2566

Pre-clinical efficacy of the Wnt pathway inhibitor RXC004 in combination with anti-cancer therapies

James R. Kelly, Simon A. Woodcock, Eimear Flanagan, Inder Bhamra, Clifford D. Jones, Richard Armer, Jane Robertson and Caroline Phillips

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 RSPO ligands[2] (**Fig. 1**).

The potent and selective PORCN inhibitor RXC004 is being investigated in Phase 1 and 2 clinical trials (NCT03447470, NCT04907851 and NCT04907539)[3], and has the potential to treat tumours dependent on Wnt ligand. Upstream Wnt pathway aberrations, including RNF43/ZNRF3 loss of function 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 cancer 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]. Inhibition of PORCN promotes differentiation of RNF43/RSPO addicted tumours and alters tumour metabolism by reduction of glucose uptake[8].

A recent publication has indicated that homologous recombination and Fanconi anaemia pathways involved in DNA damage repair (DDR), are mediated by Wnt signalling in Wnt-addicted preclinical cancer models[9]. Further, small molecule inhibition of PORCN induces a BRCA-like state in these models[9]. This finding opens opportunities for PORCN inhibition in combination with DNA damaging agents.

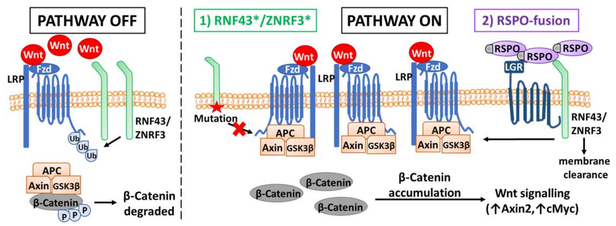

Figure 1: Upstream alterations trigger aberrant Wnt ligand-dependent signalling
RNF43/ZNRF3 keep surface Fzd receptors in check, allowing the destruction complex to phosphorylate and degrade β-catenin - Wnt pathway 'OFF'. Loss-of-function (LOF) RNF43/ZNRF3 mutations (1), or high RSPO expression through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

