Pre-clinical activity of the Wnt/Beta-Catenin pathway inhibitor RXC004 in models of Biliary Tract Cancer

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Introduction

Dysregulated Wnt signaling initiates oncogenic pathways involved in tumour initiation, growth and metastasis[1], and is more recently linked to tumour immune evasion[2,3]. Signaling through the Wnt pathway is highly regulated at the level of ligand (Wnt), receptor (Fzd/LRP) and downstream components (e.g. APC/Axin/GSK3β destruction complex).

Post-translational modification via porcupine (PORCN; a membrane bound O-acyltransferase) is essential for the secretion and activity of all 19 Wnt ligands[4]. Upstream Wnt pathway aberrations, including RNF43/ZNRF3 (E3-ubiquitin ligases) mutations and RSPO-fusions, result in high levels of surface Fzd receptors (10 known in human) and increased Wnt-ligand dependent signaling[5]. Activity of RNF43/ZNRF3 results in ubiquitination and membrane clearance of Fzd, whilst RNF43/ZNRF3 levels are kept in check via LGR and secreted RSPO ligands[6] [**Fig. 1**].

RXC004, a potent and selective inhibitor of the Wnt pathway regulator PORCN, is currently being investigated in phase 2 studies in patients with advanced cancers, including Biliary Tract Cancers (BTCs) +/- anti-PD-1 immunotherapy (NCT04907851 and NCT04907539). Whilst genetically-defined 'upstream' Wnt pathway alterations, including loss-of-function (LoF) RNF43 mutations and RSPO gene fusions, sensitize Gastrointestinal (GI) cancers to RXC004, other non-genetic factors remain to be defined. Wnt pathway mutations are rare in BTCs, however these tumors have been shown to overexpress various Wnt ligands, with evidence that this high expression is associated with worse prognosis[7,8]. This pre-clinical study aimed to identify baseline transcriptome characteristics that predict for sensitivity of BTC models to RXC004, and therefore might translate into a patient selection strategy for BTC patients in the clinic.

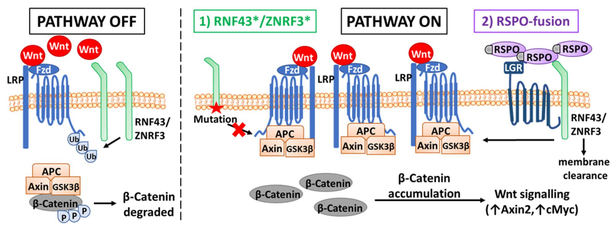


Fig. 1: Upstream alterations trigger aberrant Wnt ligand-dependent signalling RNF43/ZNRF3 keeps surface Fzd receptors in check, allowing the destruction complex to phosphorylate and degrade β-catenin - Wnt pathway 'OFF'. Loss-of-function (LOF) RNF43/ZNRF3 mutations (**1**), or high RSPO expression through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

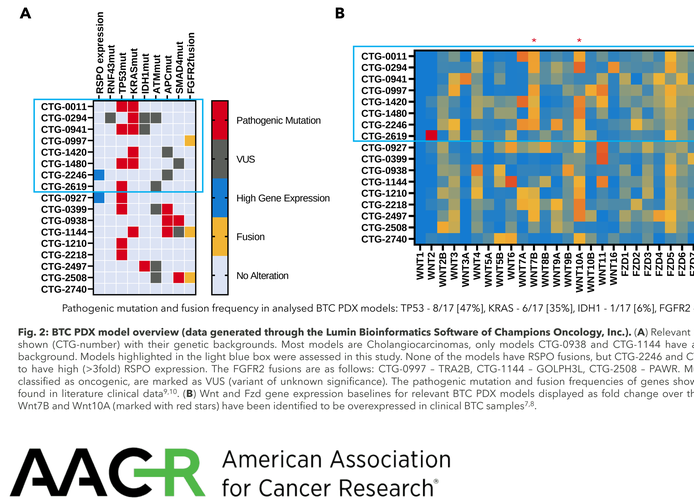
Methods

RXC004 was evaluated as a monotherapy treatment against a panel of patient-derived xenograft (PDX) models of BTC for efficacy and pharmacodynamic (PD) sensitivity. Efficacy was measured by tumor volume and tumor weight, whilst tumor PD effects were measured by quantitative Real-Time PCR (qPCR) for expression of Wnt pathway-relevant genes and by histological staining methods. Baseline transcriptome characteristics of the PDX models were determined against the PanCancer Pathways and PanCancer IO360 code sets using NanoString nCounter® analysis.

