KY1044, a novel anti-ICOS antibody, elicits long term *in vivo* anti-tumour efficacy as monotherapy and in combination with immune checkpoint inhibitors

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Introduction

ICOS is an inducible T-cell co-stimulator that is structurally and functionally related to CD28/CTLA-4.

ICOS regulates both pro- and anti-inflammatory cytokine production by effector T-cells (T_{Effs}) and regulatory T-cells (T_{Regs}), as well as T-cell proliferation and survival.

ICOS is involved in the interaction between T- and B-cells (Ig class switching), and in antibody responses to T-cell dependent antigens.

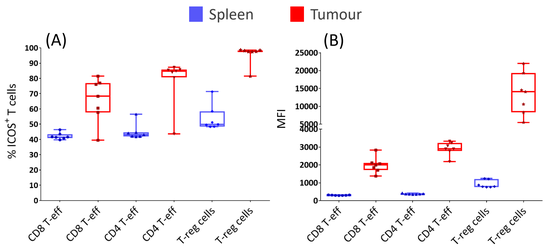


Figure 1: FACS analysis showing ICOS expression in the spleen and in CT26 tumours (n=7). Spleens and tumours (~200mm^3) were harvested and processed into single cells and stained for specific immune T-cell markers and ICOS. (A) shows the percentage in the two tissues of $CD8^+$ effector cells, $CD4^+/FOXP3^-$ effector cells and $CD4^+/FOXP3^+$ T_{Regs} expressing ICOS. (B) Relative expression of ICOS (as determine by the mean fluorescence intensity, MFI) on the cell subtypes as in (A) in spleens and tumours.

ICOS expression levels vary in different immune cell subtypes (Fig. 1), being higher on immunosuppressive T_{Regs} ($CD4^+/FOXP3^+$) than on $CD8^+$ T-Effector cells ($CD8^+$ T_{Eff}), and higher in the tumour microenvironment (TME) than in the periphery (e.g. spleen).

High levels of intratumoural $ICOS^+$ T_{Regs} have been associated with poor survival in several cancer types such as head and neck, ovarian, hepatocellular carcinoma and gastric cancer [1-4].

The preferential high expression of ICOS on intratumoural T_{Regs} makes this protein a strong candidate for a depleting antibody strategy.

KY1044

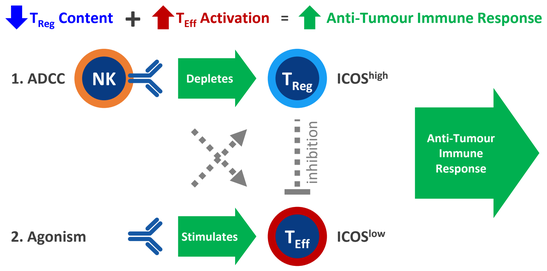
By immunising Kymouse™ in which endogenous *Icos* gene has been knocked out [5], we have identified a novel, fully human antibody, KY1044, which cross reacts with mouse ICOS facilitating in vivo studies in immune proficient mice.

KY1044 is an anti-ICOS subclass G1 kappa monoclonal antibody that selectively binds to ICOS with an affinity of less than 2nM (as determined at neutral and acidic pH as in TME).

KY1044 binds human, cynomolgus monkey, rat and mouse ICOS with similar affinity.

KY1044 has a dual mechanism of action:

(1) preferential depletion of intratumoural $ICOS^{high}$ T_{Regs} resulting in an increase in T_{Eff} : T_{Regs} ratio in the TME
(2) stimulation of $ICOS^+$ T_{Eff} cells



ICOS/FOXP3 co-expression in cancer

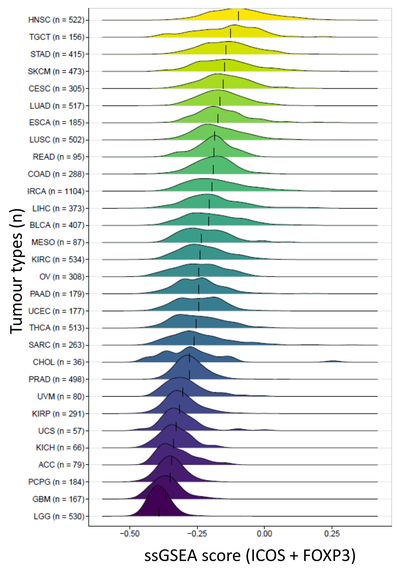


Figure 2: Joy plot showing gene set enrichment analysis of ICOS and FOXP3 expression in different tumour types (TCGA dataset). The tumour types are indicated on the left (using the abbreviation from the TCGA website (https://tcga-data.nci.nih.gov). Tumours are ranked based on high expression (yellow) to low expression (purple). The number of samples per indications is also shown in brackets.

