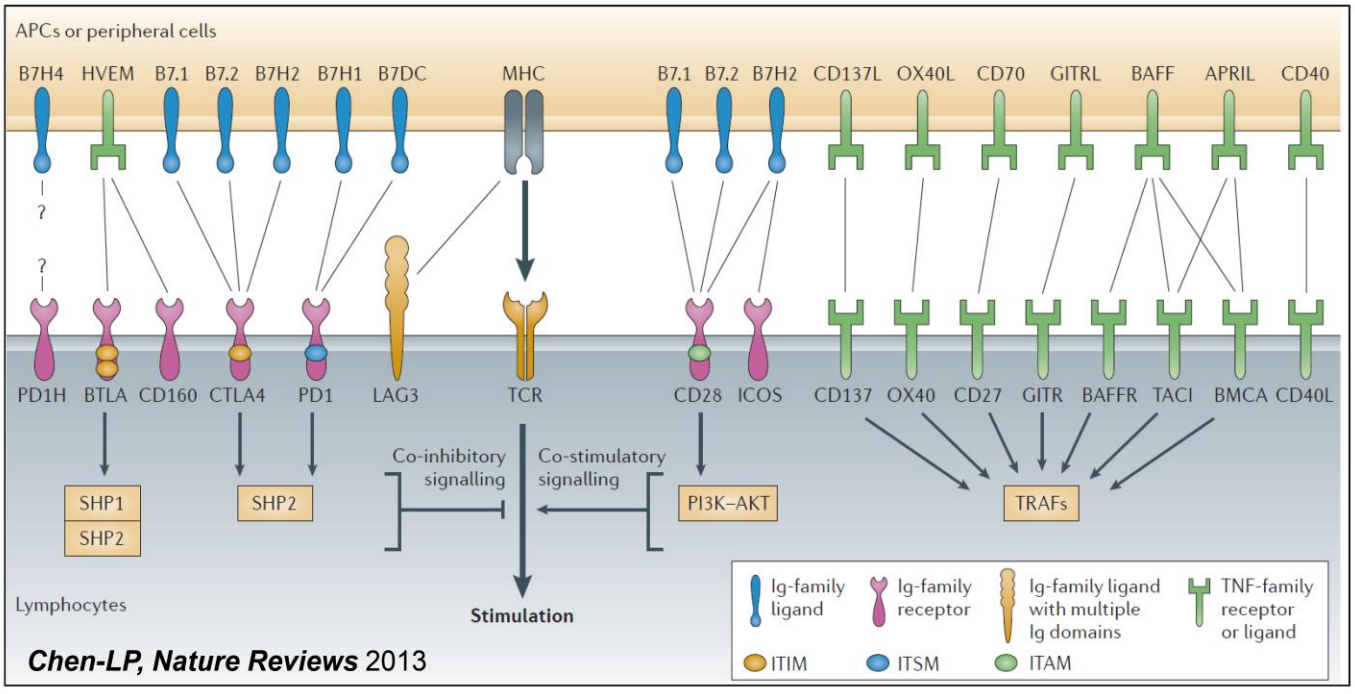
LUMINEX-BASED ASSAY FOR SOLUBLE CHECKPOINT MARKER MOLECULES

Soluble variants of checkpoint marker molecules can function as decoy receptors or as immune adjuvants interfering with the response to immunotherapeutic antibodies.

Soluble isoforms of checkpoint molecules play an active role in the T cell activation pathway.

The anticancer immunity is determined by the maintenance of an elegant balance between stimulatory and inhibitory checkpoint molecules. The simultaneous analysis of those factors is expected to yield significant insight and contribute to a better understanding of therapeutic benefit and resistance mechanisms.

To support development of cancer immunotherapeutics, along with measurement of many cytokines/chemokines secreted from immune cells, we developed Luminex-based assays to profile multiple soluble checkpoint marker molecules in human serum, plasma or cell culture supernatants such as **BTLA, GITR, HVEM, IDO, LAG-3, PD-1, PD-L1, PD-L2, TIM-3, CD28, CD80, CD137, CD27, and CD152.**



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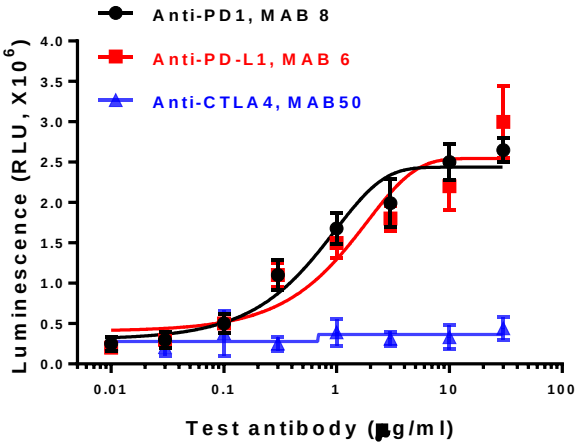
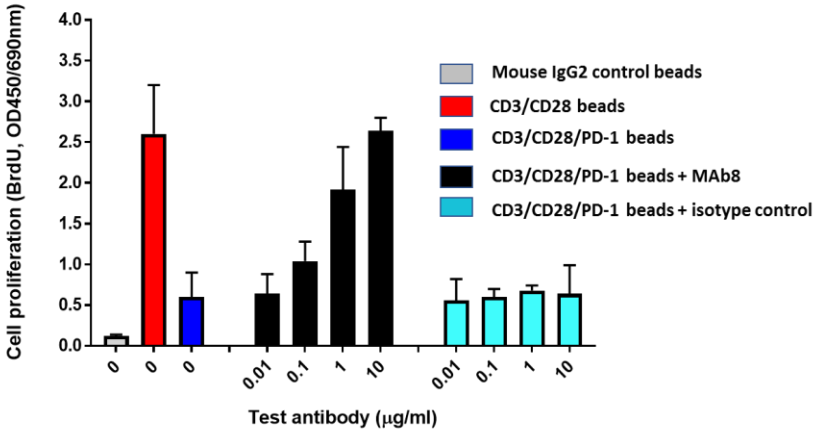
OUR SERVICE FEATURES

- ✓ High throughput: 96-well format, duplicate or triplicate
- ✓ Robust and highly reproducible assay for small to large scale screening
- ✓ Multiplex
- ✓ Fully validated and quality-controlled
- ✓ Well-validated reagents
- ✓ State-of-art platforms: Bioplex 200 Luminex Reader; CytoFlex-S Flow Cytometer
- ✓ Quick Turnaround Time
- ✓ 20+ years of experience: Expert data analysis and interpretation, and scientific support



Example 1. The anti-PD1 or anti-PD-L1 antibodies increases T cell activation.

PD-1 Effector T Cells and PD-L1 aAPC Cells were incubated for 6 hr at 37°C with increasing concentrations of either an anti-PD-1, PD-L1, or CTLA-4 antibody. The anti-PD-1 and PD-L1 antibodies, but not the anti-CTLA-4 antibody, blocked the immune checkpoint inhibitory signal resulting in increase of luciferase activity in the Effector T cells. Values are mean ± SD.



Example 2. The anti-PD1 antibody overcomes PD1–mediated inhibition of T-cell activation. The anti-PD1 antibody MAB8 can rescue T cells proliferation (measured by BrdU incorporation) in a concentration-dependent manner. Primary human T cells were stimulated with beads coated in anti-CD3, anti-CD28, and in the presence of either PD-L1 or irrelevant isotype control IgG. Values are mean ± SD.