Porcupine inhibitors demonstrate suitability for use as novel anti-fibrotic therapeutics

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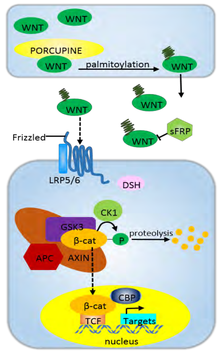
BACKGROUND

- Wnt signalling is known to be important for tissue remodelling in several pathologies including cancer, auto-immunity and fibrosis[1,2,3].
- Porcupine (PORCN) is a membrane-bound O-acyltransferase required for and dedicated to palmitoylation of Wnt ligands, an essential step in the processing of Wnt ligands for secretion[4].
- Several recent publications have shown that PORCN inhibitors (PORCNi) can ameliorate fibrosis in a number of models of fibrosis including renal, heart, lung and skin.
- Inhibition of Wnt signalling is likely to impact on several mechanisms that underpin tissue remodelling in fibrotic diseases such as suppression of inflammation, reduction of apoptosis, prevention of epithelial mesenchymal transition and inhibition of fibroblast activation[5,6].



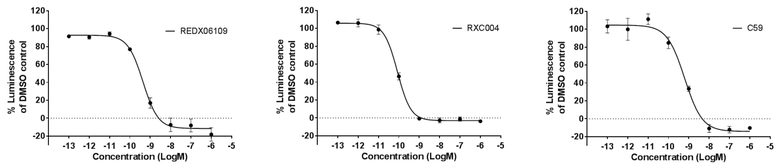
RESULTS

PORCNi display potent Wnt pathway inhibition in Wnt reporter assay and anti-proliferative activity in a pancreatic cancer cell line

In vitro cellular assays demonstrate inhibition of the Wnt signalling pathway with Redx PORCNi.

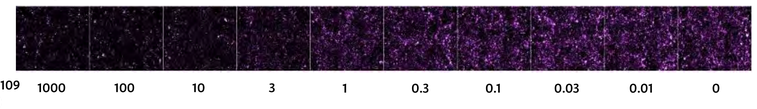
PORCNi	A: Wnt reporter gene assay IC50 (nM)	B: HPAF-II GI50 proliferation assay (nM)
RXC004	0.09	1.60
REDX06109	0.44	0.79
C59	0.62	n.d

A. Wnt reporter assay

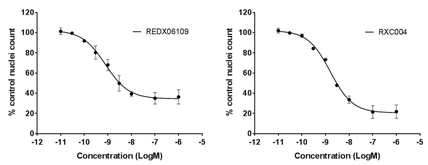


A: PORCNi inhibit Wnt secretion. Donor cells secreting Wnt3a were treated with the Redx Pharma PORCNi REDX06109, RXC004 or the PORCNi tool compound C59. Inhibition of Wnt3a secretion was detected via the transfer of conditioned media (CM), collected from donor cells, onto a β-catenin reporter cell line[7].

B. HPAF-II proliferation assay



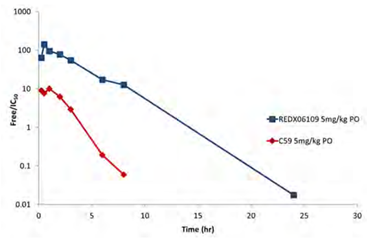
B: PORCNi inhibit cancer cell proliferation. Human pancreatic cancer HPAF-II cells showed sensitivity to PORCNi when treated for 5 d[7]. Nuclei were counted by Hoechst stain (REDX06109-treated images above) and standardised to a DMSO control (graphs right). Cell count at d0 was removed as baseline.



RESULTS

REDX06109 displays favourable properties in *in vitro* and *in vivo* ADME assays

REDX06109 and C59 CD1 mouse PK (oral, 5mg/kg)
Free drug exposure profiles (free conc/Wnt reporter IC_{50})



	REDX06109	C59
% free PPB (hu/m)	16/3	NT/<0.1
LogD	2.9	NT
Solubility pH 7.4 (mg/L) FaSSIF (pH 6.5) (mg/L) FeSSIF (pH 5.0) (mg/L)	2.2 7 153	<2.5 NT NT
Papp (AB)/ ER (BA/AB)	27 / 1.5	NT
Hu Clint (mics/heps)	44 / 4	191 / NT
Mouse Clint (mics/heps)	24 / 23	72 / 36

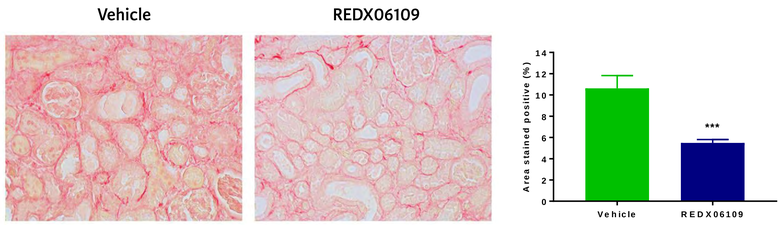
NT Not tested

In vitro metabolic stability, permeability and free fraction are supportive of oral administration. PK data across preclinical species show good bioavailability and exposure (data not shown).

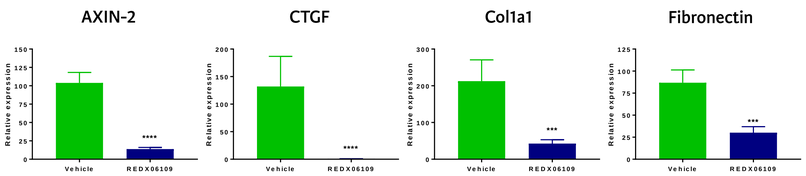
PORCNi demonstrates efficacy when dosed therapeutically in the unilateral ureteral obstruction (UUO) model

In 2016, Madan et al, reported that C59 demonstrated suppression of tubular damage and fibrosis in a murine model of fibrosis[8]. C59 was dosed prophylactically at 25mg/kg QD for 7 days. Redx Pharma sought to replicate these effects with a PORCNi dosed therapeutically.

