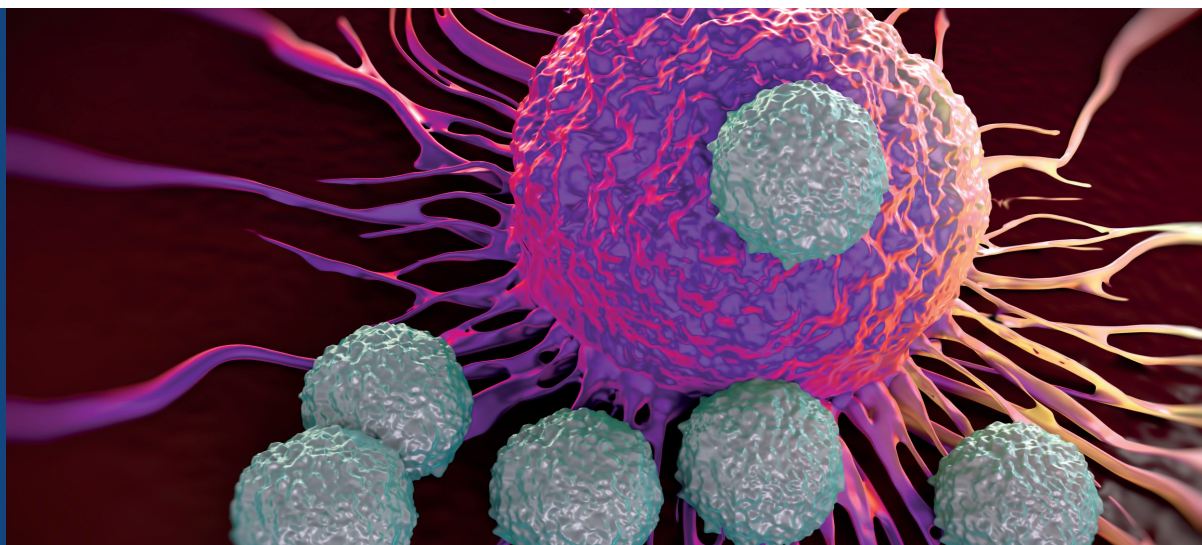


Overview

Powerful new *in vitro* assays provide a translational method to study biologics or small molecule modulators of immune responses. We've developed an optimized panel of T cell assays available to help clients better understand the complex biology of immuno-oncology.



DISCOVERY

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Complex Biology *In Vitro* Assays: Immuno-Oncology T Cell Exhaustion

The immune system is capable of distinguishing between danger and non-danger signals, thus inducing either an appropriate immune response against pathogens and [cancer](#) or inducing self-tolerance to avoid autoimmunity and immunopathology. One of the mechanisms that has evolved to prevent destruction by the immune system is to functionally silence effector T cells. Known as [T cell exhaustion](#), this mechanism is also exploited by viruses and cancers for inducing immune escape. T cell exhaustion is a state of T cell dysfunction that arises during many chronic infections and cancer. It is defined by poor effector function, sustained expression of inhibitory receptors, and a transcriptional state distinct from that of functional effector or memory T cells. Exhaustion prevents optimal control of infection and [tumors](#). Currently, multiple strategies are being explored to reinvigorate exhausted T cells by either small molecule or [therapeutic antibody](#) approaches alone or in combination. These include blocking some of the inhibitory phenotypic surface markers associated with T cell exhaustion (PD-1, PD-L1, CTLA4 LAG-3, Tim-3, and TIGIT); or activating agonistic mAbs targeting activating receptors on T cells (CD137, OX40, GITR) or APCs (anti-CD40 mAbs) and inhibitors of soluble mediators targeting IDO, A2aR, CSF1R, IL10, or TGFβ (reviewed by Zarour HM, 2016).

Staphylococcal enterotoxin B (SEB) is an enterotoxin produced by the Gram-positive bacteria *Staphylococcus aureus*. It is a common cause of food poisoning due to its stable biophysical nature. SEB used at low concentrations binds to the class II major histocompatibility complex (MHC) antigens expressed on professional antigen presenting cells (APCs) and the T cell receptor at the variable region of the β-chain. As this binding event occurs on a subset of TCR Vβ domains, a substantial number of T cells are activated by SEB, resulting in the up-regulation of activation markers and proliferation.

SEB is also known as superantigen, as it can trigger polyclonal T cell activation and trigger systemic release of pro-inflammatory cytokines such as IL-2, IL-6, TNFα and IFN-γ which can be quantified using the Meso Scale Discovery multiplex platform. Identified cytokines can be future studied [in vivo](#) across an [immunology platform](#).

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T Cell Exhaustion Assay Principle

In this assay, freshly isolated human PBMCs are stimulated with a fixed concentration of SEB for three days. The media is harvested for IL-2 and IFN- γ measurements. Cells are washed and re-suspended into assay media in the absence or presence of therapeutic molecules to evaluate T cell activation response.

