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Wnt/β-Catenin pathway inhibitor RXC004 enhances the immunity of pre-clinical models of cancer

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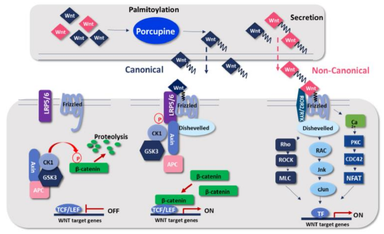
Introduction

RXC004 is a potent and selective small molecule inhibitor of the membrane bound *O*-acyl transferase Porcupine (PORCN). PORCN is required for post-translational modification of Wnt ligands, a necessary step in the initiation of canonical and non canonical Wnt signalling.

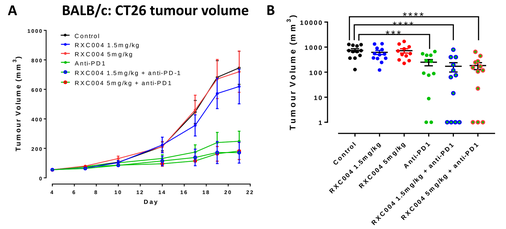


Pre-clinical studies have demonstrated the potential for PORCN inhibition to provide benefit to genetically selected cancer patient populations (see abstract #3874).[1,2] RXC004 is currently being evaluated in a first-in-human clinical study (NCT03447470). A growing body of literature suggests that Wnt signalling plays a role in the host immune response to tumours, and activation of the pathway may result in poor response and indeed resistance to immune checkpoint inhibitors.[3,4] RXC004 has undergone preliminary evaluation in syngeneic mouse models of immunotherapy demonstrating its potential to enhance immune response in the tumour microenvironment.

Results

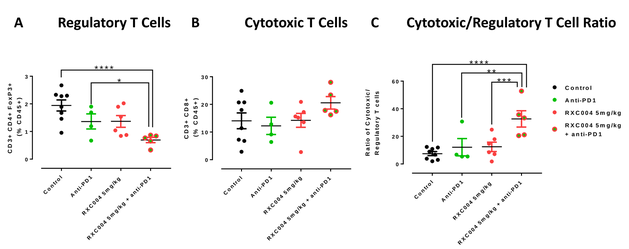
RXC004 enhances the anti-tumour effects of anti-PD-1 in the CT26 mouse colorectal cancer model: an immune checkpoint sensitive model

Figure 1. RXC004 co-operates with anti-PD1 in CT26 tumours, an immune checkpoint sensitive model. Mouse CT26 cells ($5x10^5$) were subcutaneously implanted in the flanks of female BALB/c mice. Treatment was initiated once tumours reached ~50mm^3. **A.** Mean tumour volumes of the indicated groups over time. As expected, significant tumour growth inhibition (TGI) was seen in all anti-PD1 groups at day 21 (**B**). Increased cures (i.e. tumour volume of 0mm^3) were seen in the combination groups compared to anti-PD1 alone



RXC004 in combination with anti-PD1 in the CT26 model significantly decreases Treg cells and increases the CD8:Treg ratio

Figure 2. RXC004 combines with anti-PD1 to enhance the anti-tumour immune environment. Mouse CT26 cells ($5x10^5$) were subcutaneously implanted in flanks of female BALB/c mice. Treatment was initiated once tumours reached ~50mm3. **A, B.** Flow cytometry of day 14 tumour infiltrate shows proportions in FOXP3+ and CD8+. **C.** Significant change in the ratio of CD8+ T-cells to FOXP3+ T-cells.



