Combining zelasudil, a small molecule ROCK2 inhibitor, with chemotherapy or immunotherapy improves response in preclinical models of pancreatic cancer

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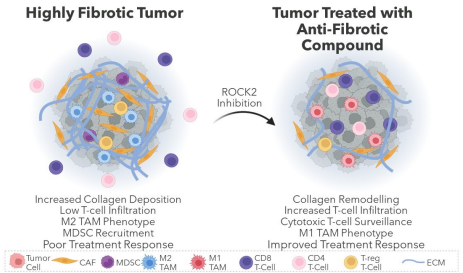
Introduction

- The heterogenous tumor microenvironment (TME) of pancreatic ductal carcinoma (PDAC) plays a pivotal role in driving tumor progression and limits the effectiveness of standard of care (SoC) treatments. Targeting the densely fibrotic TME of PDAC is an emerging strategy aiming to improve response in combination with chemo- and immunotherapy agents and overcome poor survival rates for PDAC patients.
- Previous studies have shown increased Rho-associated coiled-coil containing protein kinase (ROCK) expression in pancreatic cancer which promotes invasive tumor growth by extracellular matrix (ECM) remodelling[1]. Inhibition of ROCK1/2 by Fasudil has been shown to improve response to SoC chemotherapy in PDAC[2] but is not easily translated to the clinic due to cardiovascular effects associated with dual ROCK1/2 inhibition.
- Zelasudil (RXC007) is a small molecule highly selective inhibitor of ROCK2 which has demonstrated preclinical anti-fibrotic efficacy across multiple organs and is currently being tested in a Phase 2a clinical trial for the treatment of idiopathic pulmonary fibrosis (IPF) (NCT05570058). As a selective ROCK2 inhibitor, hypotension observed in the clinic with dual ROCK1/2 inhibitors is avoided.
- The data presented highlight the potential for targeting the fibrotic tumor microenvironment via small molecule ROCK2 inhibition with zelasudil and the opportunity to combine zelasudil with SoC chemotherapy or immunotherapy to improve treatment responses in pancreatic cancer.

Fig. 1: Targeting the fibrotic TME
Pancreatic tumor microenvironment is characterised by increased collagen deposition and a cold tumor immune microenvironment with low T cell infiltrate and high levels of MDSCs and stromal cells. Using small molecule inhibitors of ROCK2 to target fibrosis promotes ECM remodelling, infiltration of CD8+ T cells and M1 macrophage polarisation. Created with BioRender.com



(CAF) Cancer associated fibroblast; (MDSC) Myeloid derived suppressor cell; (TAM) Tumor associated macrophage; (ECM) Extracellular matrix.

Results

Zelasudil modulates the fibrotic tumor microenvironment in the KPC PDAC model

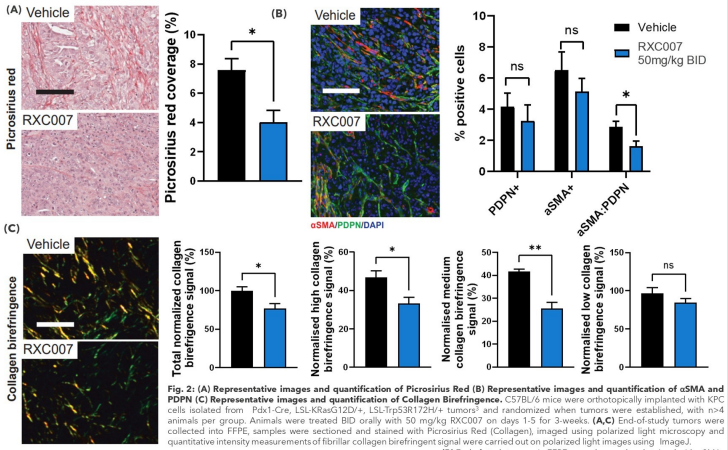


Fig. 2: (A) Representative images and quantification of Picrosirius Red (B) Representative images and quantification of αSMA and PDPN (C) Representative images and quantification of Collagen Birefringence. C57BL/6 mice were orthotopically implanted with KPC cells isolated from Pdx1-Cre, LSL-KRasG12D/+, LSL-Trp53R172H/+ tumors[3] and randomized when tumors were established, with n>4 animals per group. Animals were treated BID orally with 50 mg/kg RXC007 on days 1-5 for 3-weeks. **(A,C)** End-of-study tumors were collected into FFPE, samples were sectioned and stained with Picrosirius Red (Collagen), imaged using polarized light microscopy and quantitative intensity measurements of fibrillar collagen birefringent signal were carried out on polarized light images using ImageJ. **(B)** End of study tumors in FFPE were dewaxed and stained with αSMA and PDPN, followed by Alexa-fluor conjugated secondary antibodies. Immunofluorescent stained tissues were imaged by fluorescence microscopy and quantified using Image J. Statistical analysis was carried out using an unpaired two-tailed t-test in GraphPad Prism. * P≤0.05, ** P≤0.01.

