Pre-clinical activity of the Wnt pathway inhibitor RXC004 in combination with MAPK pathway inhibitors in GI cancer models

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Poster 944

Introduction

Gastrointestinal (GI) cancers cause more deaths (3.4 million in 2018) than any other cancer[1]. Wnt pathway activation is common in GI cancers, with the pathogenic upstream variant subgroup (RNF43 loss-of-function / RSPO gain-of-function) harboring increased Wnt ligand-dependency[2]. This subgroup has a combined prevalence of 4.7% in GI cancer patients[3], and pre-clinically shows exquisite sensitivity to RXC004 (zamaporvint), a phase 2 clinical small molecule porcupine (PORCN) inhibitor. Post-translational modification of Wnt ligands via PORCN (membrane bound O-acyltransferase) is essential for the secretion and activity of all Wnt ligands[4] [**Fig. 1**].

A striking co-occurrence of the RNF43/RSPO subgroup with MAPK pathway driver mutations (KRAS, NRAS, BRAF) occurs in 77% of Microsatellite stable (MSS) GI cancers[3], suggesting co-inhibiting these pathways could enhance clinical activity.

The aim of this study was to determine pre-clinically whether there is a beneficial effect of combining RXC004 and MAPK pathway inhibitors, providing a rationale for this combination approach in the clinic.

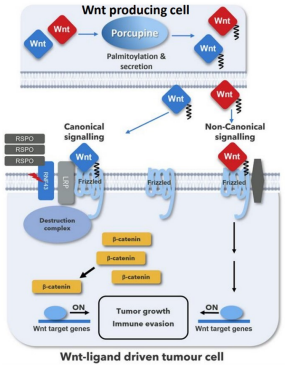


Fig. 1: Upstream alterations trigger aberrant Wnt ligand-dependent signaling. RNF43/ZNRF3 keeps surface Fzd receptors in check, allowing the destruction complex to phosphorylate and degrade β-catenin. Loss-of-function RNF43/ZNRF3 mutations or high RSPO expression through gene fusions ultimately lead to the accumulation of β-catenin and increased Wnt signaling.

Methods

Gene expression and proliferation were assessed in cell models of upstream Wnt pathway mutated GI cancer (RNF43/RSPO subgroup; see **Table 1**) using Wnt (RXC004) or MAPK (trametinib, pan-KRAS, KRAS_G12D, or undisclosed) pathway inhibitors as single agents or in combination. Cell lines with downstream or no Wnt pathway mutations were also tested. Synergistic combinations were assessed by BLISS scores. RXC004, trametinib or an undisclosed MAPK pathway inhibitor were evaluated *in vivo* in the SNU-1411. Efficacy was measured by tumor volume and weight; PD markers were assessed in end-of-study tumor samples. Relative *Axin2* (Wnt pathway) and *DUSP6* (MAPK pathway) gene expression were determined by RT-qPCR.

Cell Line	Cancer Type	RNF43/RSPO subgroup	Wnt pathway aberration	MAPK pathway aberration	MS status
SNU-1411	CRC	Yes	PTPRK-RSPO3 fusion	KRAS G12C BRAF K601N	MSS
JVE-109	CRC	Yes	RNF43 V211fs*47 & G659fs*41	BRAF V600E	MSI
HPAF-II	Panc	Yes	RNF43 E174*	KRAS G12D	MSS
AsPC-1	Panc	Yes	RNF43 S720*	KRAS G12D	MSS
HPAC	Panc	No	-	KRAS G12D	MSS
WiDr	CRC	No	HT-29: APC E853* & T1556Nfs*3	BRAF V600E	MSS
HCT-116	CRC	No	β-catenin S45del, RNF43 R117Afs*41, Axin2 G665Afs*24	KRAS G13D	MSI
PANC0403	Panc	No	APC T1556Nfs*3	KRAS G12D	MSS
H727	Lung	No	Axin2 homodeleted	KRAS G12V	MSS
AGS	Gastric	No	β-catenin G34E	KRAS G12D	MSS
H358	Lung	No	-	KRAS G12C	MSS
SAS	H&N	No	-	KRAS Amp	MSS
MKN1	Gastric	No	-	KRAS Amp	MSS
GP2d	CRC	No	APC T1445Lfs*27 & homodeleted + Axin2 G665Afs*24	KRAS G12D	MSI