KY1044 kills $ICOS^+$ cells via ADCC

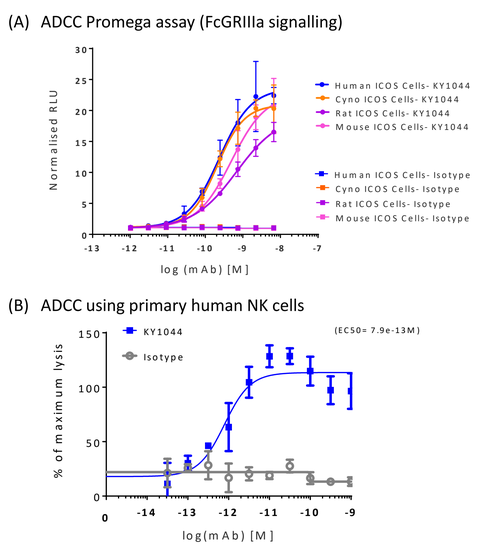


Figure 3: ADCC assays demonstrating KY1044 IgG1 killing potential in vitro. (A) KY1044 binds to CHO cells expressing ICOS proteins from different species and induces FcγRIIIa-dependent signalling, resulting in NFAT-mediated luciferase activity. (B) Human NK cells purified from PBMC (collected from healthy donors) were co-cultured for 4 hours in the presence of different concentrations of KY1044 and $ICOS^+$ CEM cells preloaded with BATDA (5:1 E:T ratio).

KY1044 induces IFNγ release from $ICOS^+$ T-cells

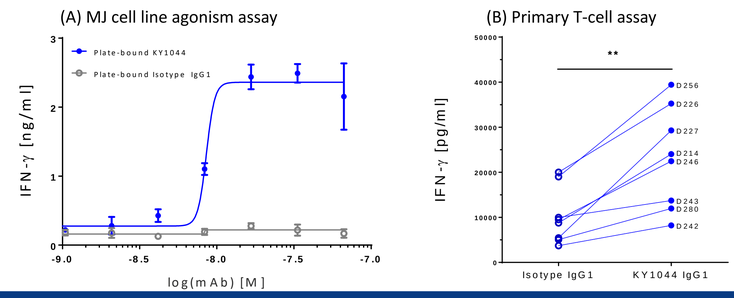


Figure 4: Plate bound KY1044 IgG1 induces IFNγ secretion by T-cells in vitro. (A) MJ cells (a T lymphoblast cell line from ATCC) which express endogenously high levels of ICOS can produce and secrete IFNγ in response to KY1044 pre-coated onto plates. (B) Primary isolated CD4/CD8 T-cells isolated from 8 healthy donors were activated with anti-CD3/CD28 (shown to induce ICOS expression) and cultured in plates pre-coated with 5ug/ml of KY1044 (n=8 donors). IFNγ levels were measured by MSD after 72 hours of culture. (HC) hybrid control. (**) p<0.01.

KY1044 triggers strong monotherapy efficacy in vivo (A20 model)

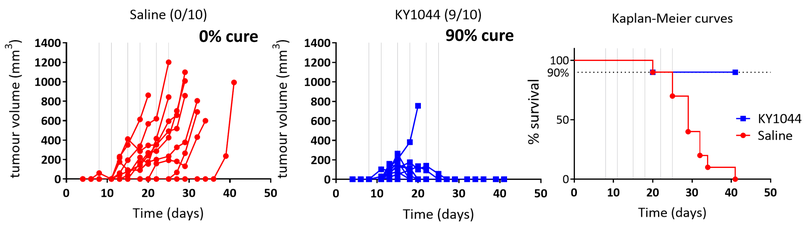


Figure 5: Spider plot and Kaplan Meier curves showing the anti-tumour efficacy of KY1044 mIgG2a in the A20 B cell lymphoma syngeneic model (sub-cut implantation of A20 and IP dosing from day 8 of KY1044 mIgG2 at 10mg/kg). Numbers in brackets indicate the number of animals showing no signs of disease at endpoint. Vertical lines indicate day of dosing.

