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Efficacy of the Wnt/Beta-Catenin pathway inhibitor RXC004 in genetically-defined models of cancer

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Introduction

Signalling through the Wnt pathway is highly regulated at the level of ligand (Wnt), receptor (Fzd/LRP) and downstream components (e.g. destruction complex – APC/Axin/GSK3β). Post-translational modification of Wnt ligands via porcupine (PORCN; a membrane bound O-acyltransferase) is essential for secretion of active Wnt[1]. Activity of RNF43/ZNRF3 (E3-ubiquitin ligases) results in ubiquitination and membrane clearance of Fzd, whilst RNF43/ZNRF3 levels are kept in check via LGR and secreted RPSO ligands[2] (**Fig. 1**).

The potent and selective porcupine (PORCN) inhibitor RXC004 is being investigated in a Phase 1 clinical trial (NCT03447470)[3], and has the potential to treat tumours dependent on Wnt-ligand. Upstream Wnt pathway aberrations, including RNF43/ZNRF3 mutations and RSPO-fusions, result in high levels of surface Fzd receptors and increased Wnt-ligand dependent signalling[4] (**Fig. 1**). These aberrations are implicated in pancreatic, gastric and colorectal cancer (CRC). Dysregulated Wnt signalling initiates oncogenic pathways involved in tumour initiation, growth and metastasis[5], and is more recently linked to tumour immune evasion[6,7] (see also abstract #506).

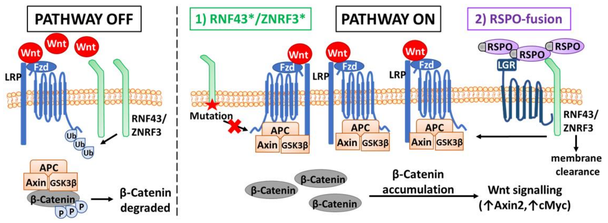


Figure 1: Upstream alterations trigger aberrant Wnt ligand-dependent signalling
RNF43/ZNRF3 keep surface Fzd in check, allowing the destruction complex to phosphorylate and degrade β-catenin - Wnt pathway 'OFF'. Loss-of-function (LOF) RNF43/ZNRF3 mutations (1), or high RSPO expression through gene fusion (2), ultimately leads to accumulation of β-catenin - Wnt pathway 'ON'.

Results

Anti-proliferative effects of RXC004 in genetically-defined tumour cell lines

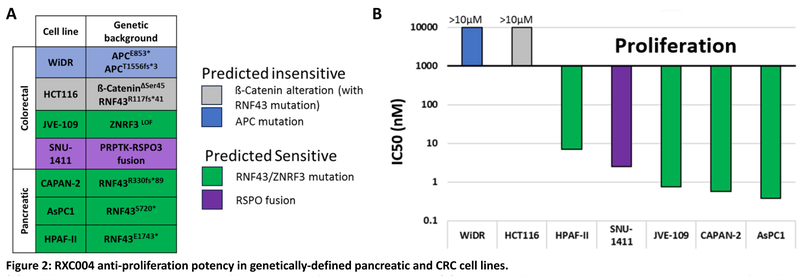


Figure 2: RXC004 anti-proliferation potency in genetically-defined pancreatic and CRC cell lines.
(A) RXC004 was evaluated across a panel of 7 genetically-defined tumour cell lines. **(B)** Indicated cell lines were treated with a dose response of RXC004 for 5 days, proliferation was measured using an ATP-lite assay. N≥3 throughout. Cell lines harbouring RNF43/ZNRF3 mutations or RSPO-fusions are sensitive to RXC004 as predicted, with anti-proliferative effects ranging from 0.3nM to 7nM.

