Immuno-Oncology Therapies: *In Vitro* Functional Assays for Drug Selection and Development

Felicity Rudge[1], Maryam Ahmadi, Rebecca Klippstein, Mark Fogg, Anaïs Manin, Gary Bembridge[1] and Campbell Bunce

Abzena plc., Babraham Research Campus, Cambridge, UK

Introduction

The immune system is at the centre of a new era in effective strategies to treat cancer leading to significant growth in the immuno-oncology sector, and the development of competing technologies to further improve patient survival rates.

Essential to the successful development of cancer immuno-therapeutics are *ex vivo* and *in vitro* assays to:

- Confirm mode of action
- Compare activity with competitors
- Identify lead candidates
- Evaluate and select combinations

At Abzena, we have developed a suite of assays to confirm and select optimally functioning, lead drug candidates for clinical development. Custom-made assays are also offered for very novel immuno-therapeutics.

Monitoring key immune signalling pathways

Selection of novel immune modulators such as TLR-agonists (e.g. adjuvants), cytokines, receptor-ligand binders *etc*. can be informed through immune signalling pathway profiles. Agonist and antagonist function can be assessed *in vitro* alongside potential off-target pathways relevant to a safety risk.

Multiple phosphorylated proteins can be measured simultaneously in cell lines and cell subsets of whole blood or PBMC by phosphoflow.

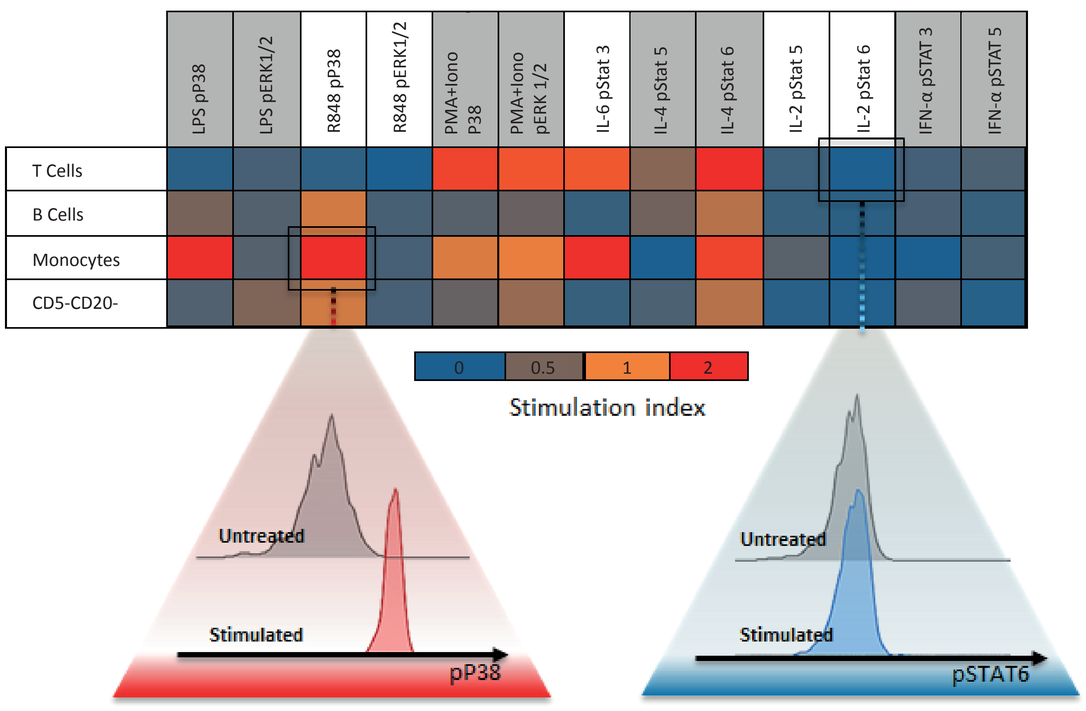


Figure 1: Simultaneous measurement of multiple phosphorylated proteins in subpopulations of whole blood.

Characterising immune checkpoint modulators

The effects of immune checkpoint modulators (ICMs) on an immune response are typically measured using two assays:

- Super Antigen Stimulation Assay: PBMC from healthy human donors are stimulated with Staphylococcal enterotoxin B (SEB) in the presence of ICMs.
- Mixed Lymphocyte Reaction (MLR): CD4+ T cells are co-cultured with allogeneic monocyte-derived dendritic cells in the presence of ICMs.

Cytokine release and T cell proliferation are measured as standard assay readouts. Phenotyping can also be performed to confirm ICM expression or further characterise immune cell populations.

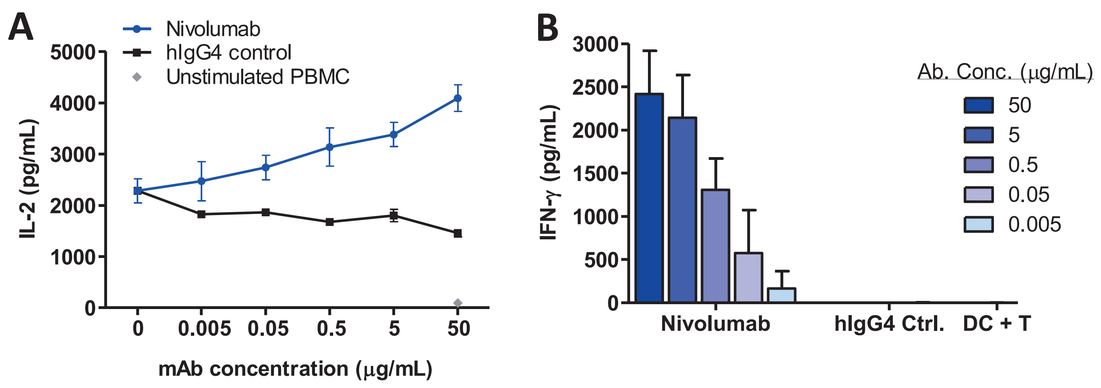


Figure 2: PD-1 blockade by nivolumab enhances cytokine secretion in both the Super Antigen Stimulation Assay (A) and the MLR (B).