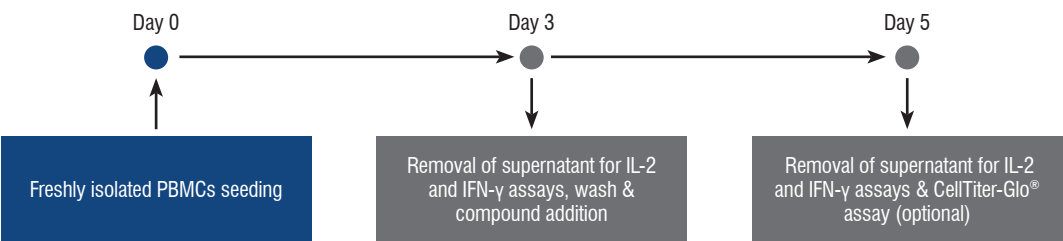
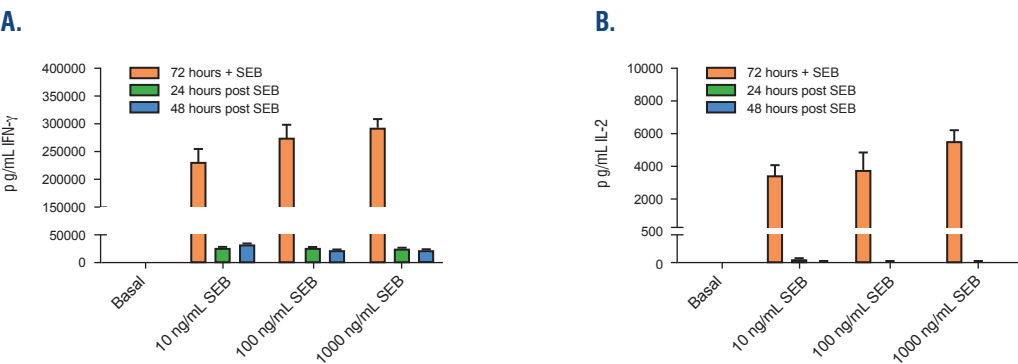
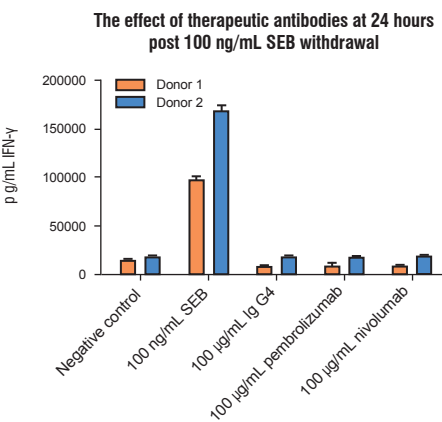


Figure 1. Representative data showing concentration dependent increase in IFN- γ (A) and IL-2 (B) with SEB stimulation (orange bars).



High level stimulation of IFN- γ and IL-2 production was measured post 3 days stimulation with 10-1,000 ng/mL SEB. Multiple donors were tested (data not shown).
A large reduction in both IFN- γ and IL-2 production observed 24 and 48 hours post SEB withdrawal.

Figure 2. Representative data showing the evaluation of two anti-PD-1 antibodies (pembrolizumab and nivolumab) in the T cell exhaustion assay at 24 hours post SEB stimulation. Similar data was also obtained at 48 hours (data not shown).



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Performance	
Donor	PBMCs
Seeding density	200,000 cells/well
Trigger (positive control)	100 ng/mL SEB or a therapeutic agent known to reverse T cell exhaustion
Negative control	No trigger
Readout	IFN- γ and IL-2 production by MSD assays

Summary

Exhaustion of T cells prevents optimal immune response and hence the control of infection and cancer. The ability to reinvigorate [T cells](#) is a therapeutic strategy that is currently being explored to help induce appropriate immune response against pathogens and cancer. It has been reported that exhausted T cells acquire an epigenetic profile distinct from effector or memory T cells (Pauken, K et al, 2016). These latter two cell types can mount effective immune responses to viruses and tumors; whereas, exhausted T cells fail, in particular, for long-lasting durable effects. Epigenetics is the way chemical modifications to DNA and the proteins binding DNA determine which genes are expressed by a cell type. Epigenetic profiles can be highly stable and confer long-term identity of a cell. The unique epigenetic profile of exhausted T cells causes these cells to express a different overall set of genes compared to memory or effector T cells. However, this epigenetic pattern was only minimally changed following the PD-L1 blockade (Pauken, K et al, 2016). This prevented these exhausted T cells from changing into the more protective effector or memory cell types.

Using this [immuno-oncology assay](#), we can monitor the ability of [therapeutic agents](#) (small molecules or antibodies) to reverse T cell exhaustion induced by low concentrations of SEB by measurement of the pro-inflammatory cytokines IL-2 and IFN- γ . An endpoint measurement of cell proliferation can also be incorporated using the CellTiter-Glo® assay.

References:

- Longoria T et al, 2016; Expert Opin Drug Metab Toxicol. 2016 Oct; 12(10): 1247–1253.
- Pauken K et al, 2016; *Science* 02 Dec 2016: Vol. 354, Issue 6316, pp. 1160-1165.
- Zarour HM; Clin Cancer Res; 22(8) April 15, 2016.
- Selby M et al, 2016; PLOS ONE September 9, 2016.

Assay Reference Code

OTS114-TCellAnergy

Complementary Immuno-Oncology Assays

[T Cell Cytokine Response Assay](#)

[T Cell Proliferation CTG Assay](#)

[T Cell-Mediated Chemotaxis Assay](#)

[3D Spheroid T Cell Cytotoxicity Assay](#)