RXC004 arrests at the G1/S and G2/M cell cycle checkpoints

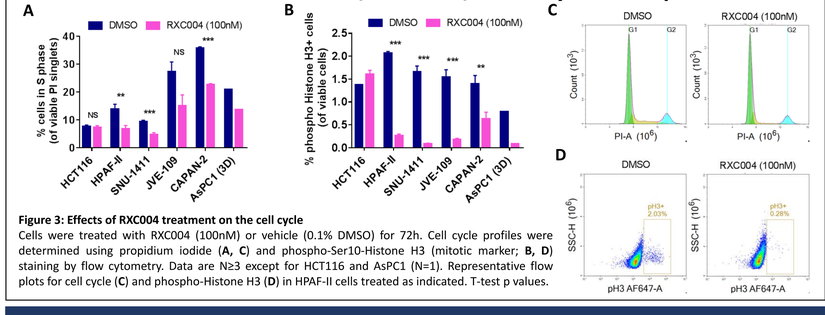


Figure 3: Effects of RXC004 treatment on the cell cycle
Cells were treated with RXC004 (100nM) or vehicle (0.1% DMSO) for 72h. Cell cycle profiles were determined using propidium iodide (**A, C**) and phospho-Ser10-Histone H3 (mitotic marker; **B, D**) staining by flow cytometry. Data are N≥3 except for HCT116 and AsPC1 (N=1). Representative flow plots for cell cycle (**C**) and phospho-Histone H3 (**D**) in HPAF-II cells treated as indicated. T-test p values.

In vitro pathway inhibition by RXC004 in genetically-defined tumour cell lines

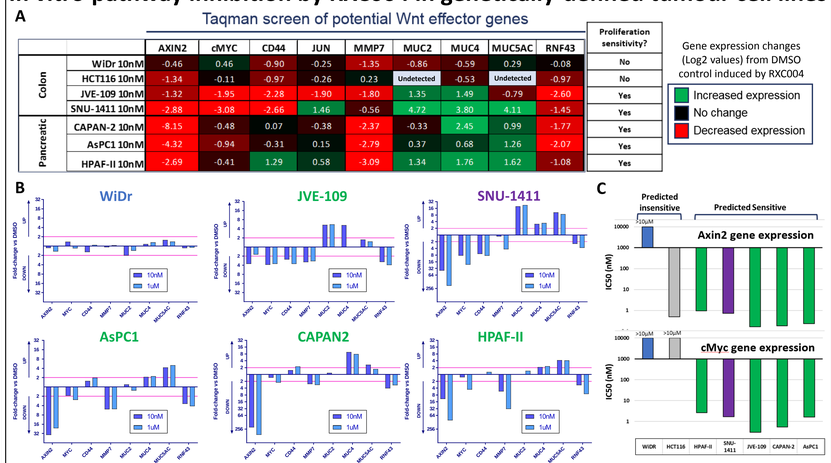


Figure 4. RXC004 regulates multiple downstream Wnt pathway effectors in genetically-defined pancreatic and CRC tumour cell lines
(A) A custom TaqMan qPCR gene array was designed based on literature evidence for genes modulated by the Wnt pathway. These custom 96-well arrays were used to screen the expression of 14 potential RXC004 effector genes (including the 9 genes indicated in **A**), across 7 genetically defined tumours lines (**Fig. 2**). Cells were treated with RXC004 (10nM) or vehicle (0.1% DMSO) for 3 days, total RNA was extracted and gene expression assessed relative to appropriate housekeepers. Heatmap **(A)** indicates the relative increase (green) or decrease (red) in gene expression induced by RXC004 treatment when compared to DMSO control. **(B)** Individual TaqMan qPCR assays for the 8 target genes indicated were confirmed separately in the specified cells lines. Cells were treated with RXC004 (10 or 1000nM) or vehicle (0.1% DMSO) for 3 days, total RNA was extracted and gene expression assessed relative to appropriate housekeepers. **(C)** Indicated cell lines were treated with a dose response of RXC004 for 3 days, total RNA was extracted and gene expression assessed relative to appropriate housekeepers. IC50 values were determined for the down-regulation of Axin2 and cMyc gene expression, N≥3.

