Novel Porcupine inhibitor RXC004: Potent efficacy in animal models of cancer through direct tumour targeting and immunomodulatory mechanisms

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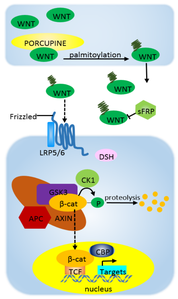
BACKGROUND

- Wnt signalling initiates key oncogenic pathways in cancer. Wnt signalling pathways are implicated in tumour initiation, growth, cell senescence, cell death, differentiation and metastasis.[1]
- Targeting the Wnt pathway is an attractive therapeutic approach to cancer treatment. Porcupine (PORCN) is a membrane-bound O-acyltransferase (MBOAT) required for and dedicated to palmitoylation of Wnt ligands, an essential step in the processing of Wnt ligands for secretion.[2]
- There is a growing body of literature suggesting that the Wnt pathway plays a role in the host immune response to tumours and that activation of the pathway may result in resistance to checkpoint inhibitors.[3] A PORCN inhibitor has the potential to benefit patients with cancers in which Wnt signalling is implicated.



References: **1.** Nusse R, Varmus H. EMBO J. 2012 Jun 13;31(12):2670-84. doi: 10.1038/emboj.2012.146. Epub 2012 May 22. **2.** Herr P, Basler K. Dev Biol. 2012 Jan 15;361(2):392-402. doi: 10.1016/j.ydbio.2011.11.003. Epub 2011 Nov 11. **3**. Spranger S., Gajewski, T. F. JITC. 2015 Sep 3;43. doi: 10.1186/s40425-015-0089-6. Epub 2015 Sep 2015.

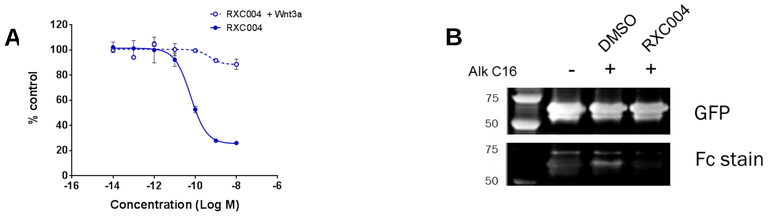
RESULTS

RXC004 displays potent Wnt pathway inhibitory activity and anti-proliferative activity in pancreatic cancer cell lines.

Wnt-Luc Reporter Gene assay IC_{50} (nM)[a]	Capan-2 GI_{50} (nM)[b]	HPAF-II GI_{50} (nM)[b]
0.06	0.49	2.66

a Dual cell assay with luciferase under control of β-catenin transcriptional response element, Wnt produced by mouse L-cells
b Proliferation assay (nuclei counting). Cytostatic effect supported by EdU staining.

RXC004 acts on the Wnt pathway upstream of Frizzled



A: Addition of recombinant human Wnt 3a restores Wnt signalling in the Wnt-Luciferase reporter assay, consistent with RXC004 inhibiting the pathway upstream of the Wnt/Fzd interaction. **B:** CHO cells expressing GFP tagged Wnt 1 incubated with alkynyl palmitic acid. Co-immunoprecipitation allows visualisation of palmitoylated Wnt ligand. Reduced levels of palmitoylated Wnt were observed in cell lysates following treatment with 10nM RXC004. This indicates that RXC004 inhibits palmitoylation of Wnt ligands.

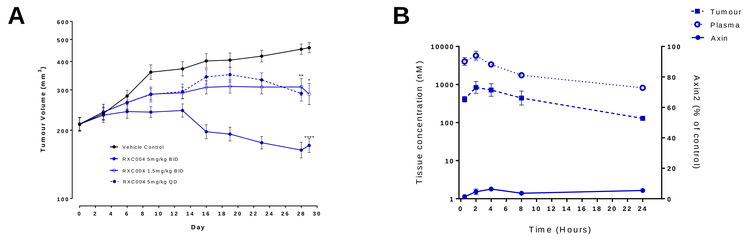
RXC004 displays a favourable *in vitro* ADME profile

Mouse Heps $T_{1/2}$ (mins)	Human Heps $T_{1/2}$ (mins)	P_{app} (x10-6 cm/s)		Mouse PPB (% Free)	Human PPB (% Free)
		MDR-MDCK	Caco-2		
>462	>462	15.0 (3.4)	14.9 (1)	7.3	4

- *In vitro* metabolic stability, permeability and free fraction are supportive of oral administration, PK data across preclinical species shows good bioavailability and exposure.

