Efficacy of the Porcupine inhibitor RXC004 in genetically-defined tumour types

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Introduction

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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 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).

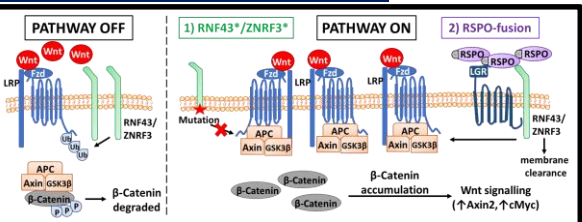

Figure 1: Upstream alterations trigger aberrant Wnt ligand-dependent signalling
RNF43/ZNRF3 keep surface Fzd in check, allowing the destruction complex to phosphorylate and degrade β-catenin - **Wnt pathway 'OFF'**. Loss-of-function (LOF) RNF43/ZNRF3 mutations **(1)**, or high RSPO expression through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

Results

In vitro pathway inhibition and anti-proliferative effects of RXC004 in genetically-defined tumour cell lines

A

	Cell line	Genetic background
Colorectal	WiDR	APC^{E853*} $APC^{T1556fs*3}$
	HCT116	ß-$Catenin^{\Delta Ser45}$ $RNF43^{R117fs*41}$
	JVE-109	$ZNRF3^{LOF}$
	SNU-1411	PRPTK-RSPO3 fusion
Pancreatic	CAPAN-2	$RNF43^{R330fs*89}$
	AsPC1	$RNF43^{S720*}$
	HPAF-II	$RNF43^{E1743*}$

Predicted insensitive
ß-Catenin alteration (with RNF43 mutation)
APC mutation

Predicted Sensitive
RNF43/ZNRF3 mutation
RSPO fusion

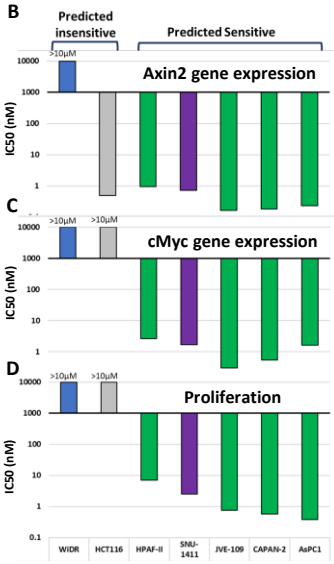

Figure 2: RXC004 potency in genetically-defined pancreatic and CRC cell lines
(**A**) A dose response of RXC004 was evaluated across a panel of genetically-defined tumour cell lines. (**B, C**) Cells were treated with RXC004 for 72h. RNA was isolated and analysed using RT-qPCR to assess mRNA expression of the downstream markers of target engagement for the Wnt pathway, Axin2 (**B**) and cMyc (**C**). (**D**) Cells were treated with RXC004 for 5 days in 2D or 3D format, proliferation was measured using an ATP-lite assay. N≥3 throughout.

RXC004 efficacy and PD in genetically-defined xenografts

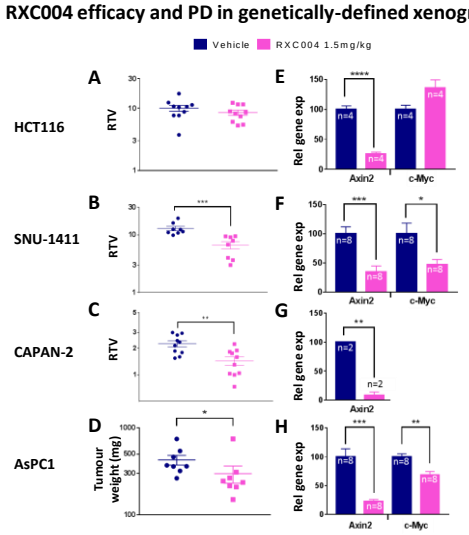

Figure 4: RXC004 efficacy and PD in human xenograft models
Relative tumour volumes (RTV) (**A-C**), end of study tumour weights (**D**; mg) and end of study relative gene expression of Axin2 and cMyc (**E-H**). HCT116 (**A**; $3x10^6$ cells; athymic nude mice), SNU-1411 (**B**; $1x10^7$ cells; NOC-SCID mice), CAPAN-2 (**C**; $3x10^6$ cells; SCID-Beige mice) and AsPC1 (**D**; $3x10^6$ cells; NOD-SCID mice) were implanted subcutaneously. Treatment was initiated once tumour volumes reached ~100-150mm^3. Tumours per group: 10 (**A**, **C**) and 8 (**B**, **D**). Dosing was 1.5mg/kg BID throughout (**A**, **C**) or BID for 7 or 13 days then QD for the remainder of study (**B**, **D**). Tumour RNA was isolated for RT-qPCR analysis of the downstream target engagement biomarkers for the Wnt pathway, Axin2 and cMyc at 8h (**G**) or 12h (**E, F, H**) post final dose. Mann-Whitney U (**A-D**) or unpaired t-test (**E-H**) p values.

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

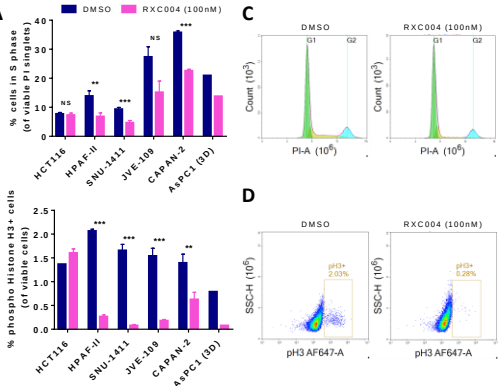

Figure 3: Effects of RXC004 treatment on the cell cycle
Cells were treated with RXC004 (100nM) or vehicle (0.1% DMSO) for 72h. Cell cycle profiles were determined using propidium iodide (**A, C**) and phospho^{Ser10}-Histone H3 (mitotic marker; **B, D**) staining by flow cytometry. Data are N≥3 except 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.

PK/PD relationship of RXC004 in the HCT116 model

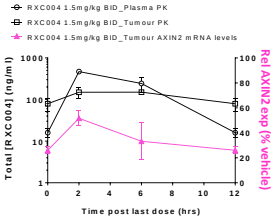

Figure 5: RXC004 levels and target engagement over time
HCT116 ($3x10^6$ cells; athymic nude mice), were implanted sub-cutaneously and dosed to steady state (1.5mg/kg BID). PK/PD post final dose shows plasma and tumour drug levels in excess of the Axin2 IC_{50} (~3ng/ml total RXC004) throughout the 12 hrs, resulting in sustained Wnt pathway inhibition as defined by Axin2 mRNA levels, measured by RT-qPCR.

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

Conclusions

- Tumour cells carrying RNF43 mutations or RSPO fusions are sensitive to RXC004 treatment both *in vitro* and *in vivo*.
- RXC004 monotherapy could benefit patients with tumours bearing RNF43 mutations or RSPO fusions.
- Data supports a genetically-defined patient selection strategy for ongoing RXC004 clinical studies.

References

1. *Dev Biol*, 2011; 355(2):275-285 **2.** *Oncogene*, 2017; 36:1461-1473
3. https://clinicaltrials.gov/ **4.** *Oncogene*, 2015; 35(17):2197-2207