RXC004 was also evaluated as combination therapy together with standard of care (SoC) first-line chemotherapy for BTC (Gemcitabine and Cisplatin) in the RXC004-responsive PDX model CTG-0011 for efficacy as measured by tumor volume and survival endpoint.

PDX models

BTC PDX models reflect clinical BTC sample characteristics



Pathogenic mutation and fusion frequency in analysed BTC PDX models: TP53 - 8/17 [47%], KRAS - 6/17 [35%], IDH1 - 1/17 [6%], FGFR2 - 3/17 [18%]

Fig. 2: BTC PDX model overview (data generated through the Lumin Bioinformatics Software of Champions Oncology, Inc.). (**A**) Relevant BTC PDX models are shown (CTG-number) with their genetic backgrounds. Most models are Cholangiocarcinomas, only models CTG-0938 and CTG-1144 have an Ampullary cancer background. Models highlighted in the light blue box were assessed in this study. None of the models have RSPO fusions, but CTG-2246 and CTG-0927 were found to have high (>3fold) RSPO expression. The FGFR2 fusions are as follows: CTG-0997 – TRA2B, CTG-1144 - GOLPH3L, CTG-2508 – PAWR. Mutations that are not classified as oncogenic, are marked as VUS (variant of unknown significance). The pathogenic mutation and fusion frequencies of genes shown match the ranges found in literature clinical data[9,10]. (**B**) Wnt and Fzd gene expression baselines for relevant BTC PDX models displayed as fold change over the pan-cancer mean. Wnt7B and Wnt10A (marked with red stars) have been identified to be overexpressed in clinical BTC samples[7,8].

Results

Efficacy of RXC004 monotherapy in PDX models of BTC

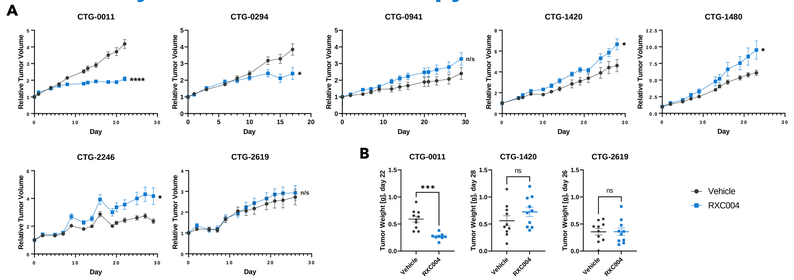


Fig. 3: (A) Relative tumor volumes compared to volumes at dosing initiation and (B) end-of-study tumor weights. *In vivo* studies were run at Champions Oncology, Inc. Athymic Nude-Foxn1nu mice were implanted with PDX tumor fragments and randomized when tumors reached an average volume of 150-300mm^3 with 10 animals per group. Animals were treated QD orally with 5 mg/kg RXC004 for timepoints indicated. Tumor volumes were measured every 3 days and normalized to the starting volume for each individual tumor. Graphs show the mean relative tumor volume ±SEM. Unpaired T test p values: * = p≤0.05, *** = p≤0.001,**** = p≤0.0001. PDX model passage numbers used: CTG-0011 p8, CTG-0294 p5, CTG-0941 p5, CTG-1420 p4, CTG-1480 p7, CTG-2246 p4, CTG-2619 p5.

Sensitivity of Wnt pathway-associated genes to RXC004 monotherapy in BTC PDX models