Table 1: Genetic background and microsatellite status of cell lines used in this study. CRC: colorectal, Panc: pancreatic, H&N: head and neck, MSI: microsatellite instable

Results

Reciprocal Wnt and MAPK Pathway Signaling in SNU-1411 Cells

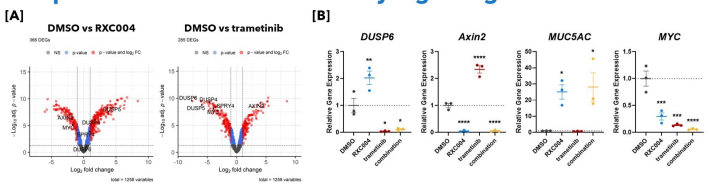


Fig. 2: RXC004 or trametinib induced gene expression changes. RXC004 (100nM; 72hrs) and/or trametinib (100nM; MEKi) for the final 2hrs. [**A**] DEGs assessed using the PanCancer and IO360 NanoString code sets. Differential expression analysis was performed. Benjamini-Hochberg was used for p-value adjustment. [**B**] Target gene expression relative to 2 reference genes determined via RT-qPCR and normalized to DMSO controls (N=3). Significance determined by one-way ANOVA with Dunnett's

Combining RXC004 with a MAPK Pathway Inhibitor Overcomes Reciprocal Signaling in the RNF43/RSPO GI Cancer Subgroup

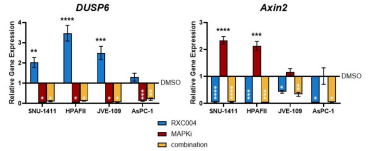


Fig. 3: Gene expression changes in RNF43/RSPO subgroup cell lines in response to RXC004 and/or MAPK pathway. Cell lines were dosed with 100nM RXC004 for 72 hours and MAPKi for the final 2 hours: SNU-1411 and HPAF-II cells with 100nM trametinib (MEKi); JVE-109 cells with 20nM trametinib, and AsPC-1 cells with 4μM BI2493 (pan-KRASi). Target gene expression relative to 2 reference genes was determined via RT-qPCR. Graphs show the mean ±SEM (N=3). Significance of treatment effect via one-way ANOVA with Dunnett's.

Beneficial Effects of Combining RXC004 with MAPK Pathway Inhibitors on Proliferation of RNF43/RSPO GI Cancer Subgroup

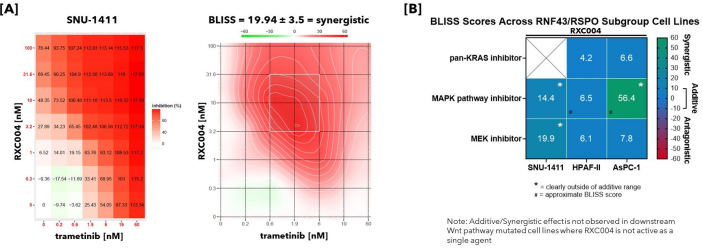


Fig. 4: [A] Exemplary proliferation data from SNU-1411 cells for BLISS score determination and [B] BLISS scores determined across the RNF43/RSPO subgroup GI cell models. Cell lines were treated with RXC004 and trametinib, or a MAPK pathway inhibitor, or BI2493. Cell proliferation was determined. Synergistic effects were determined using BLISS scores[5] (-10 to 10 = likely additive, >10 = likely synergistic, <-10 = likely antagonistic). [**A**] Exemplary dose-response matrix and synergy distribution contour plot in the SNU-1411 cell line. Graphs show averaged data from 3 biological replicates. SNU-1411 cells grown over a total of 7 days, dosed on day 2 and ATPlite™ endpoint taken on day 7. [**B**] BLISS scores for combinations of RXC004 with the indicated MAPK pathway inhibitors (each BLISS score from N=3).