RXC004 efficacy and pathway inhibition translates *in vivo*

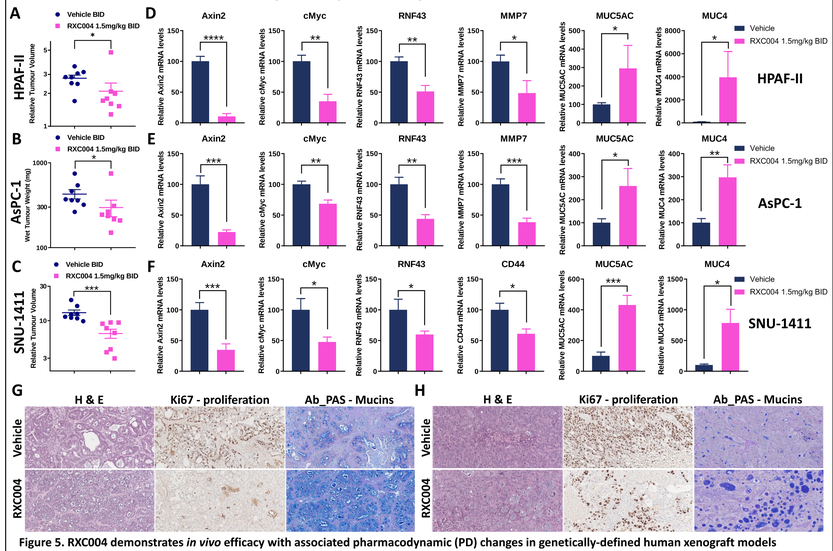
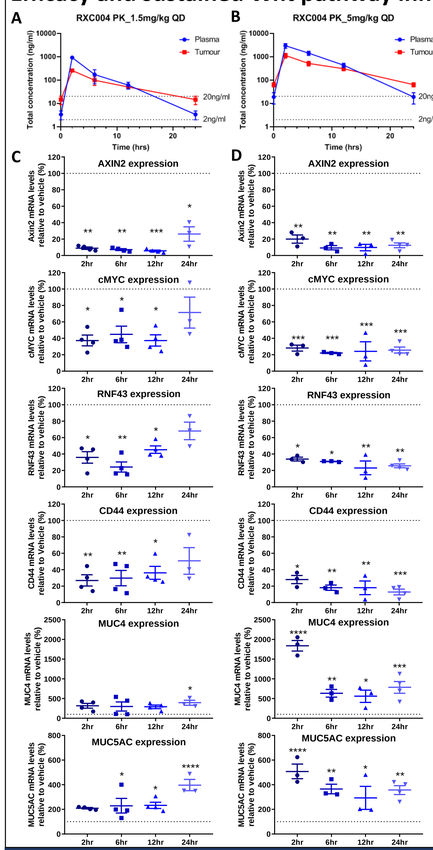


Figure 5. RXC004 demonstrates *in vivo* efficacy with associated pharmacodynamic (PD) changes in genetically-defined human xenograft models
Relative tumour volumes (**A, C**), end of study tumour weights (**B**; mg), end of study relative gene expression of Axin2, cMyc, RNF43, MMP7, CD44, MUC4 and MUC5AC (**D-F**), and histology (**G, H**) were analysed. HPAF-II (**A, D, G**; 5x10^6 cells; athymic nude mice), AsPC1 (**B, E**; 3x10^6 cells; athymic nude mice), and SNU-1411 (**C, F, H**; 1x10^7 cells; NOC-SCID mice) were implanted subcutaneously. Treatment was initiated once tumour volumes reached ~100-150mm^3 (**A-H**). Dosing was 1.5mg/kg BID RXC004 for 7-13 days then QD for the remainder of study (**A-C**). Tumour RNA was isolated for RT-qPCR expression analysis of the indicated genes, relative to appropriate housekeepers, at 12h post final dose (**D-F**). End of study HPAF-II (**G**) and SNU-1411 (**H**) tumours were formalin-fixed paraffin-embedded (FFPE) and sections stained with haematoxylin and eosin (H&E), anti-Ki67 (proliferation marker) or combined Alcian blue-PAS (Ab_PAS; Mucin differentiation marker) as indicated. Mann-Whitney U (**A-C**) or unpaired t-test (**D-F**) p values.