Zelasudil induces positive changes in the immune microenvironment in a Syngeneic KPC PDAC model

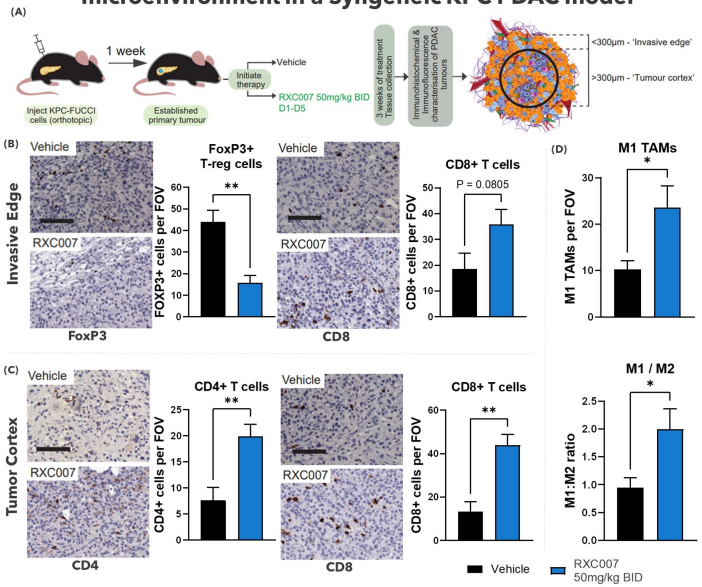
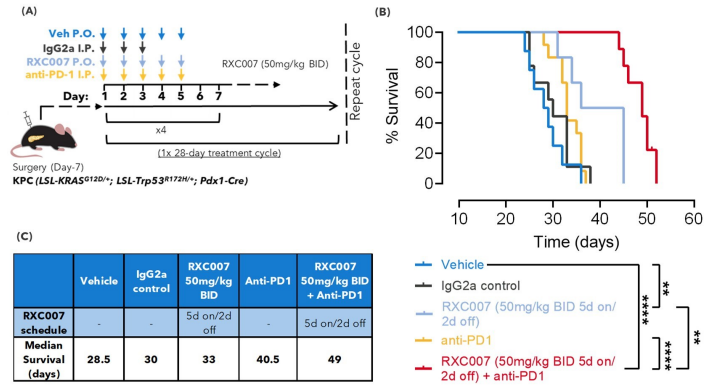


Fig. 3: (A) Schematic representing study design in the KPC RXC007 study (B-D) Quantification of the number of positively stained CD4+ and CD8+ T cells, FOXP3+ T-regulatory cells and M1 and M2 polarized macrophages. (B, C) Representative images of CD4+ and CD8+ T cells and T regulatory cells at the Tumor Invasive Edge and within the Tumor Cortex. C57BL/6 mice were orthotopically implanted with KPC cells isolated from Pdx1-Cre, LSL-KRasG12D/+, LSL-Trp53R172H/+ tumors[3] and randomized when tumors were established, with n>4 animals per group. Animals were treated BID orally with 50mg/kg RXC007 on days 1-5 for 3-weeks. End-of-study tumors were collected into FFPE, samples were sectioned and stained with CD8, CD4, FoxP3 (T-reg), F4/80 & CD68 (M1) or F4/80 & CD206 (M2). Quantification of the number of the indicated cell type per field of view was carried out in ImageJ. Statistical analysis carried out using two-tailed t-test in GraphPad Prism. * P≤0.05, ** P≤0.01.

Zelasudil increases survival in combination with anti-PD1 in the Syngeneic KPC PDAC model

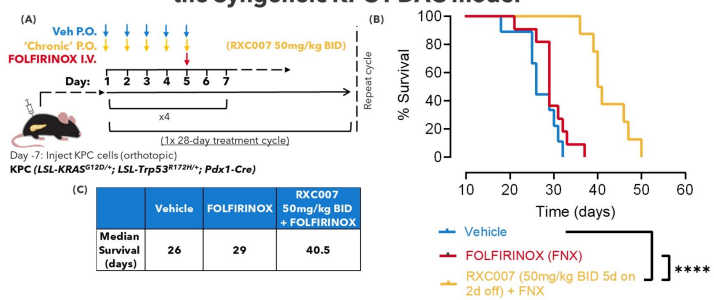


(C)

	Vehicle	IgG2a control	RXC007 50mg/kg BID	Anti-PD1	RXC007 50mg/kg BID + Anti-PD1
RXC007 schedule	-	-	5d on/2d off	-	5d on/2d off
Median Survival (days)	28.5	30	33	40.5	49

Fig. 4: (A) Schematic representing dose scheduling in the KPC RXC007 + anti-PD1 study. (B) Kaplan-Meier survival plot of RXC007 + anti-PD1 KPC survival study (C) Table of the median survival of each treatment group. C57BL/6 mice were orthotopically implanted with the KPC ((LSL-KrasG12D/+; LSL-Trp53R172H/+; Pdx1-Cre) cell line into the pancreas. Mice were randomised one-week post-implantation, following confirmation of a single luciferase positive mass in the pancreas by IVIS imaging. Animals were treated BID orally with 50mg/kg RXC007 for 5 days on 2 days off and twice weekly by intraperitoneal injection with 200ug anti-PD1 or 10mg/kg isotype control (IgG2a). Vehicle n=8; Isotype control n=9; anti-PD1 n=12; RXC007 n=6; RXC007 + anti-PD1 n=9. Statistical analysis of survival carried out using Mantel-Cox test in GraphPad Prism. ** P≤0.01, **** P≤0.0001.

