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

<table>
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<th>Cell line</th>
<th>Genetic background</th>
<th>Predicted insensitive</th>
<th>Predicted Sensitive</th>
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<td>β-Catenin/ APC/ Fzd (II)</td>
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<tr>
<td>SNL-5</td>
<td>SNL-5/ TF/RSPO fusion</td>
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<tr>
<td>AsPC1</td>
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<td>1.5</td>
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<td>JVE-109</td>
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**Results**

RXC004 efficacy and PD in genetically-defined xenografts

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; atrophic nude mice), SNU-1413 (B; 1x10^6 cells; NOD-SCID mice), CAPAN-2 (C; 1x10^6 cells; SCID-Beige mice) and AsPC1 (D; 3x10^6 cells; NOD-SCID mice) were implanted subcutaneously. Treatment was initiated once tumour volumes reached ~100-150mm³. 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 12h (E, F, H) post final dose. Mann-Whitney U (A-D) or unpaired t-test (E-H) p-values.

**Conclusions**

- Tumour cells carrying RXF43 mutations or RSPO fusions are sensitive to RXC004 treatment both in vitro and in vivo.
- RXC004 monotherapy could benefit patients with tumours bearing RXF43 mutations or RSPO fusions.
- Data supports a genetically-defined patient selection strategy for ongoing RXC004 clinical studies.

**References**


**Data**

- RXC004 arrests at G1/S and G2/M cell cycle checkpoints
- PK/PD relationship of RXC004 in the HCT116 model

**Figure 1**: Upstream alterations trigger aberrant Wnt ligand-dependent signalling RXF43/ZNRF3 keep surface Fzd in check, allowing the destruction complex to phosphorylate and degrade β-catenin - Wnt pathway ‘OFF’. Loss-of-function (LOF) RXF43/ZNRF3 mutations (1), or high RSPO expression through gene fusion (2), ultimately leads to accumulation of β-catenin - Wnt pathway ‘ON’.

**Figure 2**: RXC004 potency in genetically-defined pancreatic and CRC cell lines (A) A panel 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 Myc (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.

**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-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.

**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; atrophic nude mice), SNU-1413 (B; 1x10^6 cells; NOD-SCID mice), CAPAN-2 (C; 1x10^6 cells; SCID-Beige mice) and AsPC1 (D; 3x10^6 cells; NOD-SCID mice) were implanted subcutaneously. Treatment was initiated once tumour volumes reached ~100-150mm³. 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 12h (E, F, H) post final dose. Mann-Whitney U (A-D) or unpaired t-test (E-H) p-values.

**Figure 5**: RXC004 levels and target engagement over time HCT116 (1x10^6 cells; atrophic nude mice), were implanted subcutaneously 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 IC₅₀ (~3ng/ml total RXC004) throughout the 12 hours, resulting in sustained Wnt pathway inhibition as defined by Axin2 mRNA levels, measured by RT-qPCR.

**Data**

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