KY1055, a novel ICOS/PD-L1 bispecific antibody, efficiently enhances T cell activation and delivers a potent anti-tumour response in vivo

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Introduction

Considerable progress has been made using monoclonal antibodies to modulate T cell function in the Tumour Microenvironment (TME) by blocking the PD-1/PD-L1 axis, resulting in control of tumour growth and improvement of the clinical outcomes for many cancer indications.

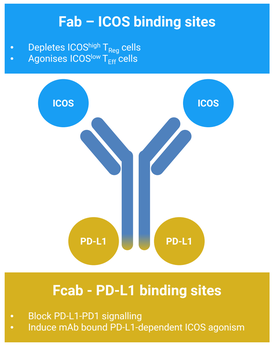
Further improvement of durability and anti-tumour response rates is likely to result from the combination of therapies targeting, for example, different modulators of T cell function.

One of such targets is ICOS, which regulates both pro- and anti-inflammatory cytokine production by effector T-cells (T_{Eff}) and regulatory T-cells (T_{Reg}), as well as T-cell proliferation and survival.

The rationale for targeting of PD-L1 and ICOS using a bispecific antibody is supported by the analysis of the TCGA database, showing the co-expression of both targets in many cancers, including breast , head and neck and melanoma.

KY1055

KY1055 is a human IgG1 mAb[2] antibody that binds to ICOS (~2nM) via the Fabs and PD-L1 (~0.3nM) via the Fcab (Fc-region with antigen-binding activity).



- KY1055 modulates T cell function as measured by the increase of IFNγ in cell-based assays developed to investigate the individual contribution of PD-L1 and ICOS arms of the bispecific molecule.
- KY1055 depletes ICOS+ CCRF-CEM cells by ADCC using NK cells isolated from PBMC of healthy donors.
- KY1055 brings together ICOS and PD-L1 expressing cells.
- KY1055 demonstrates monotherapy efficacy in several syngeneic models resistant to PD-L1 treatment and improves anti-tumour efficacy when combined with anti-CTLA-4 or anti PD-1.
- KY1055 promotes *in vivo* T_{Reg} depletion and increase of $CD8^+T_{Eff}$:T_{Reg} ratio by preferentially depleting high ICOS expressing T_{Reg}.

KY1055 induces ICOS$^+$ CEM cells killing by ADCC

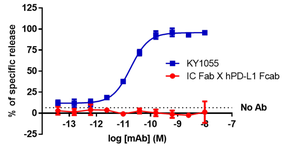


Figure 1: ICOS$^+$ CCRF-CEM cells and human NK cells isolated from healthy PBMCs were added to assay plates in 5:1 effector : target cell ratio. Target cells are loaded with DELFIA®BATDA dye, which is released as cells are killed forming a fluorescent chelate. Controls for calculation of the percentage of specific release: 1) CEM cells only (minimum killing) 2) CEM +lysis buffer (maximum killing). Data shown for one donor.

KY1055 bridges ICOS and PD-L1 expressing cells

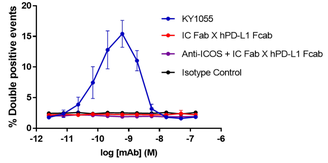


Figure 2: Ability of KY1055 to bind to PD-L1 and ICOS in a cell based assay was investigated by flow cytometry. PD-L1 and ICOS **positive** CHO cells were labelled respectively with CellTrace Far Red and Cell Trace Violet and incubated with KY1055, IC Fab X hPD-L1 Fcab, anti-ICOS combined with IC Fab X hPD-L1 Fab and isotype control.
PD-L1 and ICOS expressing cells treated with KY1055 are in close proximity as a result of the ability of the bispecific molecule to bind to PD-L1 and ICOS simultaneously, as demonstrated by an increase of the percentage of double positive events. Effect is concentration-dependent.

KY1055 demonstrates anti-tumour efficacy in CT26 model

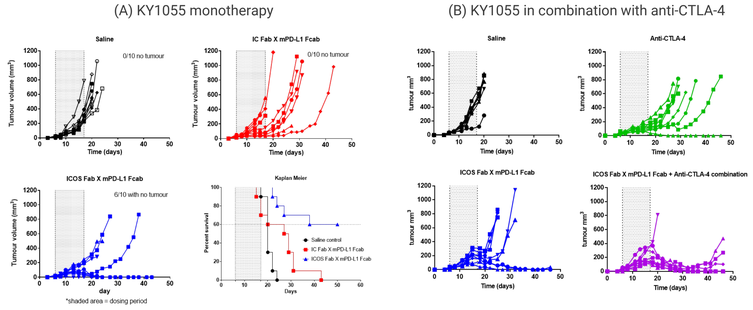


Figure 5: CT26 mice were treated with ICOS Fab X mPD-L1 Fcab alone (A) or in combination with anti-CTLA-4 (B) at 200 µg/dose, I.P. 3 times a week for 2 weeks (shaded area).