Combining RXC004 with MAPK Pathway Inhibitors Abolishes Reciprocal Signaling and Drives Regressions *In Vivo*

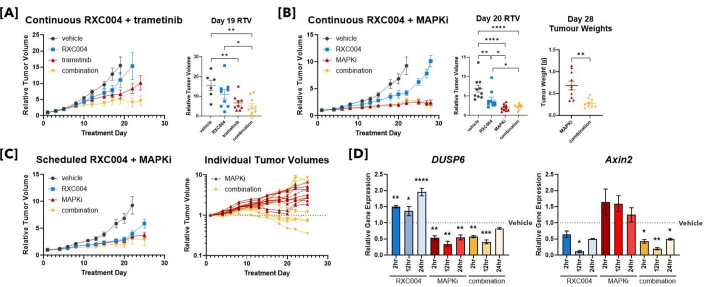


Fig. 5: Anti-tumor efficacy and end-of-study gene expression profiles of SNU-1411 xenografts; mice treated orally [A] continuously with RXC004 and/or trametinib, or [B & D] continuously with RXC004 and/or a MAPK pathway inhibitor, or [C] scheduled with RXC004 and/or a MAPK pathway inhibitor. Mice were implanted with SNU-1411 cells and randomized at an average tumor volume (TV) of 150mm^3 (N=10). Animals were dosed for up to 28 days or until TV exceeded 1700mm^3. End-of-study tumor weight (TW) measurements were taken. Animals were treated with: [**A**] vehicle, 1.5mg/kg RXC004 QD, 0.3mg/kg trametinib QD, or both; [**B**] vehicle, 1.5mg/kg RXC004 QD, 3mg/kg MAPK pathway inhibitor BID, or both; [**C**] vehicle, 5 days on / 2 days off schedule with either 5mg/kg RXC004 QD, or 3mg/kg MAPK pathway inhibitor BID, or both. [**D**] End-of-study tumors collected into RNAlater® 2, 12, or 24 hours post final dose of RXC004, MAPK pathway inhibitor, or both for the study described in [**B**]. Target gene expression determined via RT-qPCR. Significance of target gene expression changes upon treatment compared to vehicle determined via one-way ANOVA with Dunnett's.

Wnt and MAPK Reciprocal Signaling and Combination Therapy Potential Extends Beyond the RNF43/RSPO GI Cancer Subgroup

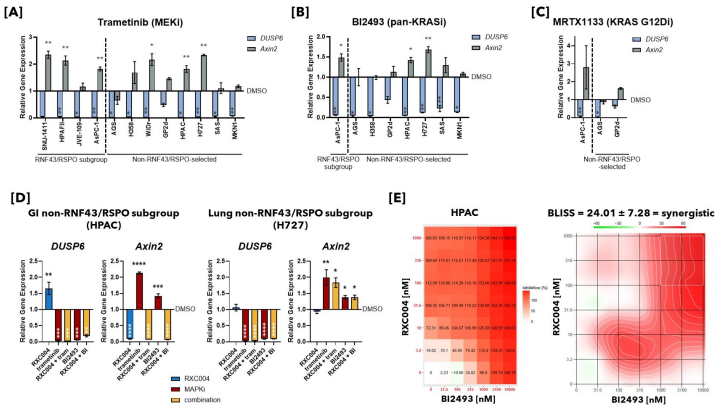
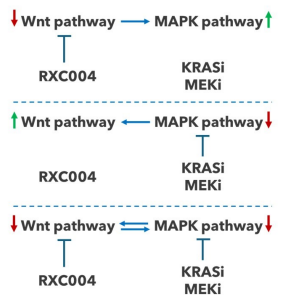


