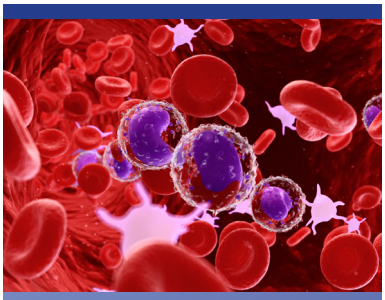
cyprotex AN EVOTEC COMPANY

*In vitro* **Toxicology**

# PBMC Proliferation

## Background Information



'*In vitro* high throughput assays utilising primary human immune cells will significantly enhance our capabilities to predict candidate drugs with potentials to cause rare but occasionally fatal hypersensitivity reactions during early stages of preclinical drug development.'

- Drug hypersensitivity reactions are rare off-target, immune-mediated and mostly delayed-type reactions involving drug-specific T-lymphocytes.
- T-lymphocyte mediated drug hypersensitivity reactions mainly target the skin and liver[1,2]. These reactions are dependent on antigen presentation to drug-specific T-lymphocytes which result in their activation, expansion, differentiation and the targeting of tissues and organs by activated T-lymphocytes.
- Common classes of drugs implicated in hypersensitivity include antibiotics, anticonvulsants, antivirals, non-steroidal anti-inflammatory drugs and biologics.
- These reactions are difficult to predict during preclinical drug discovery due to the involvement of specific individual characteristics (genetic and non-genetic) that are present in only susceptible individuals[3].
- Current animal models of immunogenicity fail to recapitulate the complexity of the human immune system.
- *In vitro* drug-induced T-lymphocyte proliferation using PBMC isolated from drug hypersensitive individuals have provided supporting evidence for the diagnosis of clinical cases of drug hypersensitivity reactions[4,5].
- Our high throughput PBMC proliferation assay evaluates the immunogenic potential of candidate drugs using multiple HLA-typed PBMC donors cryopreserved in our immune cell biobank.

## Protocol

**Cell Type**
Human peripheral blood mononuclear cells (PBMC).

**Donors**
>6 donors available for multi-donor studies.

**Analysis Platform**
PBMC proliferation – fluorescent detection using Alamar blue cell viability reagent.
Cellular ATP – CellTiter-Glo® (Promega) with a Cytation 3 Cell Imaging Multi-Mode Reader (BioTek).

**Test Article Concentrations**
8 point dose response curve with top concentration based on 100x $C_{max}$ or solubility limit.
3 replicates per concentration*.

**Test Article Requirements**
Maximum (dependent upon number of repeat doses) 150 µL of a DMSO* solution to achieve 200x top concentration maintained at 0.5% DMSO or equivalent amount in solid compound.

**Time Points**
24-72 hour pre-incubation*.

**Quality Control**
Negative control: 0.5% DMSO (vehicle)*.
Positive controls: 2 appropriate compounds.

**Data Delivery**
Minimum effective concentration (MEC) and $AC_{50}$ value for PBMC proliferation and cellular ATP content.

*Other options available on request.

The PBMC proliferation assay is non-radioactive and assesses the immunogenic potential of candidate drugs (biologics and small chemicals molecules) based on antigen-induced PBMC proliferation and corresponding increase in cellular ATP.

