Primary Human Immune Cell Assays for the Evaluation of New Immuno-Oncology Therapies

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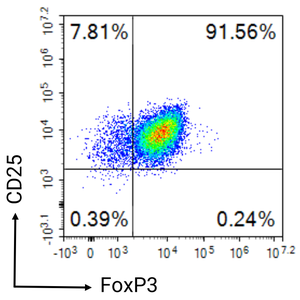
INTRODUCTION

The field of immuno-oncology has gained significant attention in recent years due to the ability of the immune system to recognise and eliminate cancer cells. Different subtypes of primary human immune cells, such as regulatory T cells, NK cells, macrophages, T cells and exhausted T cells, play important roles in the tumour microenvironment. *In vitro* assays using these cells can provide useful insight in the evaluation of new immuno-oncology therapies.

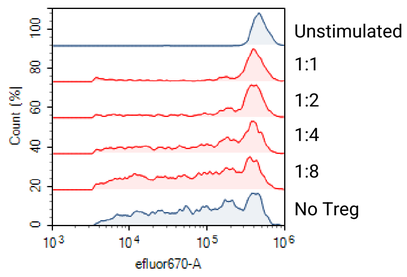
REGULATORY T CELLS

Regulatory T cells (Treg) can be isolated directly from PBMCs (nTreg) or naïve CD4 T cells can be induced to differentiate into Treg (iTreg). These can be used to suppress effector T cell responses such as proliferation and pro-inflammatory cytokine secretion. Isolated nTreg can be employed in defined Treg to target effector cell ratios and used to suppress anti-CD3 effector CD4 and CD8 T cell proliferation. Candidate therapeutics can be assessed for their ability to reverse regulatory T cell activity. Such assays can be performed at greater scale using iTreg, differentiated from naïve CD4 T cells in the presence of a proprietary cytokine cocktail.

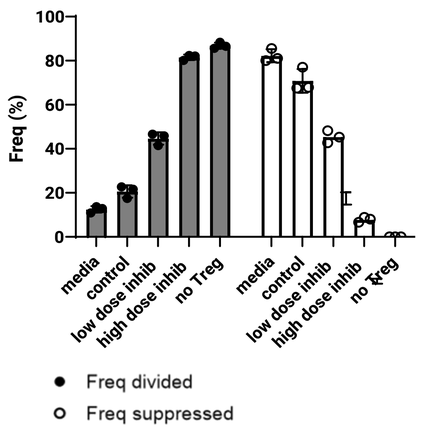
Isolated nTreg



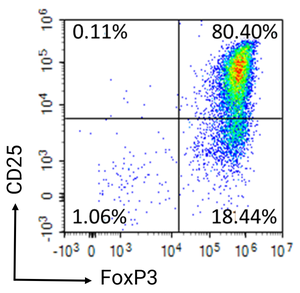
Dose dependent nTreg activity



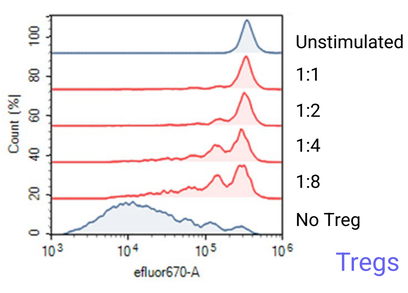
Reversal of nTreg activity by cbl-b antagonism

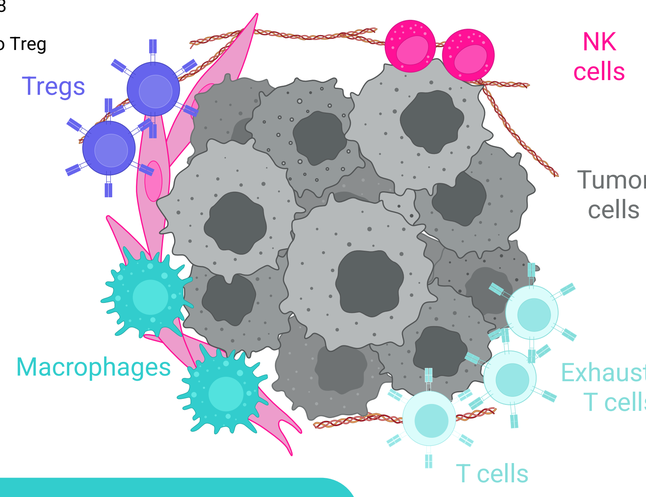


Induced Treg (iTreg)