RXC004 monotherapy efficacy in B16F10 melanoma model: an immune checkpoint resistant model

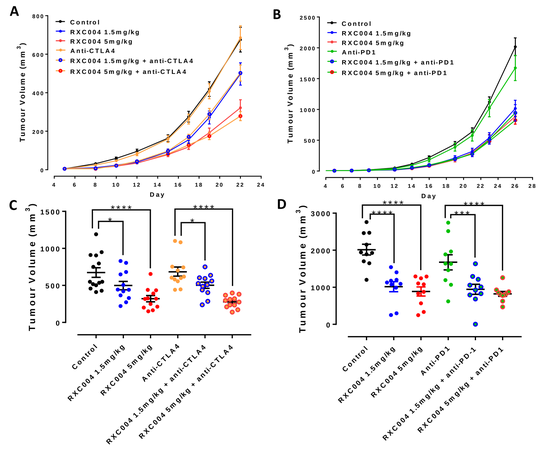


Figure 3. RXC004 shows monotherapy efficacy and increased survival in B16F10 tumours, a model non-responsive to immune checkpoint inhibition. Mouse B16F10 cells ($2x10^5$) were subcutaneously implanted in flanks of male C57BL/6 mice. Treatment was initiated once tumours were palpable, 3 days later. **A, B.** Mean tumour volumes over time shows no effect of either anti-CTLA4 or anti-PD1 immune checkpoint inhibitors in this model; as confirmed with Day 22 **(C)** or Day 26 **(D)** tumour volumes. Significant TGI was seen in all RXC004 treated groups, as either a monotherapy or in combination with anti-CTLA4 **(C)** or anti-PD1 **(D)**.

RXC004 has monotherapy efficacy in B16F10 melanoma model at lower and scheduled doses

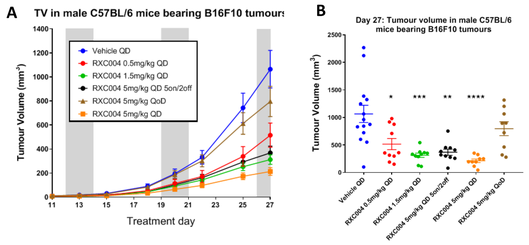


Figure 4. RXC004 shows monotherapy efficacy in B16F10 tumours, a model non-responsive to immune checkpoint inhibition. Mouse B16F10 cells ($2x10^5$) were subcutaneously implanted in flanks of male C57BL/6 mice. Treatment was initiated once tumours were palpable, 3 days later. **A, B.** Mean tumour volumes over time shows significant TGI was seen in all RXC004 treated groups, except the every other day schedule (5mg/kg QoD) as a monotherapy. Grey bars show drug dosing holidays for 5 on 2 off.

RXC004 has a confirmed immune MoA in the B16F10 melanoma model

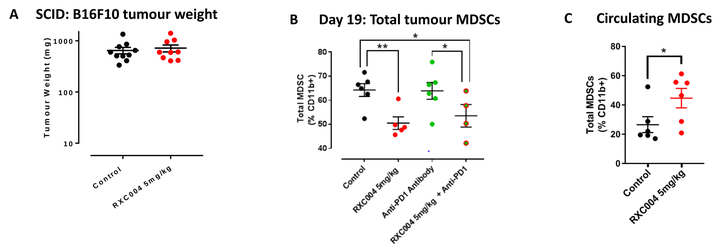


Figure 5. RXC004 has a confirmed immune mechanism of action in the B16F10 melanoma model Mouse B16F10 cells ($2x10^5$) were subcutaneously implanted in the flank of immunocompromised male SCID-Beige mice. Treatment was initiated 3 days later. **A.** Mean tumour weights at the end of study (21 days) confirmed no significant effects of RXC004 on B16F10 growth in the SCID mouse strain. SCID-Beige mice animals have severely reduced T and B-lymphocytes, along with defects in macrophages. In addition they have impaired NK cell function. RXC004 has no effect on the proliferation of B16F10 cells *in vitro* (not shown). **B.** At 2 hours post final dose (Day 19) tumours were resected, weighed **and** digested and stained for flow cytometry analysis. A myeloid analysis panel included viability dye, CD45, CD11b, Ly6C and Ly6G. Myeloid MDSCs (M-MDSC) were gated as $CD45^+CD11b^+$ $Ly6G^-Ly6C^{high}$ and polymorphonuclear MDSCs (PMN-MDSC) were gated as $CD45^+CD11b^+$ $Ly6G^+Ly6C^{med}$. Quantification of total-MDSCs. **C.** At 2hrs post final dose terminal blood was taken and stained and analysed as described for B and C. Quantification of circulating total MDSCs.