Fig. 6: RXC004 and MAPKi induced gene expression changes and cell proliferation Cell lines dosed as indicated with MAPK pathway inhibitors for 2 hours [**A**] - [**C**] and/or RXC004 for 72 hours [**D**] - [**E**]. Target gene expression relative to 2 reference genes determined via RT-qPCR. Graphs show mean relative gene expression ±SEM (N=3). [**A**], [**B**] & [**C**] Cells treated with trametinib (tram) or BI2493 (BI) or MRTX1133 (MRTX): SNU-1411, HPAF-II, and AsPC-1 - 100nM tram; JVE-109 - 20nM tram; AsPC-1 - 4μM BI or 100nM MRTX; AGS - 90nM tram, 15μM BI or 30nM MRTX; H358 - 3nM tram or 3.5μM BI; WiDr - 20nM tram; GP2d - 10nM tram, 3μM BI or 6nM MRTX; HPAC - 12nM tram or 2μM BI; H727 - 40nM tram or 10μM BI; SAS - 7nM tram or 250nM BI; MKN1 - 10nM tram or 250nM BI. Treatment effects determined via paired two-tailed T-test. [**D**] HPAC cells dosed with 100nM RXC004 and/or 12nM tram or 2μM BI2493. H727 cells dosed with 100nM RXC004 and/or 40nM tram or 10μM BI2493. Treatment effects via one-way ANOVA with Dunnett's. [**E**] HPAC cells treated with RXC004 and BI2493, 3D cell proliferation determined after 8 days. Synergistic effects determined using BLISS scores[5] (-10 to 10 = likely additive, >10 = likely synergistic, <-10 = likely antagonistic).

Conclusions and Future Perspectives

- Wnt and MAPK pathways are frequently co-activated in GI cancers, particularly in the upstream Wnt pathway mutated subgroup[3].
- Pre-clinically we show that Wnt and MAPK pathways act as potential reciprocal resistance mechanisms following single agent inhibition of either pathway; monotherapy MAPK inhibition up-regulates the Wnt pathway, whilst monotherapy Wnt inhibition up-regulates the MAPK pathway.
- Co-inhibition of these pathways abolishes this reciprocal resistance and leads to synergistic effects *in vitro* and enhanced efficacy *in vivo*, including tumor regression, in models of the RNF43/RSPO GI cancer subgroup.
- We demonstrate this reciprocal resistance extends beyond this genetic subgroup, suggesting wider utility of combining Wnt and MAPK pathway inhibitors.



- This data is consistent with *in vitro* proliferation data shown for a PORCN inhibitor in combination with BRAF inhibition in BRAF-mutant cell lines[6] and data showing MEK inhibition activates Wnt signaling and stemness *in vivo*[7].
- Therefore, this study provides a clear rationale to assess combining RXC004 (zamaporvint) with MAPK pathway inhibitors in the clinic, and suggests this combination has potential benefit in GI cancers beyond the genetically defined RNF43/RSPO subgroup.

References

1. Arnold *et al.* (2020), *Gastroenterology*, 159:335-349; **2. Madan *et al.* (2016)**, Oncogene, 35(17):2197-2207; **3. Cook *et al.* (2023)**, *Annals of Oncology*, 34: supplement 2; **4. Biechele *et al.* (2011)**, *Dev. Biol.*, 355(2):275-285; **5. Ianevski *et al.* (2020)**, *NAR*, 48(w1):W488-W493; **6. Russo et al. (2018)**, *Nature Communications*, 9:2287; **7. Zhan *et al.* (2019)**, *Nature Communications*, 10:2197.

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p value significance: *p≤0.05, **p≤0.01, ***p≤0.001, ****p≤0.0001

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