Results

RXC004 treatment induces BRCAness in Wnt-ligand dependent cell lines

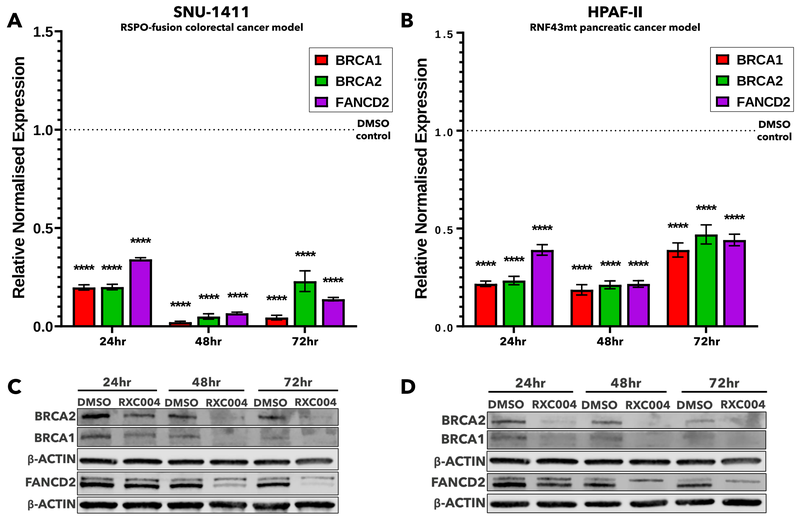

Figure 2. RXC004 significantly downregulates the expression of BRCA1, BRCA2 and FANCD2 in SNU-1411 and HPAF-II cells. (**A**, **B**) RT-qPCR analysis of (**A**) SNU-1411 cells or (**B**) HPAF-II cells dosed with DMSO or 100nM RXC004 for 24, 48 or 72 hours. ΔΔCq values relative to ACTIN and GUSB (SNU-1441) or GUSB and RPLPO (HPAF-II) housekeeping genes were normalised to respective DMSO timepoint. As controls, significant downregulation of AXIN2 and MYC, Wnt pathway targets, and significant upregulation of MUC5AC, differentiation marker, was seen with RXC004 treatment for both cell lines (data not shown). Ordinary one-way ANOVA p values versus DMSO control are shown (n=3), **** = p<0.0001. (**C**, **D**) Western blot analysis of (**C**) SNU-1411 cells or (**D**) HPAF-II cells treated with DMSO or 100nM RXC004 for 24, 48 or 72 hours. β-actin was used as a loading control.

RXC004 reduces tumour volume, induces BRCAness and reduces proliferation in RSPO-fusion *in vivo* model

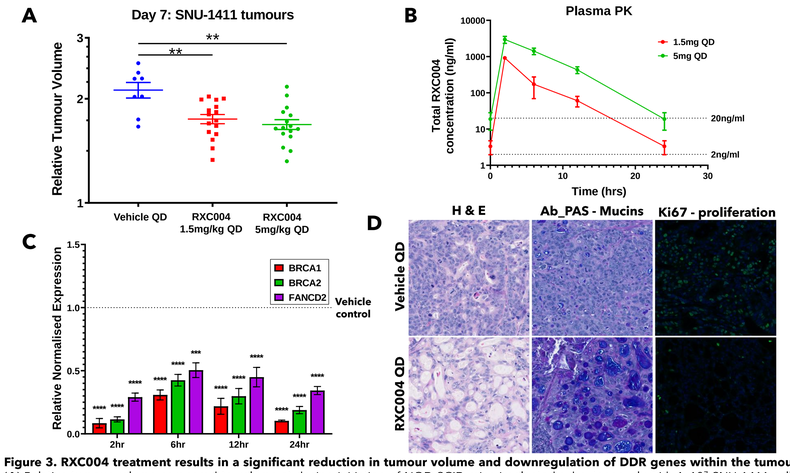

Figure 3. RXC004 treatment results in a significant reduction in tumour volume and downregulation of DDR genes within the tumour. (**A**) Relative tumour volume compared to volume at dosing initiation of NOD-SCID mice implanted subcutaneously with 1x10^7 SNU-1411 cells. Mice were dosed for 7 days as indicated. Ordinary one-way ANOVA p values, ** = p<0.005. (**B**) Total plasma concentrations of RXC004 at 0, 2, 6, 12 and 24 hours after final dose. Dotted lines at 2 and 20ng/ml indicate coverage over cellular corrected IC50 and 10 x IC50. (**C**) RT-qPCR of SNU-1411 tumours dosed at 5mg/kg and sampled at timepoints indicated. ΔΔCq relative to ACTIN and GUSB housekeeping genes were normalised to vehicle samples. As controls, significant downregulation of AXIN2 and MYC and significant upregulation of MUC5AC was seen with RXC004 treatment (data not shown). Ordinary one-way ANOVA p values versus respective vehicle timepoint, *** = p<0.0005, **** = p<0.0001. (**D**) Immunohistochemistry of Vehicle QD or RXC004 5mg/kg QD treated tumours 24 hours post final dose and stained with H&E, Ab_PAS (Alcian blue PAS) for Mucins or anti-Ki67 as a marker of proliferation (Ki67 = Green, DAPI = Blue).