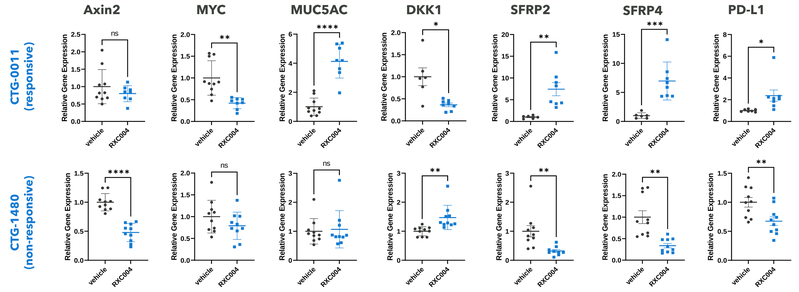


Fig. 4: (A) Exemplary gene expression data across two PDX models shown in figure 3. (B) Significant changes in the expression of Wnt pathway relevant genes upon treatment with RXC004 across all tested BTC PDX models. *In vivo* studies were run at Champions Oncology, Inc. as described in figure 3. Animals were treated QD orally with 5 mg/kg RXC004 for 17 to 29 days, or 6 days for CTG-0997 (PD assessment only). End-of-study tumors were collected into RNAlater® 6 hours post final dose. Target gene expression relative to 2 housekeeping genes was determined via RT-qPCR, they are shown normalized to vehicle control per model. Significance of target gene expression changes upon RXC004 treatment was determined via unpaired T-test with Welch's correction using GraphPad Prism software. (**A**) Graphs show the mean relative gene expression ± SEM. IO = Immuno-Oncology. T-test p values * = p≤0.05, ** = p≤0.01, *** = p≤0.001,**** = p≤0.0001. (**B**) Gene expression changes were plotted when determined as significant via unpaired T test with p≤0.05.

Effects of RXC004 monotherapy on the histology of BTC PDX models

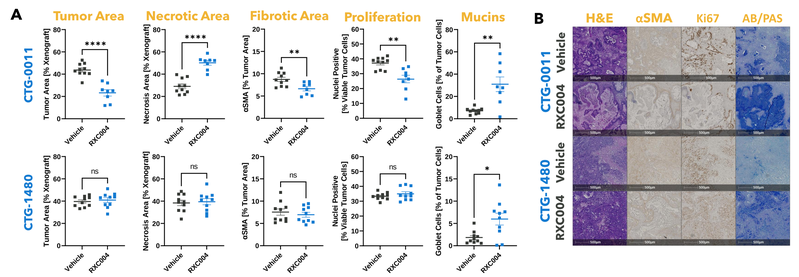


Fig. 5: CTG-0011 (RXC004-responsive model) and CTG-1480 (RXC004-non-responsive model) histology results (A) and representative images (B). *In vivo* studies were run at Champions Oncology, Inc. as described in figure 3. End-of-study tumors were collected into FFPE 6 hours post final dose, samples were stained with Hematoxylin/Eosin (H&E; tumor and necrosis areas), anti-αSMA (fibrotic area; CAF), Ki67 (proliferation), or Alcian blue/periodic acid Schiff (AB/PAS; mucins). (**A**) Quantitation of digital images was performed with Visiopharm software using bespoke machine learning-based algorithms. Significance of changes upon RXC004 treatment was determined via unpaired T-test with Welch's correction using GraphPad Prism software. T test p values: * = p≤0.05, ** = p≤0.01, *** = p≤0.001, **** = p≤0.0001.

Baseline gene expression of Wnt pathway drivers defines two distinct clusters of BTC PDX models

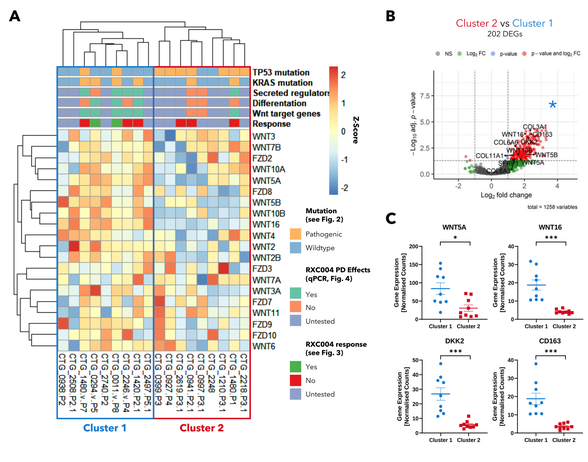


Fig. 6: (A) Hierarchical clustering established on baseline gene expression of Wnt ligands and Fzd receptors in BTC PDX models. (B) Differential gene expression between the two clusters identified in (A) in BTC PDX models. (C) Potential markers of RXC004 sensitivity. Clustering analysis of Wnt driver genes was performed on baseline transcriptomes of the PDX models defined by the PanCancer Pathways and PanCancer IO360 NanoString codesets using nCounter® and R analysis, and is shown as a heatmap (**A**; Z scores of gene expression, $\log_{10}$ normalized counts). Two clearly defined clusters of equal size were identified; Cluster 1 contains both RXC004-responsive PDX models (see efficacy Fig. 3) and all 5 PDX models that demonstrated RXC004-dependent gene expression changes (PD by qPCR, Fig. 4), whereas Cluster 2 contains RXC004 non-responsive PDX models and the 2 PDX models that had no RXC004-dependent changes in gene expression. Passage numbers are annotated next to the model numbers. For CTG-1480 the early passage sample (P1) did not cluster with later passage sample (P7) of the same model - this might be due to a change in stroma over passage. (**B**) 202 differentially expressed genes were identified as significant when comparing Cluster 2 vs Cluster 1. Section marked with * contains the genes that are significantly higher expressed in Cluster 1 compared to Cluster 2. (**C**) Example of differentially expressed genes that link to published clinical BTC data[7,8]. Significance was determined via the 'limma' R package which uses linear models and empirical Bayes methods to make comparisons between experimental groups. Adjusted p values: * = p≤0.05, *** = p≤0.001.