KY1044 and anti-PD-L1 strongly synergise, curing up to 100% of tumours

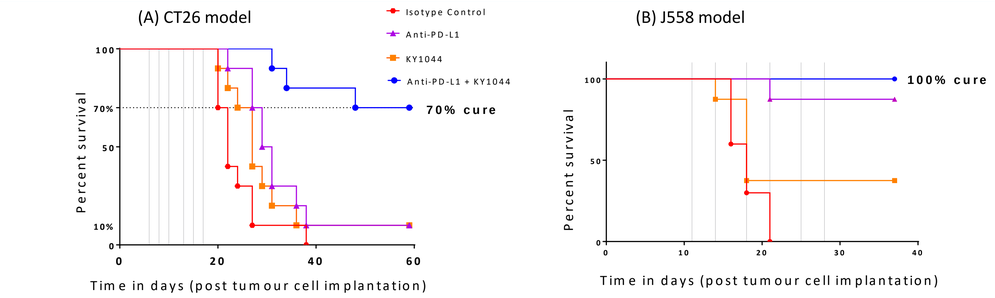


Figure 6: Kaplan Meier graphs demonstrating anti-tumour efficacy of KY1044 mIgG2a in combination with anti-PD-L1 in the CT26 and J558 syngeneic tumour models. (A) CT26 cells were implanted sub-cut in the right flank of 8-10 week old Balb/c mice and treated with the isotype control, anti-PD-L1, KY1044 mIgG2a or a combination of both antibodies. Animals (n=10 per treatment groups) were dosed IP 3 times a week for two weeks (vertical lines) from day 6 post tumour cells implantation. KY1044 was dosed at 10mg/kg TIW and anti-PD-L1 at 10mg/kg. (B) J558 cells were implanted sub -cut in the right flank of 8-10 week old Balb/c mice and treated with the isotype control, anti-PD-L1, KY1044 mIgG2a or a combination of both. Animals (n=10 per treatment groups) were dosed IP twice a week for three weeks (vertical lines) from day 11. KY1044 was dosed at 3mg/kg and anti-PD-L1 at 10mg/kg.

KY1044 depletes intratumoural T_{Regs} in vivo

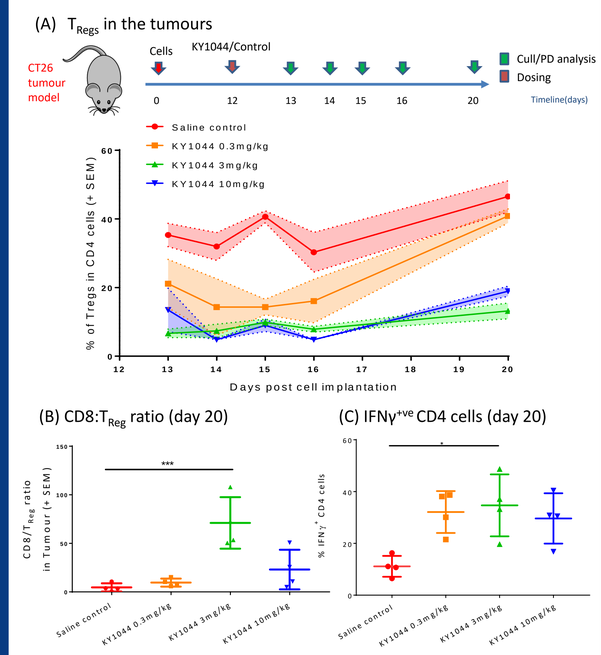


Figure 7: *(A)* Graph showing the effect of KY1044 mIgG2a on T_{Regs} levels in tumours (n=4 per time points/conditions, at different doses and at different times post dosing). (B) An intermediate dose of KY1044 mIgG2a (3mg/kg) was associated with the highest increase in CD8: T_{Regs} ratio by day 20. (C) The intermediate dose of KY1044 was also associated with the highest increase in CD4 helper (and CD8, data not shown) T-cells expressing IFNγ in the TME. (*) p<0.05, (***) p<0.001.

Increased efficacy at an intermediate dose

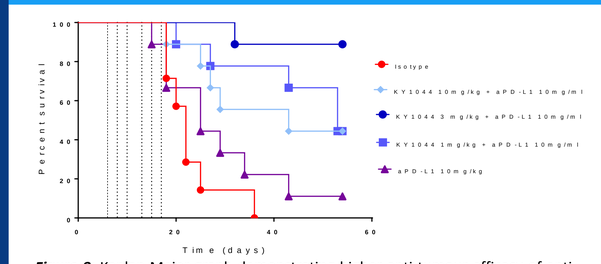


Figure 8: Kaplan Meier graph demonstrating higher anti-tumour efficacy of anti-ICOS/anti-PD-L1 at the intermediate dose of KY1044 (3mg/kg). CT26 cells were implanted sub-cut in the right flank of 8-10 week old Balb/c mice and treated with the isotype control, anti-PD-L1, or a combination of KY1044 mIgG2a (1, 3 or 10mg/kg) and anti-PD-L1 (10mg/kg). Animals (n=7-9 animals per treatment group) were dosed IP 3 times a week for two weeks (vertical lines) from day 8.

Conclusions

Intratumoural T_{Regs} express high level of ICOS on their surface. ICOS/FOXP3 expression varies in human tumour types, showing high expression in head and neck cancers, and low expression in glioblastoma/glioma.

KY1044, a novel fully human anti-ICOS antibody has a dual mechanism of action. KY1044 has the ability of killing $ICOS^{high}$ cells via ADCC. KY1044 also acts as an agonist antibody on $ICOS^+$ effector cells in vitro (IFNγ release).

As shown in different models, KY1044 strongly inhibits tumour growth as monotherapy and in combination with checkpoint inhibitors such as anti-PDL1.

KY1044 depletes intratumoural T_{Regs}, improves the effector to T_{Regs} ratio and also induces the up-regulation of inflammatory cytokines in vivo.

In summary, our data demonstrates that targeting ICOS with KY1044 is a valid approach for manipulating the immune system and for inducing a strong anti-tumour response. The data presented here also warrant the assessment of KY1044 in cancer patients in a clinical trial.

References
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