RXC004 treatment results in increased immune-related gene expression by Nanostring IO-360 panel in B-16F10 melanoma model

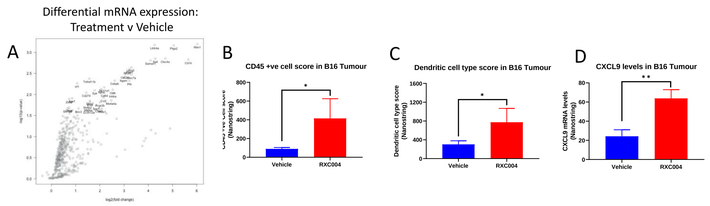


Figure 6. Vehicle and RXC004 treated (5mg/kg QD) B16F10 tumours were snap frozen, total RNA was purified and mRNA expression levels were analysed using Nanostring. **A.** Volcano plot displaying each genes –log10 (p-value) and log2 fold change. Highly statistically significant genes fall at the top of the plot and highly differentiated genes at either side. The 40 most statistically significant genes are labelled. **B** RXC004 treatment results in an increase in CD45 cell score, **C.** dendritic cell score and **D.** an increase expression of CXCL9 in the tumour. CXCL9 was reported to be be produced by CD103+ve dendritic cells. The absence of this cell type has been linked to immune evasion by tumours.[5]

RXC004 treatment results in changes in immune-related cell surface markers on sensitive tumour cell lines only

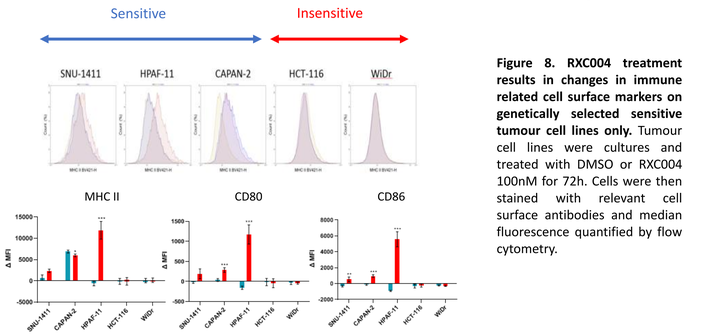
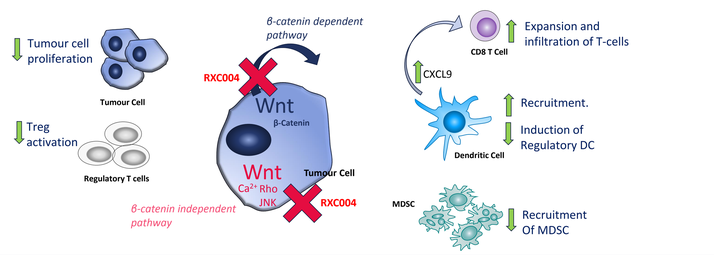


Figure 8. RXC004 treatment results in changes in immune related cell surface markers on genetically selected sensitive tumour cell lines only. Tumour cell lines were cultures and treated with DMSO or RXC004 100nM for 72h. Cells were then stained with relevant cell surface antibodies and median fluorescence quantified by flow cytometry.

Summary

RXC004 inhibits tumour proliferation and stimulates a tumour fighting microenvironment



References

1. Madan B *et al; Oncogene*, 2015, 35 (17): 2197 **2.** Bhamra I *et al; J Clin Oncol*, 2017, 35 (15);**3.** Wang *et al; TIPs,* 2018, 39(7):648: **4.** Spranger *et al; Nature Reviews 18:139* 2018. **5.** Spranger *et al; Cancer Cell, 2017, 31:711.*

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AACR American Association for Cancer Research