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Porcupine inhibitor RXC004 enhances immune response in pre-clinical models of cancer

Inder Bhamra, Caroline Phillips, Richard Armer, Matilda Bingham, Catherine Eagle, Alicia Edmenson Cook and Simon Woodcock

Redx Pharma, Block 33S, Mereside, Alderley Park, Macclesfield, Cheshire, SK10 4TG, UK. e: i.bhamra@redxpharma.com t: +44(0)1625 469943

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 Wnt signalling (Figure 1).[1] Aberrant Wnt signalling initiates key oncogenic pathways in cancer, implicated in tumour initiation, growth, cell senescence, cell death, differentiation and metastasis.[2] Pre-clinical studies have demonstrated the potential for PORCN inhibition to provide benefit to molecularly selected cancer patient populations.[3,4] 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.[5] RXC004 has undergone preliminary evaluation in syngeneic mouse models of immunotherapy demonstrating its potential to enhance immune response in the tumour microenvironment. Early *in vitro* work using human primary cells is consistent with this hypothesis.

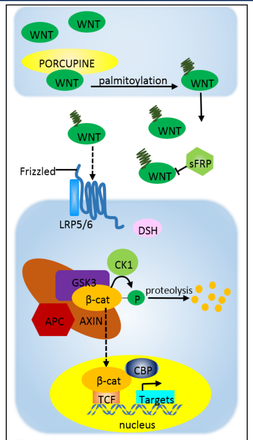

Figure 1. Canonical Wnt Pathway.
The activity of Porcupine within Wnt expressing cells is required for secretion of active Wnt. This active Wnt can in turn bind Frizzled receptors to initiate signalling of the canonical Wnt pathway.

Results

RXC004 enhances the anti-tumour effects of anti-PD-1

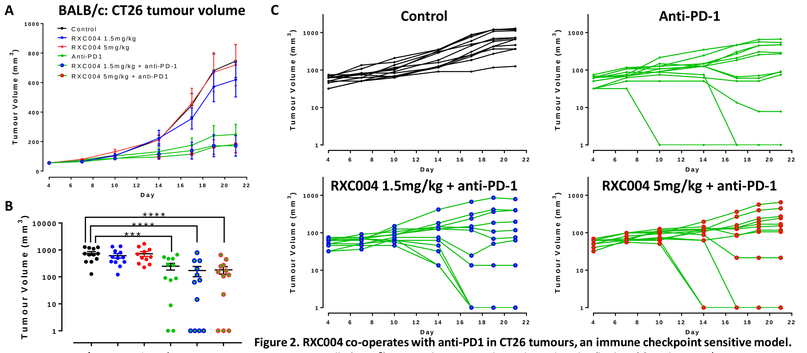

Figure 2. RXC004 co-operates with anti-PD1 in CT26 tumours, an immune checkpoint sensitive model.
Mouse CT26 cells ($5x10^5$) were subcutaneously implanted in the flanks of female BALB/c mice. Treatment was initiated once tumours reached ~50mm³. **A.** Mean tumour volumes of the indicated groups over 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 0mm³) were seen in the combination groups compared to anti-PD1 alone. **C.** Individual animal tumour growth curves. Clear tumour regression is observed in the RXC004 + anti-PD1 combination groups. RXC004 does not affect proliferation of CT26 cells *in vitro* (not shown).

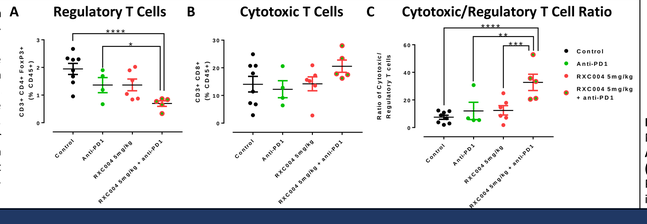

Figure 3. RXC004 combines with anti-PD1 to enhance the anti-tumour immune environment.
Mouse CT26 cells ($5x10^5$) were subcutaneously implanted in flanks of female BALB/c mice. Treatment was initiated once tumours reached ~50mm3. **A, B.** Flow cytometry of day 14 tumour infiltrate shows proportions in FOXP3+ and CD8+. **C.** Significant change in the ratio of CD8+ T-cells to FOXP3+ T-cells.

