Cell based screening cascade to select anti-human PD-L1 antibodies

P074

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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[1].

Using the Kymouse™ platform, we have identified a panel of potent fully human, antagonistic 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-PDL1 antibodies

Primary screening assessed antibody binding to human PD-L1 using standard recombinant protein based assays such as HTRF and ELISA **(Figure 1).** Primary hits were analyzed 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.

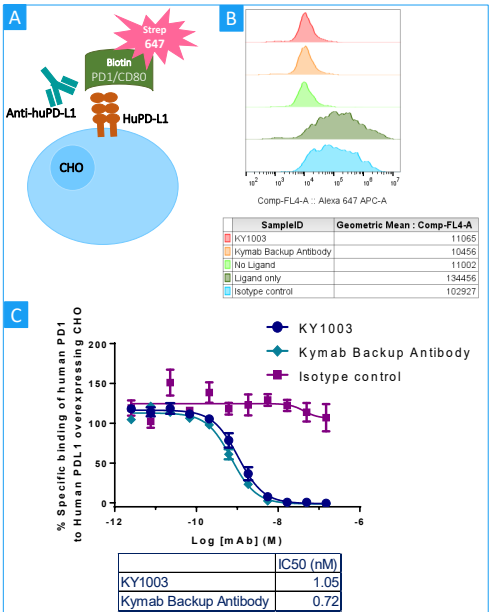
Primary screening – Binding Assays		Secondary Screening Binding/Neutralisation Assays
Recombinant protein Human/Cyno PDL1 binding HTRF	Recombinant protein PD1/CD80 neutralisation ELISA	Transfected Cells PD1/CD80 neutralisation Flow cytometry
Functional Characterisation Assays		
Surrogate Mouse Antigen Presenting cell-T cell Assay	Autologous primary monocyte and T cell co-culture	

Figure 1 **Schematic of screening cascade utilised to select anti human PD-L1 antibodies**

Anti-human PD-L1 antibodies were shown to neutralise PD1 & CD80 binding to human PD-L1

Specific binding of recombinant PD1 to human PD-L1 overexpressing CHO cells was reduced in a dose dependant manner with Kymab anti-human PD-L1 antibodies **(Figure 2)** .

Anti human PD-L1 antibodies were selected based on potency as measured by IC_{50} values in the low nM range.



SampleID	Geometric Mean : Comp-FL4-A
KY1003	11065
Kymab Backup Antibody	10456
No Ligand	11002
Ligand only	134456
Isotype control	102927

	IC50 (nM)
KY1003	1.05
Kymab Backup Antibody	0.72

Figure 2 ***Neutralisation of recombinant PD1 binding to human PD-L1 transfected CHOs.***
A) Schematic of Human PD-L1-PD1/CD80 neutralisation as measured by flow cytometry. Human PD-L1 overexpressing CHO cells were incubated with biotinylated recombinant PD1/CD80 and anti-human PD-L1 antibodies. To determine specific binding of PD1 after antibody treatment, unbound PD1/CD80 was washed off and remaining PD1/CD80 was detected using Streptavidin Alexa-647. Cells were analysed on the Cytoflex (Beckmann coulter) flow cytometer and binding of PD1/CD80 was quantified by measuring Alexa-647 geometric mean. B) Histograms showing anti-human PD-L1 (50nM) neutralising PD-L1-PD1 interaction and reducing the geometric mean comparable to no ligand control. C) B) Concentration response curves for anti-human PD-L1 antibodies represented as % specific binding of PD1 and calculation of IC_{50} values for each antibody.

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 antigen presenting 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).**