RXC004 increases survival of RSPO tumour bearing mice when combined with standard of care chemotherapy

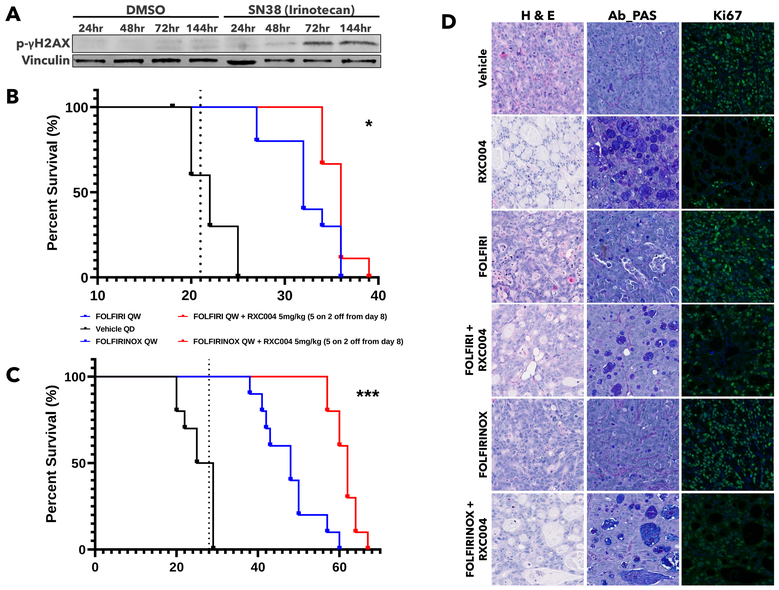

Figure 4. SOC chemotherapy induces double strand breaks and dosing with RXC004 significantly improves survival of SNU-1411 tumour bearing mice. (**A**) Western blot analysis of SNU-1411 cells treated with DMSO or 25μM SN38, the active metabolite of Irinotecan, over a 144hr period. Vinculin was used as a loading control. (**B**) Survival of NOD-SCID mice implanted subcutaneously with 1x10^7 SNU-1411 cells and treated with either; Vehicle for 5 days with 2 days off (5/2), FOLFIRI (25mg/kg 5-FU and 50mg/kg Irinotecan) QW or a combination of FOLFIRI and 5mg/kg RXC004 for 5/2 (dosing from day 8). (**C**) Survival of NOD-SCID mice implanted with 1x10^7 SNU-1411 cells and treated with either; Vehicle QD, FOLFIRINOX (25mg/kg 5-FU, 50mg/kg Irinotecan and 5 mg/kg Oxaliplatin) QW, FOLFIRINOX with 5mg/kg RXC004 for 5/2 (dosing from day 8) or RXC004 from day 1. Dotted line indicates final day of dosing. Log-rank (mantel-Cox) test p values of monotherapy versus combination are shown, * = p<0.05, *** = p<0.0005. (**D**) Immunohistochemistry of treated tumours (Vehicle for 5/2, FOLFIRI QW, FOLFIRINOX QW, 5mg/kg RXC004 for 5/2 or a combination of FOLFIRI / FOLFIRINOX with RXC004) sampled on Day 16 and stained with H&E, Ab_PAS (Alcian blue PAS) for Mucins or anti-Ki67 as a marker of proliferation (Ki67 = Green, DAPI = Blue), as indicated.

Combination of RXC004 with PARP inhibition enhances the anti-proliferative effect of RXC004 and induces apoptosis