A. Sirus Red staining

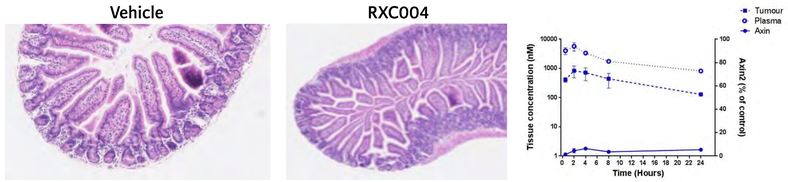


B. RT PCR



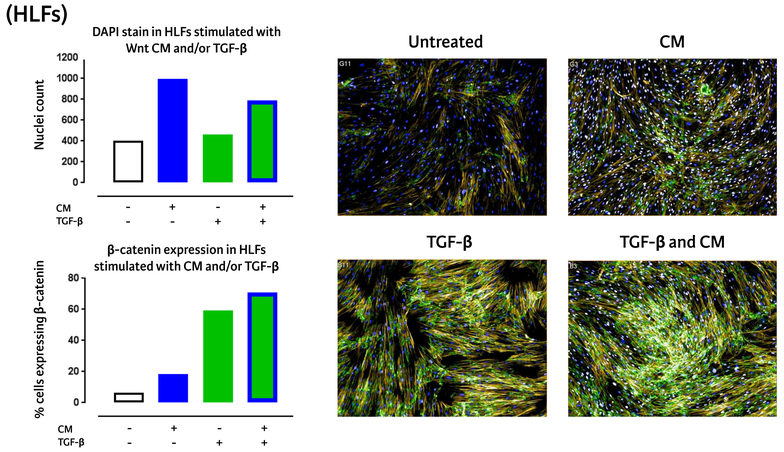
PORCNi is efficacious in UUO model of kidney fibrosis.
C57BL/6 mice aged 8-12 weeks were subject to ureteral ligation for 11 days. Vehicle (carboxymethylcellulose + 0.1% Tween-80) or REDX06109 (5mg/kg QD) were dosed from day 5 to 11 before the animals were sacrificed.
A: Collagen deposition in the kidney was measured by Sirus Red staining (representative images). The percentage area of positive staining was assessed by double blind analysis (mean data n=10).
B: RT PCR was performed on kidney tissue samples to show the effect of REDX06109 on markers of pathway activation (AXIN-2) and fibrosis (connective tissue growth factor (CTGF), type 1 collagen (Col1a1) and fibronectin). Genes of interest were normalised to GAPDH.
Significant effects of treatment ***p<0.001, ****p<0.0001.

RXC004 does not show GI toxicity when dosed at efficacious concentrations



RXC004 was dosed orally at 5mg/kg QD for 28 days in a human pancreatic cancer xenograft[9]. Tumour growth was suppressed. Measurement of AXIN-2 by RT PCR of tumour samples showed 24h pathway engagement. Despite 24h coverage, analysis of micro-villi integrity in the ileum showed no adverse effects.

Wnt3a and TGF-b induce proliferation and activation of human lung fibroblasts (HLFs)



Wnt signalling induces proliferation in human primary fibroblasts. HLFs were cultured for 48h in the presence of TGF-β 5ng/ml and/or Wnt3a CM. Image analysis was performed using an Operetta™ with algorithms to assess surface area stained. Cell proliferation was measured using DAPI (blue); β-catenin (green) and actin (yellow) expression were detected by specific antibodies.

SUMMARY

- Redx PORCNi exhibit potent suppression of the Wnt signalling pathway and are able to suppress tumour cell proliferation. RXC004 has been nominated as a CD for oncology indications.
- REDX06109 demonstrates a robust anti-fibrotic response when dosed therapeutically into a murine model of renal interstitial fibrosis.
- Suppression of fibrosis is achieved at *in vivo* concentrations that will have minimal impact on gastro-intestinal Wnt related pathologies.
- Preliminary data show Wnt ligand is a potent stimulator of HLF proliferation and is likely to synergise with other pro-fibrotic mediators to induce an aggressive fibrotic response to tissue injury.
- RXC004 has been nominated as a candidate for development and CTA enabling studies have been successfully completed with First Time in Humans studies expected to begin mid-2017.

References: **1.** Nusse R, Varmus H. EMBO J. 2012 Jun 13;31(12):2670-84. **2.** Castellone M, Laukkanen M. Front Biosci (Schol Ed). 2017 Jan 1;9:31-45. **3.** Miao CG, Yang Y. Cell Signal. 2013 Oct;25(10):2069-78. **4.** Herr P, Basler K. Dev Biol. 2012 Jan 15;361(2):392-402. **5.** Moon J, Zhou H. Proc Natl Acad Sci U S A. 2017 Feb 14;114(7):1649-1654. **6.** Chen CW, Beyer C. Ann Rheum Dis. 2016 Apr;76(4):773-778. **7.** Thompson, BA. Cancer Res 2015;75(15 Suppl):Abstract nr 5071. **8.** Madan B 2016 May;89(5):1062-74. doi: 10.1016/j.kint.2016.01.017 **9.** Bhamra, I. European Journal of Cancer , Volume 69 , S100 - S101

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