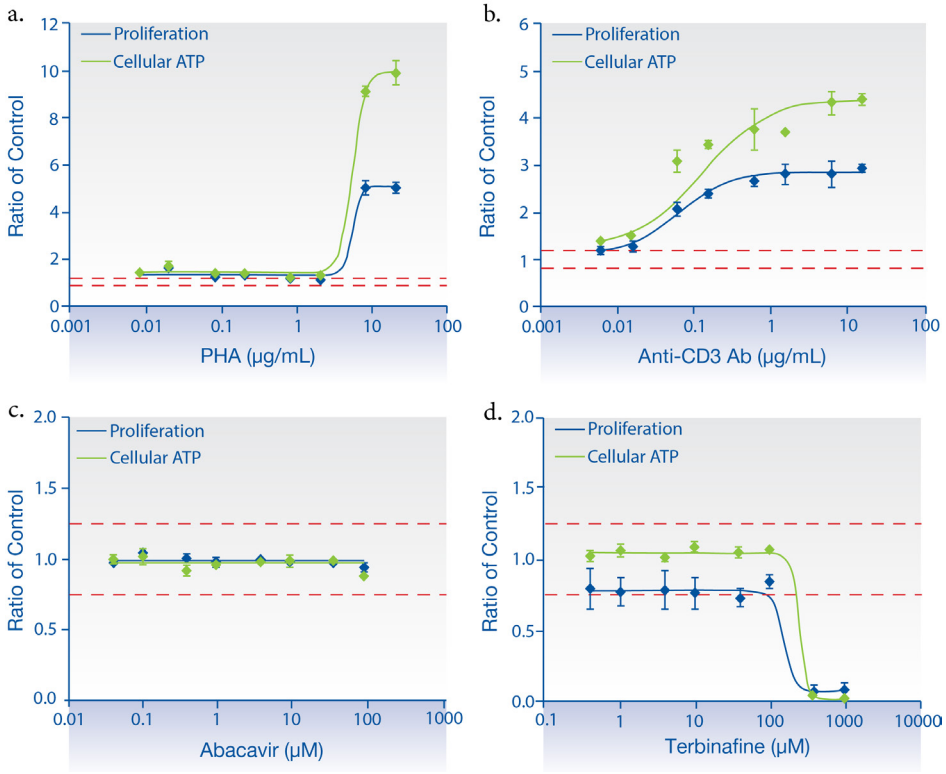



**Figure 1**

Representative PBMC proliferation (blue line) and cellular ATP (green line) dose response graphs for (a) phytohemagglutinin (PHA), (b) purified anti-CD3 antibody, (c) abacavir and (d) terbinafine.

Treatments with PHA ($AC_{50}$= 2.86 µg/mL; MEC = 1.97 µg/mL) and purified anti-CD3 ($AC_{50}$ = 0.012 µg/mL; MEC = <0.006 µg/mL) for 72 hours resulted in an increase in PBMC proliferation with a corresponding increase in cellular ATP. Abacavir had no effect on either PBMC proliferation or cellular ATP levels at 100 µM while terbinafine caused a decrease in PBMC viability at >100 µM.

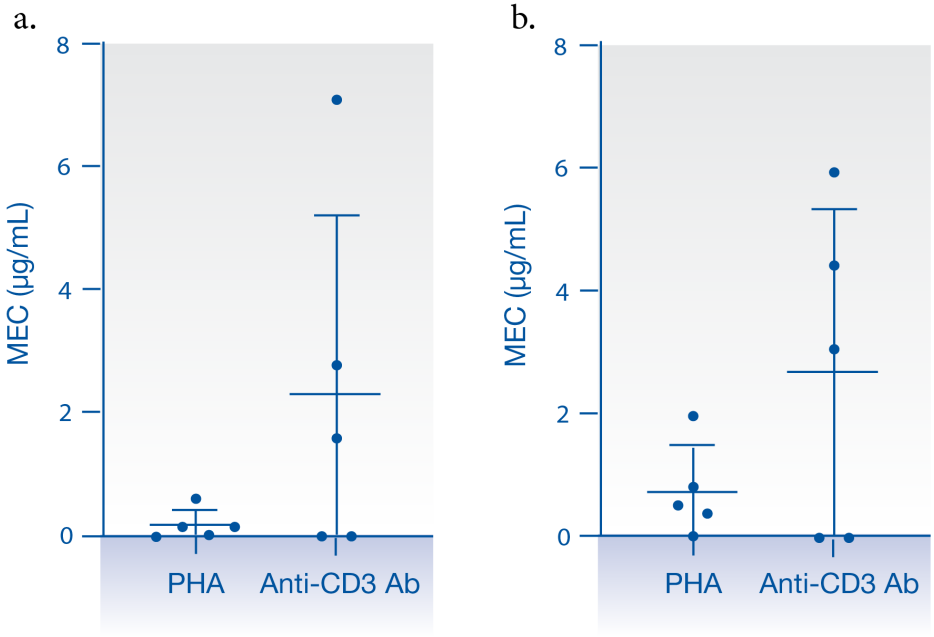



**Figure 2**

Dot plots demonstrating the MEC values collected for PHA and purified anti-CD3 antibody treatments across 5 individual PBMC donors.

Figures 2a and 2b show data obtained for PBMC proliferation and cellular ATP assays respectively.

Solid blue lines represent the average of the measurement while error bars represent ± standard deviation.


**References**

[1] Sullivan A *et al.*, (2018). ß-Lactam hypersensitivity involves expansion of circulating and skin-resident $T_H22$ cells. J *Allergy Clin Immunol* **141(1)**; 235-249

[2] Mennicke M *et al.*, (2009). Fulminant liver failure after vancomycin in a sulfasalazine-induced DRESS syndrome: fatal recurrence after liver transplantation. *Am J Transplant* **9(9)**; 2197-2202.

[3] Gibson A *et al.*, (2018). Genetic and nongenetic factors that may predispose individuals to allergic drug reactions. *Curr Opin Allergy Clin Immunol* **18(4)**; 325-332.

[4] Pichler WJ and Tilch J, (2004). The lymphocyte transformation test in the diagnosis of drug hypersensitivity. *Allergy* **59(8)**; 809-820

[5] Nyfeler B and Pichler WJ, (1997). The lymphocyte transformation test for the diagnosis of drug allergy: sensitivity and specificity. *Clin Exp Allergy* **27(2)**; 175-181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