RESULTS

RXC004 is efficacious in a pancreatic cancer Capan-2 xenograft

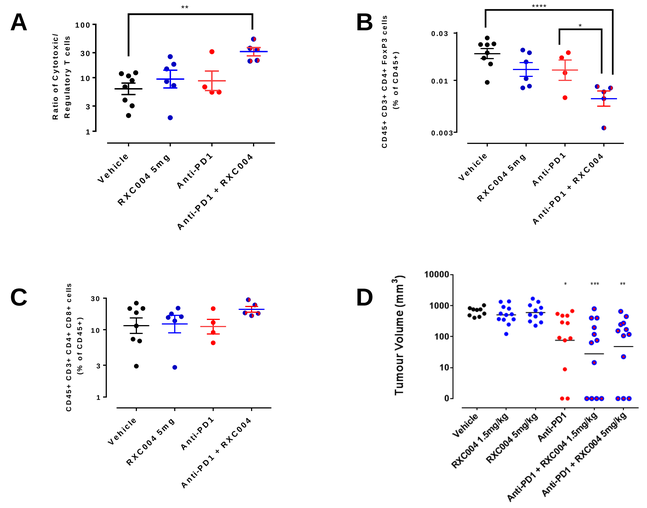


Capan-2 cells were subcutaneously implanted into the flanks of SCID Bg. mice. Once tumours reached 200mm^3 treatment with RXC004 was initiated orally for 28 days following both QD and BID regimens.

A: RXC004 treatment results in significant TGR and TGI following both BID and QD regimens.

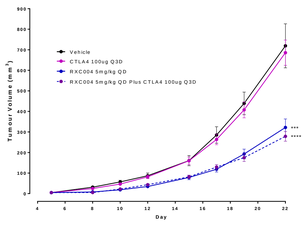
B: PK/PD post final dose (5mg/kg) show tumour drug levels in excess of the IC_{50} for 24 hrs resulting in Wnt pathway inhibition. Axin2 mRNA levels measured by qPCR.

RXC004 improves the anti-tumour effect of anti-PD1 treatment in a mouse syngeneic CT26 model



RXC004 has no effect on the proliferation of CT26 cells *in vitro.* To investigate its immunomodulatory effect *in vivo* CT26 were subcutaneously implanted in the flanks of female BALB/c mice. Treatment initiated once tumours reached 50mm^3.
A: FACS analysis of tumour infiltrate at day 14 shows a significant change in the ratio of $CD8^+$ cytotoxic T-cells to $FOXP3^+$ regulatory T-cells.
B and C: Changes in $CD8^+$ and $FOXP3^+$ proportion in tumour infiltrates.
D: The increased ratio of cytotoxic to regulatory T cell resulted in increased cures (defined by tumour volume of 0mm^3) in animals treated with the combination compared to PD1 inhibition alone at day 21**.**

RXC004 displays anti-tumour activity in a mouse syngeneic B16 melanoma model



- RXC004 has no effect on the proliferation of B16 mouse melanoma cells *in vitro.* To investigate its potential immunomodulatory effect *in vivo* B16 cells were subcutaneously implanted in the flanks of C57/BL6 mice. Treatment was initiated once tumours were palpable and anti-CTLA4 antibody treatment (alone and in combination with RXC004) was assessed.
- Treatment with RXC004 alone led to significant tumour growth inhibition (55% TGI). Anti-CTLA4 antibody alone had no effect on tumours and no significant synergy was observed in the combination arm (61% TGI).
- Studies are ongoing to determine whether the efficacy of RXC004 in this model is attributable to immunomodulatory effects of Wnt pathway inhibition.

In vivo PK and early safety data

Species	Dose (mg/kg)	Dosing Route	Cmax (μM)	$T_{1/2}$ (hrs)	Vd_{ss} (L/kg)	CL (mL/min/kg)	AUC (ng.hr/mL)	F (%)
Mouse	2	IV	13.9	1.8	0.64	2.9	17181	-
	5	PO	11.4	3.4	-	-	10978	62.6
Rat	2	IV	7.9	2.5	0.64	5.8	5689	-
	3	PO	1.7	3.7	-	-	5907	69.1
Dog	2	IV	10.5	0.8	0.4	8	3780	-
	5	PO	10.4	1	-	-	12930	137

- PK profile of RXC004 across pre-clinical species.
- IC_{50} of the hERG ion channel is 12.7μM and in a dog CV safety study RXC004 displayed no adverse effects at all dose levels tested.
- RXC004 shows no inhibition of major CYP isoforms or activation of PXR *in vitro.*
- RXC004 is non-mutagenic in mini-Ames and micronucleus assays *in vitro*.
- RXC004 displays excellent selectivity in a CEREP safety panel, no counter-activities observed.
- RXC004 is deemed non-cytotoxic in a HepG2 cell cytotoxicity assay.

$CYP1A2$ IC_{50} (μM)	$CYP2C19$ IC_{50} (μM)	$CYP2C9$ IC_{50} (μM)	$CYP2D6$ IC_{50} (μM)	$CYP3A4$ IC_{50} (μM)	$CYP3A4T$ IC_{50} (μM)
>30	>30	>30	>30	>30	>30

SUMMARY

- Redx porcupine inhibitor RXC004 exhibits potent and selective inhibition of the Wnt pathway in *in vitro* and *in vivo* models of Wnt dependant pancreatic cancer.
- Preliminary results from both CT26 and B16 syngeneic mouse models indicate that RXC004 may enhance the efficacy of checkpoint inhibitors such as anti-PD1 antibodies by reducing the proportion of regulatory T-cells in the tumour microenvironment and enhancing the ratio of CD8 +ve to FOXP3 +ve T-cells in tumour infiltrates.
- Pre-clinical studies are ongoing to determine the effect of RXC004 on immune response to cancer. RXC004 has been nominated as a candidate for development and IND enabling studies are in progress with First Time in Humans studies expected to begin early in 2017.

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