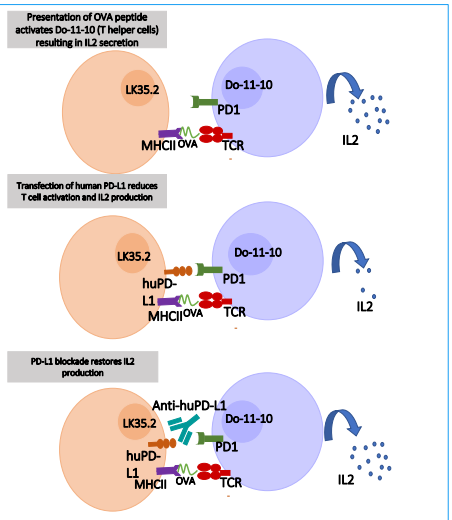
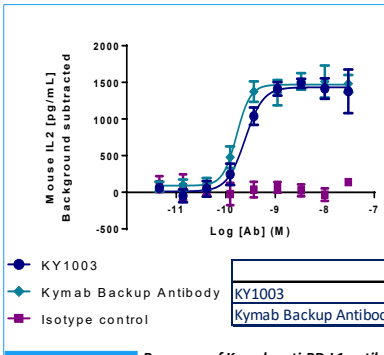


Figure 3 ***Schematic of surrogate mouse APC-T cell assay.*** *Do-11-10 is a murine T helper cell line which is activated when $OVA_{323-329}$ peptide is presented by LK35.2 (APCs). Activated Do-11-10 cells produce murine IL2 which is quantifiable by ELISA. Production of IL2 can be modulate in this system by the transfection of human PD-L1 into LK35.2 cells. Once expressed on the cell surface human PD-L1 can bind to endogenous mouse PD1 on Do-11-10 cells, reducing T cell activity and IL2 production by delivering inhibitory signals. Addition of neutralising anti-human PD-L1 antibodies results in blockade of the PD-L1-PD1 axis and restoration of T cell activity, measured by increased murine IL2 production.*

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 an increase in murine IL2 production **(Figure 4)**.



	EC50 (nM)
KY1003	0.25
Kymab Backup Antibody	0.17

Figure 4 ***Response of Kymab anti-PD-L1 antibodies in a surrogate murine APC-T cell assay.*** *Do-11-10 cells and $OVA_{323-329}$ peptide loaded LK35.2 cells are co-cultured at a 1:1 ratio and treated with anti-human PDL1 antibodies/isotype control. After 24 hours supernatants are harvested and Mouse IL2 is quantified by murine IL2 Duoset ELISA (R&D systems). Cytokine release (pg/mL) increased in a concentration dependant manner with increasing concentrations of Kymab anti-human PD-L1 antibodies compared to isotype control. Plot represents mean of 3 individual experiments +/- SEM.*

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

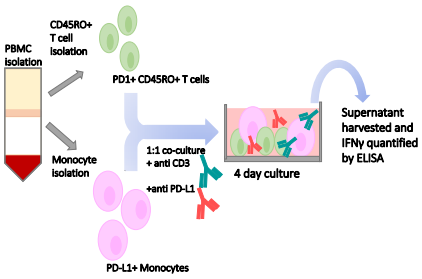
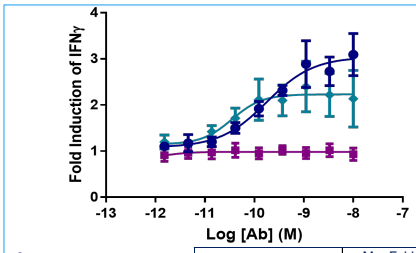


Figure 5 ***Schematic of autologous monocyte and T cell co-culture assay.*** *Fresh peripheral blood mononuclear cells (PBMCs) were isolated from leukocyte cones via density centrifugation using Ficoll. Isolated PBMCs then underwent further rounds of isolations to obtain autologous monocytes and $CD45RO^+$ T cells either via negative or positive selection using Miltenyi magnetic bead separation kits. Monocytes and T cells were co-cultured at a 1:1 ratio with anti-CD3 and anti-human PD-L1 antibodies. After 4 day incubation supernatants were harvested and assessed for IFNγ release by Duoset ELISA (R&D systems)*

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.



	Max Fold Induction
KY1003	3.04
Kymab Backup Antibody	2.23

Figure 6 ***Response of Kymab anti-PD-L1 antibodies in a autologous monocyte and T cell co-culture assay.*** TCR (anti-CD3) stimulation induces basal induction of IFNγ, which is increased in a concentration dependant manner after PD-L1 blockade. Plot represents mean +/- SEM fold induction of IFNγ across 4/5 healthy donors.

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-tumour 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.

References: 1) Iwai, Y., et al. *J Biomed Sci. 2017;24(1):26*

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