Wnt pathway activation induces immunosuppressive human moDCs

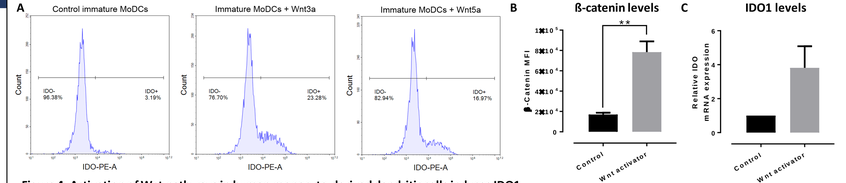

Figure 4. Activation of Wnt pathways in human monocyte-derived dendritic cells induces IDO1.
Human monocytes were purified from peripheral blood mononuclear cells (PBMCs) using CD14 positive selection and cultured with GM-CSF and IL-4 for 6-7 days to derive immature dendritic cells (iDCs). **A.** iDCs were cultured with recombinant human Wnt3a or Wnt5a ligands (100 ng/ml) as indicated for 24hrs. IDO1 protein expression was analysed by flow cytometry; a donor responding to Wnt ligands is shown. **B.** Analysis of β-Catenin protein levels by flow cytometry. Donor iDCs (n=6) were treated with Wnt pathway activator LY2090314 (10 nM), a GSK3β inhibitor, for 24hrs. GSK3β inhibition increased the median fluorescence intensity (MFI) of β-Catenin in iDCs. **C.** Analysis of IDO1 mRNA by RT-PCR. Donor iDCs (n=6), treated as in **B**, demonstrate increased IDO1 mRNA expression when treated with GSK3β inhibitor. IDO1 gene expression was normalised to housekeeping genes Actin and GAPDH.

RXC004 blocks secretion and function of Wnts

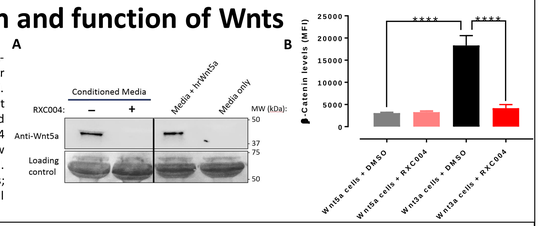

Figure 5. RXC004 blocks Wnt3a and Wnt5a secretion.
A. Western blot of conditioned media (CM) from L-Wnt5a cells. Cells were treated with 300nM RXC004 for 48hrs, equal volumes of CM were loaded (lower panel). Cell-free media or media containing 15ng recombinant human Wnt5a were loaded as controls. **B.** L-Wnt3a and L-Wnt5a cells were treated with 100nM RXC004 for 24 hours. Cells were stained with anti-ß-Catenin for flow analysis; ß-catenin levels were determined by MFI. Canonical Wnt3a expression increased ß-catenin levels; RXC004 blocked this effect. As expected, non-canonical Wnt5a expression did not alter ß-catenin levels.

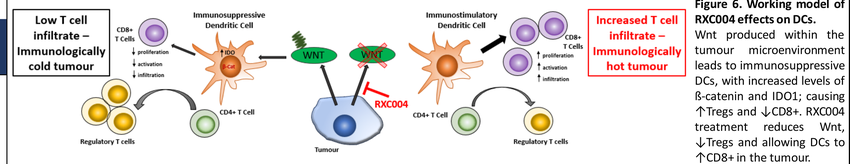

Figure 6. Working model of RXC004 effects on DCs.
Wnt produced within the tumour microenvironment leads to immunosuppressive DCs, with increased levels of ß-catenin and IDO1; causing ↑Tregs and ↓CD8+. RXC004 treatment reduces Wnt, ↓Tregs and allowing DCs to ↑CD8+ in the tumour.

RXC004 monotherapy efficacy in immunologically 'cold' tumours

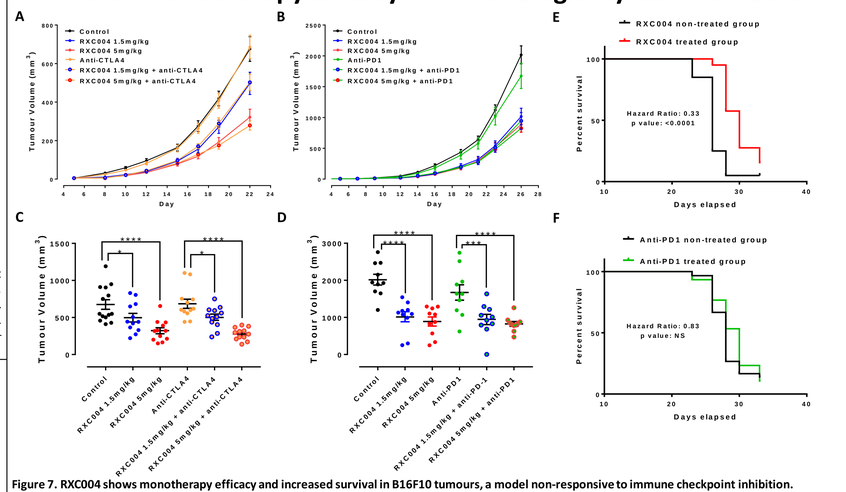

Figure 7. RXC004 shows monotherapy efficacy and increased survival in B16F10 tumours, a model non-responsive to immune checkpoint inhibition.
Mouse B16F10 cells ($2x10^5$) were subcutaneously implanted in flanks of male C57BL/6 mice. Treatment was initiated once tumours were palpable, 3 days later. **A, 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)**. **E ,F.** Kaplan-Meier survival plots from a time-to-event B16F10 study (tumour volume cut-off 1500mm³). RXC004-treated group shows significantly increased survival **(E)**, whereas anti-PD1-treated group shows limited effects **(F)**. RXC004 has no effect on the proliferation of B16F10 cells *in vitro* (not shown).