CT26 syngeneic colon murine model is relatively resistant to anti-PD-L1 and anti-ICOS monotherapies (Poster 2792).

ICOS Fab X mPD-L1 Fcab presents a strong anti-tumour efficacy and increased survival compared to IC Fab X mPD-L1 Fcab and saline control.

Improved efficacy is observed when ICOS Fab X mPD-L1 Fcab is combined with anti-CTLA-4 and anti-PD1 (data not shown).

KY1055 elicits IFNγ release by blocking PD-L1/PD-1 and driven by ICOS agonism

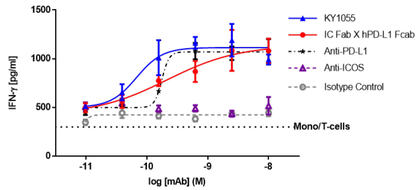


Figure 3: Fresh peripheral blood mononuclear cells (PBMCs) isolated from leukocyte cones underwent multiple isolations to obtain autologous monocytes and CD45RO+ T cells. Monocytes and T cells were co-cultured at a 1:1 ratio with anti-CD3, test molecules and isotype control.

Cells treated with KY1055 induce IFNγ release at comparable levels to those observed in cells cultured with PD-L1 antibody, demonstrating that the PD-L1 arm of the bispecific molecule is functional. Data shown is representative of a total of 6 donors.

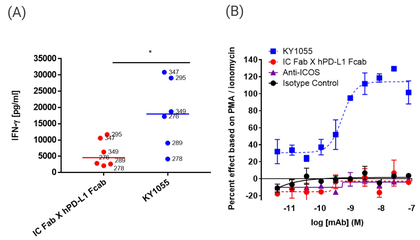


Figure 4: IFNγ quantification measured in ICOS driven agonism assays.

A) T cells isolated from PBMCs were treated with CD3/CD28 Dynabeads and cultured for 3 days with 5µg/ml plate-bound IC Fab X hPD-L1 Fcab and KY1055. IFNγ levels in response to KY1055 are significantly higher than IFNγ produced by IC Fab X hPD-L1 treated T cells. Each dot corresponds to an individual donor.

B) KY1055, anti-ICOS, IC Fab X hPD-L1 and isotype control were added to PD-L1 coated plates and incubated with ICOS$^+$ MJ cells (ATCC®CRL-8294™) for 3 days. Crosslinking of KY1055 by binding to plate-bound PD-L1 resulted in an increase of IFNγ. Plot shows percentage of effect calculated based on IFNγ release measured for PMA/ionomycin positive control.

In all assays, quantification in supernatants was performed using the Duoset ELISA Kit (R&D Systems).

KY1055 depletes T_{Reg} and improves $CD8^+$:T_{Reg} ratio in the TME

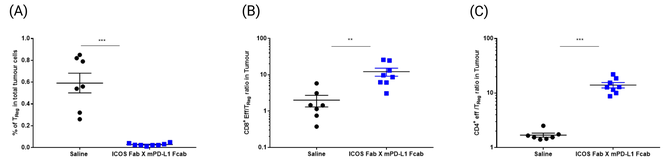


Figure 6: CT26 mice received 200 µg/dose, I.P. at days 13 and 15. Tumour isolation was performed at day 16 post-tumour implantation and processed for flow cytometry analysis.

A significant reduction of the percentage of T_{Reg} (A) in ICOS Fab X mPD-L1 Fcab compared to saline control group results in higher $CD8^+/T_{Reg}$ (B) and $CD4^+/T_{Reg}$ (C) ratios in the TME.

No depletion is seen in spleen and in tumour draining lymph nodes (data not shown).

Conclusions

- KY1055 is a mAb[2] bispecific antibody that binds with high affinity to PD-L1 and ICOS and modulates T cell function as measured by:
  1. increasing levels of IFNγ as a result of PD-1/PD-L1 blocking, but also as a direct effect of PD-L1 binding dependent ICOS-driven agonism.
  2. modulation of immune-suppressive bias in the TME by selectively targeting high-ICOS expressing T_{Reg} (depletion).
- KY1055 anti-tumour efficacy was demonstrated in several syngeneic models that are poorly responsive to the respective monotherapies and added potency is seen when used in combination with anti-CTLA-4 and PD-1.
- These data support further development of KY1055 for the treatment of solid tumours.

References:

1. Amatore F, et al. Expert Opin Ther Targets. 2018.
2. Vanella V et al. Oncoimmunology. 2017.

Acknowledgements:

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