# Wnt/β-Catenin pathway inhibitor RXC004 enhances the immunity of pre-clinical models of cancer

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## Introduction

RXC004 is a potent and selective small molecule inhibitor of the membrane bound O-acetyl transferase Porcupine (PORCN). PORCN is required for post-translational modification of Wnt ligands, a necessary step in the initiation of canonical and non canonical Wnt signalling.

Pre-clinical studies have demonstrated the potential for PORCN inhibition to provide benefit to genetically selected cancer patient populations (see abstract #3874). RXC004 is currently being evaluated in a first-in-human clinical study (NCT03447470). A growing body of literature suggests that Wnt signalling plays a role in the host immune response to tumours, and activation of the pathway may result in poor response and instead resistance to immune checkpoint inhibitors. RXC004 has undergone preliminary evaluation in syngeneic mouse models of immunotherapy demonstrating its potential to enhance immune response in the tumour microenvironment.

## Results

### RXC004 enhances the anti-tumour effects of anti-PD-1 in the CT26 mouse colorectal cancer model: an immune checkpoint sensitive model

Figure 1. RXC004 co-operates with anti-PD-1 in CT26 tumours, an immune checkpoint sensitive model. Mouse CT26 cells (1x10^5) were subcutaneously implanted in the flanks of female NOD-SCID mice. Treatment was initiated once tumours reached ~50mm. A. Mean tumour volumes of the indicated groups over time. B and C. Tumour volumes after 21 and 26 days, respectively. Significant tumour growth inhibition (TGI) was seen in all anti-PD1 groups at day 21 (B). Tumour volumes at day 26 (C) were also reduced compared to control. *p<0.05, **p<0.01, ***p<0.001

Figure 3. RXC004 combines with anti-PD1 to enhance the anti-tumour immune environment. Mouse CT26 cells (1x10^5) were subcutaneously implanted in the flanks of female NOD-SCID mice. Treatment was initiated once tumours reached ~50mm. A. Flow cytometry of day 14 tumour infiltrating lymphocytes (TILs) stained for FoxP3 (Treg) and CD3 (CD3+). B. FoxP3 expression in CD3+ T cells is reduced in the CD8+ T cell population.

### RXC004 has monotherapy efficacy in B16F10 melanoma model at lower and scheduled doses

Figure 4. RXC004 monotherapy efficacy in B16F10 melanoma model: an immune checkpoint resistant model. Mouse B16F10 cells (5x10^6) were subcutaneously implanted in the flanks of male C57BL/6 mice. Treatment was initiated once tumours were palpable, 3 days later. A. Mean tumour volumes over time show no effect of anti-PD1 or anti-PD-1/PD-L1 immune checkpoint inhibitors in this model, as confirmed with Day 22 (J) or Day 26 (K) tumour volumes. Significant TGI was seen in all RXC004 treated groups, either a monotherapy or in combination with anti-PD1 (L) or anti-PD-1 (M).

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Figure 5. RXC004 has a confirmed immune MoA in the B16F10 melanoma model. A. Schematic of RXC004 treatment response in B16F10 melanoma model. B. Flow cytometry of day 7 untreated tumours. C. Flow cytometry of day 7 untreated tumours. D. Flow cytometry of day 7 untreated tumours. E. Flow cytometry of day 7 untreated tumours. F. Flow cytometry of day 7 untreated tumours. G. Flow cytometry of day 7 untreated tumours. H. Flow cytometry of day 7 untreated tumours. I. Flow cytometry of day 7 untreated tumours. J. Flow cytometry of day 7 untreated tumours. K. Flow cytometry of day 7 untreated tumours. L. Flow cytometry of day 7 untreated tumours. M. Flow cytometry of day 7 untreated tumours. N. Flow cytometry of day 7 untreated tumours. O. Flow cytometry of day 7 untreated tumours. P. Flow cytometry of day 7 untreated tumours. Q. Flow cytometry of day 7 untreated tumours. R. Flow cytometry of day 7 untreated tumours. S. Flow cytometry of day 7 untreated tumours. T. Flow cytometry of day 7 untreated tumours. U. Flow cytometry of day 7 untreated tumours. V. Flow cytometry of day 7 untreated tumours. W. Flow cytometry of day 7 untreated tumours. X. Flow cytometry of day 7 untreated tumours. Y. Flow cytometry of day 7 untreated tumours. Z. Flow cytometry of day 7 untreated tumours.

### RXC004 treatment results in changes in immune-related cell surface markers on sensitive tumour cell lines only

Figure 6. RXC004 treatment results in changes in immune related cell surface markers on genetically selected sensitive tumour cell lines only. Tumour cell lines were cultured and treated with DMSO or RXC004 (10µM) for 72h. Cells were then stained with relevant cell surface antibodies and median fluorescence quantified by flow cytometry.

## Summary

RXC004 inhibits tumour proliferation and stimulates a tumour fighting microenvironment.

## References