**Introduction**

Anti-programmed death ligand 1 (PD-L1) antibodies are one of the leading immune checkpoint inhibitors on the market with several molecules already FDA approved for cancer treatment. Using the Kymouse™ platform, we have identified a panel of potent fully human, antigenic antibodies which bind human and cynomolgus PD-L1 and enhance T cell activity. This research describes a cell based in vitro screening cascade which enabled functional characterisation of anti-human PD-L1 antibodies.

**Screening cascade for the selection of anti-PD-L1 antibodies**

Primary screened antibody binding to human PD-L1 using standard recombinant protein based assays such as HTRF and ELISA (Figure 1). Primary hits were analysed by flow cytometry to confirm ability to bind to PD-L1 expressed on cells and neutralize the interaction with its cognate receptors (PD1 and CD80) (Figure 2). This reduced the number of clones for further characterisation in functional assays.

**Neutralising anti-human PD-L1 antibodies restore murine IL2 production in a surrogate APC-T cell assay**

Biological activity was confirmed using a surrogate murine IL2 producing cell (APC)-T cell assay, which addressed key biological questions, namely the ability of antibodies to neutralise PD-L1-PD1 interaction in a cell-cell context and improve T cell activity (Figure 3).

Lead and back up antibodies were further selected on their functional activity based on their ability to block the interaction between human PD-L1 and murine PD1 and restore T cell activity, measured by increase in murine IL2 production (Figure 4).

**PD-L1 Blockade by Kymab anti-Human PD-L1 antibodies increases human IFNγ in a autologous monocyte and T cell co-culture assay**

To confirm that our anti-PD-L1 antibodies enhanced human T cell activation antibodies were assessed in a primary autologous human monocyte and T cell co-culture assay (Figure 5).

This allowed investigation into the ability of antibodies to bind endogenously expressed human PD-L1 and modulate IFNγ production in a primary cell based assay in a concentration dependent manner (Figure 6).

Noteworthy, varying levels of IFNγ upregulation were detected for different clones allowing ranking and selection of the most potent antibodies, illustrating the importance of these biologically relevant assays to select the most potent clones.

**Conclusions**

Altogether, identification of a potent, selective fully human monoclonal anti-PD-L1 antibody has been facilitated through an appropriate in vitro screening cascade.

Kymouse™ derived antibodies selected using primary function assays have demonstrated strong anti-lumour activity in a human T cell Xenograft co-transplant in vivo model, confirming that this screening cascade can efficiently select very potent clones. Further characterisation of KY1003 can be found in P075 & P077.

In conclusion, we have identified a lead clone KY1003, that has the characteristics of a clinically relevant PD-L1 antibody.