RXC004 efficacy in B16F10 tumours requires an intact immune system

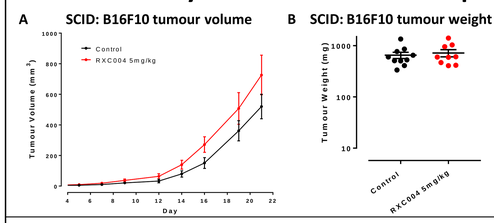

Figure 8. RXC004 efficacy requires the immune system
Mouse B16F10 cells ($2x10^5$) were subcutaneously implanted in the flank of immunocompromised male SCID-Beige mice. Treatment was initiated once tumours were palpable, 3 days later. **A.** Mean tumour volumes of the indicated groups over time shows no effect of RXC004 in this mouse strain. **B.** Mean tumour weights at the end of study (21 days) confirmed no significant effects of RXC004 on B16F10 growth in this 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).

RXC004 reduces myeloid-derived suppressor cells from 'cold' tumours

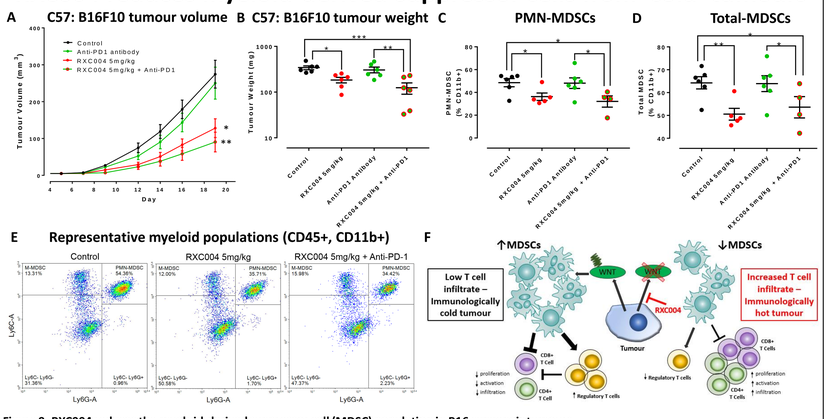

Figure 9. RXC004 reduces the myeloid-derived suppressor cell (MDSC) population in B16 syngeneic tumours.
A-E. Mouse B16F10 cells ($2x10^5$) were subcutaneously implanted in flanks of male C57BL/6 mice. Treatment was initiated once tumours were palpable, 3 days later. **A.** Mean tumour volumes over time shows RXC004 efficacy. At 2 hours post final dose (Day 19) tumours were resected, weighed **(B)**, 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 PMN-MDSCs **(C)** and total MDSCs (M + PMN; **D**) in all animals across treatment groups, confirming a significant decrease in PMN and total MDSCs in tumours of RXC004 and RXC004 + anti-PD-1 treated animals. **E.** Representative MDSC gating plots from control, RXC004 alone and RXC004 + anti-PD-1 treated animals. **F.** Working model of RXC004 effects on MDSC tumour infiltrate. MDSCs suppress T cell immune responses via multiple mechanisms; for example increased Arginase-I activity, depletion of arginine and T cell cycle arrest. Through reducing tumour MDSCs, we propose RXC004 increases immune response to the tumour.

Conclusions

- Consistent with the proposed role of the Wnt pathway in host immune response,[6] RXC004 potently inhibits Wnt ligand secretion and thus enhances the immune response in the tumour microenvironment.
- Wnt pathway activation in immature human MoDCs results in the formation of an immunosuppressive dendritic cell phenotype with increased IDO expression.
- Two immunomodulatory mechanisms have been observed and are being further investigated:
  1. RXC004/anti-PD1 combination in a CT26 syngeneic mouse colon tumour model (responsive to immune checkpoint inhibition) significantly increases the ratio of cytotoxic to regulatory T cells in tumour infiltrate *via* reduction of FOXP3+ Tregs.
  2. RXC004 monotherapy in a B16F10 syngeneic mouse melanoma model (immunologically cold) results in tumour growth inhibition by reducing myeloid derived suppressor cell populations in the tumour microenvironment.
- RXC004 is currently under evaluation in a first-in-human clinical study and early human PK indicates drug exposure levels predicted for a therapeutic effect are achievable.

References

1. Biechele S, Cox BJ, Rossant J; *Dev Biol,* 2011, 355 (2): 275-285; **2.** Nusse R, Varmus H; *EMBO J,* 2012, 31 (12) : 2670-2684; **3.** Madan B *et al; Oncogene*, 2015, 35 (17): 2197-2207 **4.** Bhamra I *et al*; *J Clin Oncol*, 2017, 35 (15); **5.** Gopalkrishna Pai S *et al*; *J Haematol Oncol*, 2017, 10:101; **6.** Holtzhausen A, Hanks BA*; Cancer Immunol Res*, 2015, 3 (9): 1082-1095.

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AACR American Association for Cancer Research