Investigating immune cell migration

Molecules recruiting immune cells to the tumour site can be characterised *in vitro* using trans-well migration or invasion assays.

- Fluorescently labelled immune cells migrate through a porous membrane in response to a chemoattractant.
- Migrated cells are then counted using image analysis.

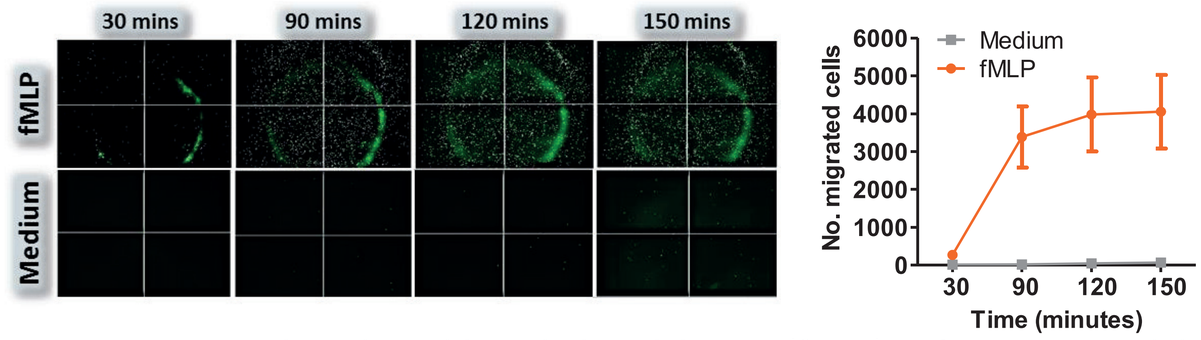
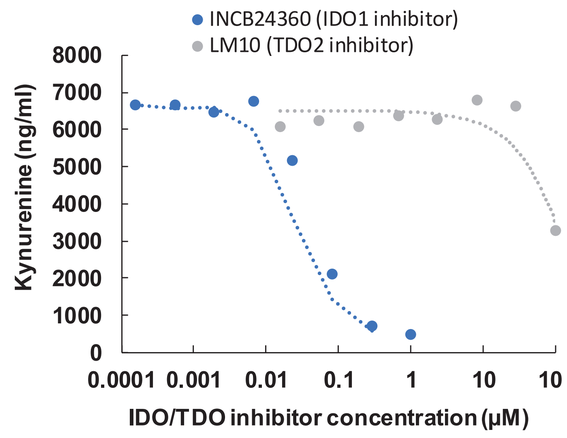


Figure 3: Formyl-methionyl-leucyl-phenylalanine (fMLP) induces neutrophil migration in a trans-well migration assay.

Assessing IDO/TDO inhibitors

The effect of IDO/TDO inhibitors on the catabolism of tryptophan and on the maintenance of immune suppression can be assessed by directly quantifying kynurenine production or by measuring T cell proliferation in a co-culture system.



Cell line-based assay:

- A172 or IFN-γ stimulated HeLa cells are treated with TDO2 or IDO1 inhibitors, respectively.
- Kynurenine production is measured after 2 days as an indicator of IDO/TDO activity.

Figure 4: IDO1 inhibition in HeLa cells leads to decreased levels of kynurenine.

PBMC-based assay:

IDO1 inhibition reverses T cell suppression mediated by IDO-expressing HeLa or dendritic cells (DC).

- In a co-culture assay, T cells are mixed with allogeneic DC or HeLa cells.
- Kynurenine production and T cell proliferation are measured as an indicator of IDO activity.

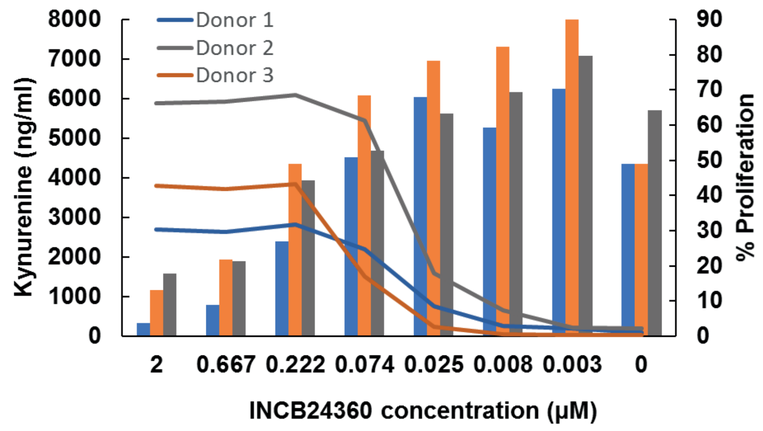


Figure 5: IDO1 inhibition of HeLa:T cell co-culture enhances T cell proliferation and decreases kynurenine production.

Summary

- Abzena has developed a panel of assays designed to enable successful characterisation, selection and development of your immuno-oncology therapeutic candidates.
- Each assay can be tailored to the specific requirements of your project.
- Where standard solutions are not an option for very novel therapeutic candidates or combinations, we also offer custom-made assays. Our team of experts will work with you to provide solutions to suit your specific needs.

[1]Contact: felicity.rudge@abzena.com or gary.bembridge@abzena.com