Dose dependent iTreg activity

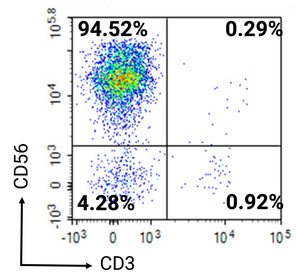




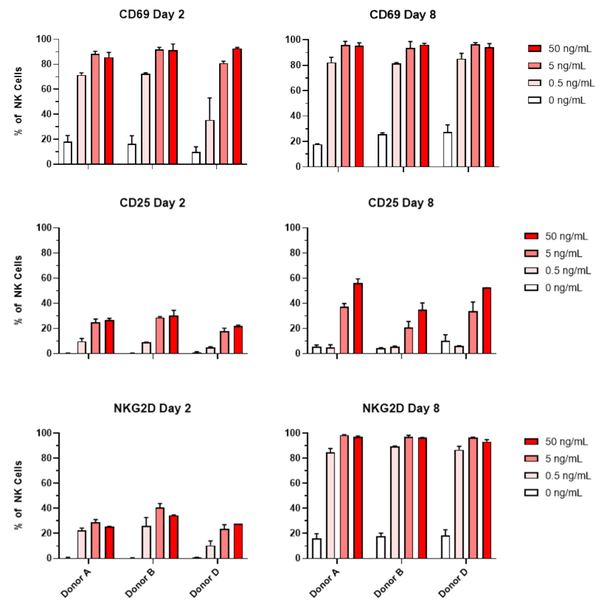
NK CELLS

Natural killer (NK) cells isolated from freshly collected whole blood or cryopreserved PBMCs can be assessed by flow cytometry for phenotypic analysis and used in killing assays in various formats e.g. xCELLigence, luciferase or flow cytometry readouts.

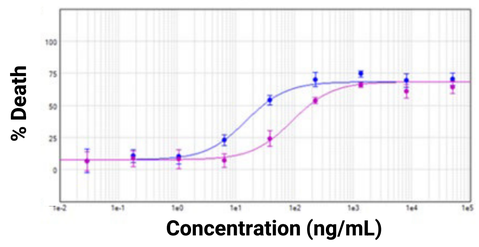
Isolated NK cells



Phenotypic analysis following cytokine stimulation

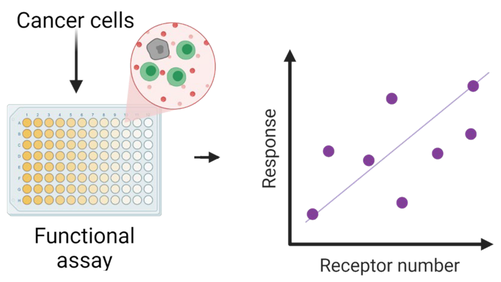


Antibody-dependent cellular cytotoxicity



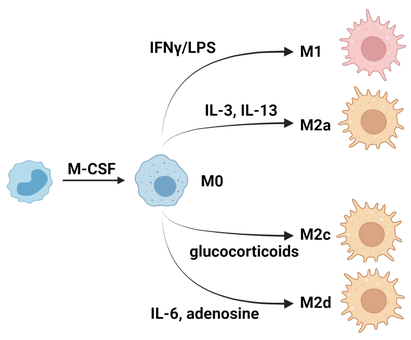
TUMOUR CELLS

We have developed a novel, model cell line platform that enables precise control of target antigen expression. This can be used in primary cell assays to determine activation thresholds and identify the potential "on-target, off tumour" side effects.



MACROPHAGES

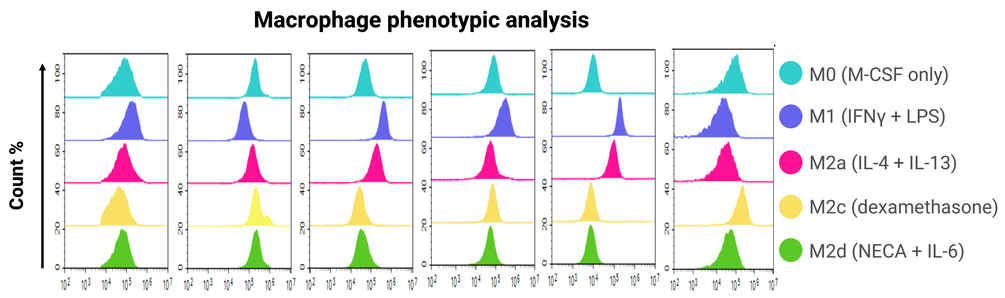
Isolated monocytes can be differentiated into macrophages for polarisation from M0 macrophages to M1 or M2 populations depending on what best reflects the in vivo conditions being modelled.