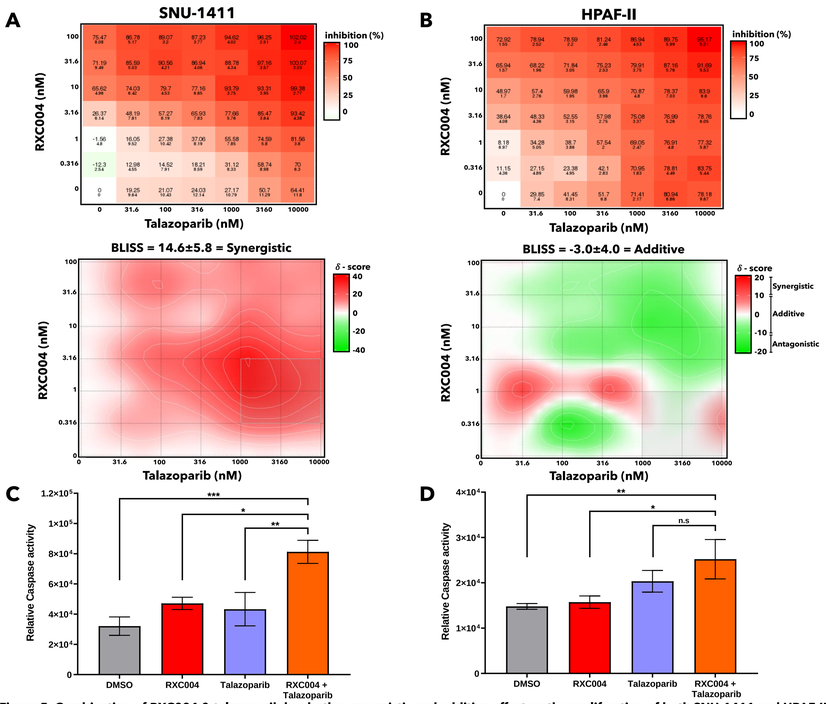

Figure 5. Combination of RXC004 & talazoparib has both a synergistic and additive effect on the proliferation of both SNU-1411 and HPAF-II cells. Combination induces a significant increase in apoptosis compared to RXC004 treatment alone. (**A**, **B**) The inhibition of proliferation of (**A**) SNU-1411 cells or (**B**) HPAF-II cells dosed with RXC004 and talazoparib in combination. Cells were grown for a total of 8 days, with cells dosed on day 1 and ATPLite endpoint taken on day 8. Synergy score represented as a BLISS score[10] = 14.6±5.8 (SNU-1441) and -3.0±4.0 (HPAF-II) (n=4). (**C**, **D**) Caspase activity of (**C**) SNU-1411 cells or (**D**) HPAF-II cells dosed with 100nM RXC004, 10nM talazoparib or both in combination. Measured by CaspaseGlo and normalised to cell number (ATPLite) (n=3). Ordinary one-way ANOVA p values shown, * = p<0.05, ** = p<0.005, *** = p<0.0005.

Summary

Exploiting the BRCAness induced by RXC004 to develop combination therapies for Wnt ligand driven tumours

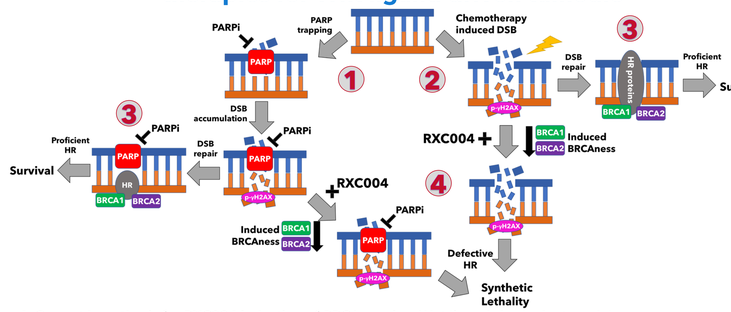

Figure 6. Current hypothesis for RXC004 induction of BRCAness in a Wnt ligand-dependent model.
1. Inhibition of PARP (PARPi) results in the accumulation of double strand breaks (DSB) by PARP trapping.
2. Treatment with standard of care chemotherapy agents results in DSB.
3. Proficient homologous recombination (HR) and alternate DDR pathways repair DSB leading to survival.
4. RXC004 induces BRCAness in which DSB cannot be repaired leading to synthetic lethality.

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*, 2018; 18:139. **8. Phillips *et al***; *Cancer Res.*, 2021, 81(13)998. **9. Kaur *et al***; *EMBO Mol. Med.*, 2021; 13:e13349. **10. Ianevski *et al***; *NAR*, 2020; 48(w1):W488-W493.

Redx Pharma, Block 33S, Mereside, Alderley Park, Cheshire, SK10 4TG, UK
e: s.woodcock@redxpharma.com, www.redxpharma.com

AACR American Association for Cancer Research