RXC004 delays re-growth of BTC PDX tumor-bearing mice when combined with SoC chemotherapy

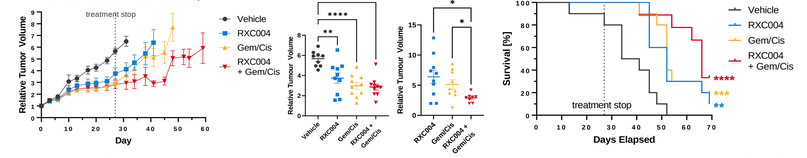


Fig. 7: (A) Relative tumor growth + re-growth after end of weekly treatment cycles and day 27 and 41 tumor volumes, as well as (B) survival of CTG-0011 tumor bearing mice treated with RXC004 and/or Gem/Cis. *In vivo* study was run at Champions Oncology, Inc. Athymic Nude-Foxn1nu mice were implanted with PDX tumor fragments and randomized when tumors reached an average volume of 150-300mm^3 (p6) with 10 animals per group. For the RXC004 dose group the animals were treated on a weekly cycle with 5 mg/kg RXC004 daily for 5 days followed by a two-day dosing holiday (5on + 2off). For the Gem/Cis group the animals were treated on a weekly cycle with 60 mg/kg Gemcitabine plus 3.2 mg/kg Cisplatin (Q7D). For the combination group the animals were concurrently treated with equivalent weekly cycles and dosages to the monotherapy groups. For the vehicle group, the animals were treated with RXC004 vehicle, Gemcitabine vehicle and Cisplatin vehicle according to the combo treatment dosing schedules. Four weekly cycles of treatment were completed for all groups, then treatment was stopped. All treatments were well tolerated across the groups. (**A**) Individual tumor volumes were measured every three days and normalized to the starting volume for each individual tumor. Datapoints over time are included for days where the individual groups still had 7 or more animals. Graphs show the mean relative tumor volume ±SEM for each group. Welch's Anova p values: ** = p≤0.01, *** = p≤0.001,**** = p≤0.0001. (**B**) Animals were culled when the tumor reached a size of ≥2000mm^3. Median overall survival: vehicle: 37.5 days, RXC004: 52 days, Gem/Cis: 52 days, RXC004+Gem/Cis: 66 days. Mantel-Cox test p values vs. vehicle: ** = p≤0.01, *** = p≤0.001,**** = p≤0.0001.

Conclusions and Future Perspectives

Panels of BTC PDX models available at Contract Research Organizations (CROs) show similar characteristics to clinical BTC samples in terms of both their mutation profile and baseline Wnt driver transcriptome.

The PORCN inhibitor RXC004 was efficacious as a monotherapy in two out of seven BTC PDX models (~29%).

RXC004 treatment induced gene expression changes, including decreases in direct Wnt pathway regulated genes, in five out of seven BTC PDX models (~71%). This is consistent with literature data that indicates ~70% of BTC have a high Wnt-ligand levels[7,8].

PDX models of BTC could be clustered based on baseline transcriptomic profiles of Wnt pathway driver genes. All five models with a PD effect (RXC004-induced gene expression changes), including the two models that were responsive, located to Cluster 1. Differentially expressed genes that were higher in Cluster 1 included Wnt5a, Wnt16, DKK2 and CD163.

Wnt pathway activation is well known to induce immune evasion[11]. In this study, CD163 expression (Immunosuppressive M2 macrophages[12]) was shown to be higher in the cluster containing the RXC004-sensitive PDX models, consistent with previous studies linking M2 macrophages to Wnt-ligand expression in BTC[8]. Moreover, RXC004 promoted TME changes to a less fibrotic profile. These findings both support the hypothesis of exploring the efficacy of RXC004 in combination with anti-PD1 therapy in BTC (NCT04907851, module 3).

Lastly, combination of RXC004 with SoC chemotherapy (Gem/Cis) delayed the re-growth of CTG-0011 tumor bearing mice, giving an opportunity for further RXC004 combination therapies in the future.

References

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