Efficacy and sustained Wnt pathway inhibition by low and scheduled RXC004



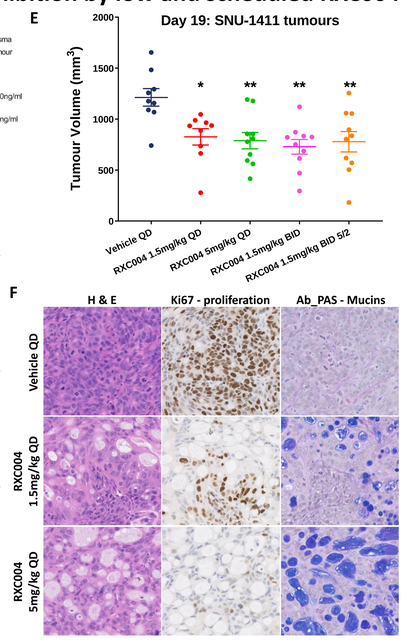
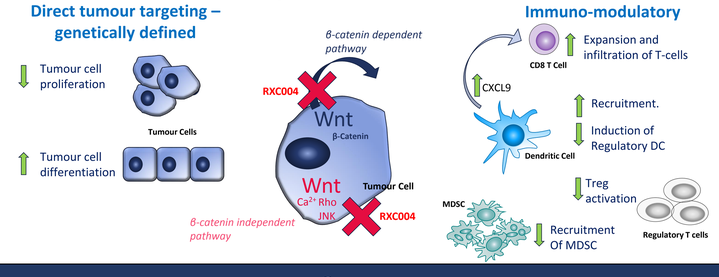


Figure 6. RXC004 displays sustained *in vivo* PD effects and efficacy at low doses in the human SNU-1411 colorectal xenograft model
Pharmacokinetic (PK) analysis of RXC004 (**A, B**), pharmacodynamic (PD) analysis of gene expression (**C, D**), tumour volume measurements (**E**), and histology (**F**). SNU-1411 (1x10^7 cells; NOC-SCID mice) were implanted subcutaneously, treatment was initiated at 200mm^3 (**A-D**) or 125mm^3 (**E**). Dosing of RXC004 was 1.5mg/kg (**A, C**) or 5mg/kg (**B, D**) continuous for 7 days, or as indicated for 19 days (**E**; 5/2 – 5 days on, 2 days off dosing). Tumour RNA was isolated for RT-qPCR expression analysis of the indicated genes at the specified timepoints post final dose (**C, D**). End of study tumours from (**A-D**; 7 days dosing) were FFPE and sections stained with H&E, anti-Ki67 or Ab_PAS as indicated (**F**). N≥3 per timepoint (**A-D**), N≥9 per group (**E**). Ordinary one-way ANOVA p values (**A-E**).

Summary

RXC004 inhibits tumour proliferation and increases differentiation



References

1. *Biechele et al*; *Dev Biol*, 2011; 355(2):275-285. **2. *Zhan et al***; *Oncogene*, 2017; 36:1461-1473. **3.** https://clinicaltrials.gov/. **4. *Madan et al***; *Oncogene*, 2016; 35(17):2197-2207. **5. *Anastas et al***; *Nat. Rev. Cancer*, 2013, 13 (1): 11-26. **6. Wang *et al***; *TIPs*, 2018, 39(7):648. **7. Spranger *et al***; *Nature Reviews 18:139* 2018.

Data represent Mean ±SEM. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001

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