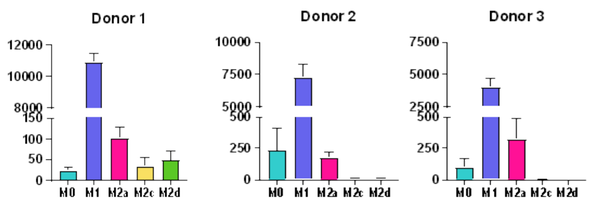
Macrophages can be applied to;

- Phagocytosis assays
- Suppression assays
- Macrophage phenotyping & cytokine release
- Mixed lymphocyte reactions (MLR)

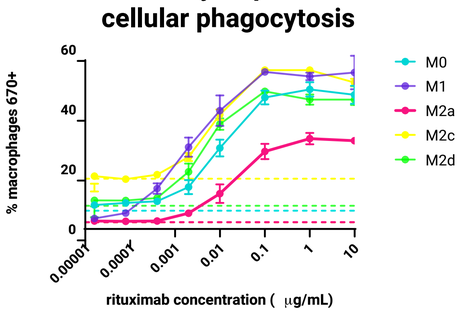
Macrophage phenotypic analysis



TNF secretion by macrophage populations

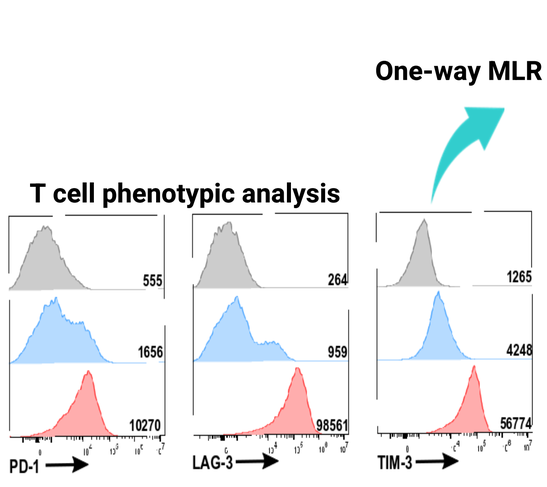


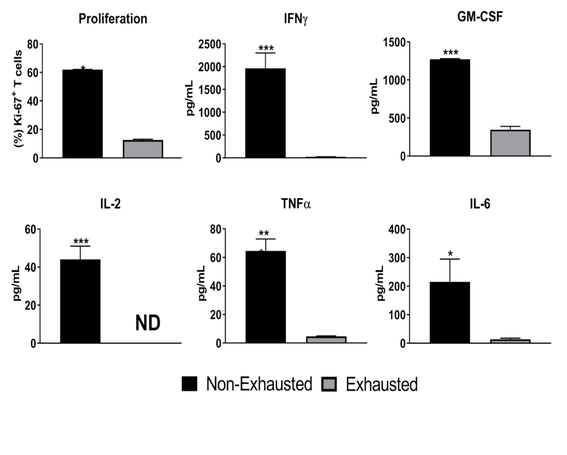
Antibody-dependent cellular phagocytosis



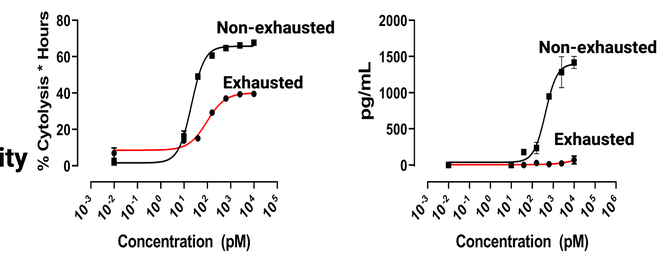
EXHAUSTED AND EFFECTOR T CELLS

One-way MLR



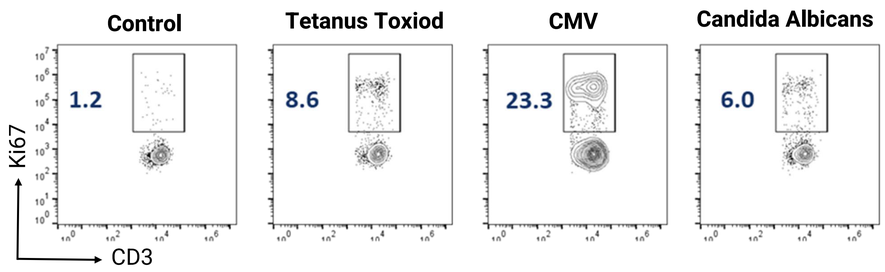


Cytotoxicity



Exhausted T cells are generated from human T cells isolated from PBMCs. These can be employed in a variety of functional assays to assess reinvigoration by test molecules. We are also able to study primary T cell responses employing model antigens (e.g. CMV), alloresponses (MLRs) or polyclonal stimulation utilising a variety of readouts e.g. flow cytometric phenotyping, proliferation, cytokine responses and target cell killing.

Antigen specific T cell responses



SUMMARY

In vitro assays with primary human immune cells are powerful tools for evaluating the impact of new immuno-oncology therapies on immune cell types that are relevant to cancer. These assays can help elucidate the mechanisms of action of the new therapies and assess their potential for clinical benefit, facilitating the discovery and development of new immuno-oncology therapies that can improve cancer treatment quality for patients.

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