Zelasudil increases survival in combination with FOLFIRINOX in the Syngeneic KPC PDAC model

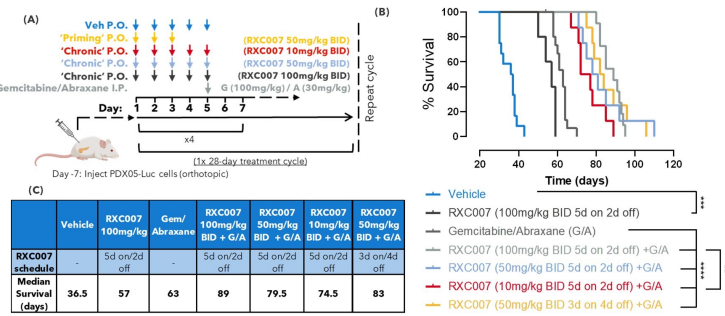


(C)

	Vehicle	FOLFIRINOX	RXC007 50mg/kg BID + FOLFIRINOX
Median Survival (days)	26	29	40.5

Fig. 5: (A) Schematic representing dose scheduling in the KPC RXC007 + FOLFIRINOX study (B) Kaplan-Meier survival plot of RXC007 + FOLFIRINOX KPC study (C) Table of the median survival of each treatment group. C57BL/6 mice were orthotopically implanted with the KPC ((LSL-KrasG12D/+; LSL-Trp53R172H/+; Pdx1-Cre) cell line into the pancreas. Mice were randomised one-week post-implantation, following confirmation of a single luciferase positive mass in the pancreas by IVIS imaging. Animals were treated BID orally with 50mg/kg RXC007 5 days on 2 days off and once weekly by intravenous injection with FOLFIRINOX (25mg/kg Leucovorin, 25mg/kg 5-Fluorouracil; 5mg/kg Oxaliplatin; 15mg/kg Irinotecan). Vehicle n=9; FOLFIRINOX n=11; RXC007 + FOLFIRINOX n=8. Statistical analysis of survival carried out using Mantel-Cox test in GraphPad Prism. **** P≤0.0001.

Zelasudil improves survival in combination with Gemcitabine/Abraxane in a fibrotic patient-derived orthotopic model of PDAC



(C)

	Vehicle	RXC007 100mg/kg	Gem/ Abraxane	RXC007 100mg/kg BID + G/A	RXC007 50mg/kg BID + G/A	RXC007 10mg/kg BID + G/A	RXC007 50mg/kg BID + G/A
RXC007 schedule	-	5d on/2d off	-	5d on/2d off	5d on/2d off	5d on/2d off	3d on/4d off
Median Survival (days)	36.5	57	63	89	79.5	74.5	83

Fig. 6: (A) Schematic of the orthotopic implantation and treatment groups in the PDX05-Luc mouse model (B) Kaplan-Meier survival plot of RXC007 + Gem/Abraxane PDX05 study (C) Table of the median survival of each treatment group. NOD-SCID mice were orthotopically implanted with the PDX05-Luc patient derived cell line and randomized when tumors were established. Animals were treated BID orally with indicated dose of RXC007 and QW by intraperitoneal injection with 30mg/kg Abraxane and 100mg/kg Gemcitabine. Vehicle n=12; RXC007 (100mg/kg) n=5; Gem/Abraxane (G/A) n=15; RXC007 (10mg/kg BID 5 d on, 2 d off) + G/A n=11; RXC007 (50mg/kg BID 5d on, 2d off) + G/A n=8; RXC007 (10mg/kg BID 5d on, 2 d off) + G/A n=8; RXC007 (50mg/kg BID 3d on, 4d off) + G/A n=8. **(B,C)** Statistical analysis of survival carried out using Mantel-Cox test in GraphPad Prism. ** P≤0.01, *** P ≤ 0.001, **** P≤0.0001.

Conclusions

Zelasudil (RXC007) is a highly selective small molecule inhibitor of Rho-associated coiled-coil containing protein kinase 2 (ROCK2), developed in-house at Redx and is currently being tested in the clinic for the treatment of fibrotic lung disease. The anti-fibrotic efficacy of RXC007 highlights an opportunity to explore combinations with standard of care chemo- and immunotherapies to improve treatment responses in fibrotic tumors, such as PDAC.

Anti-fibrotic efficacy of RXC007 monotherapy was demonstrated in the KPC model via a decrease in collagen content and organization, effects associated with CAF reprogramming and reduced intra-tumoral αSMA+ PDPN+ myofibroblast-like CAFs. Selective inhibition of ROCK2 by RXC007 alone elicited positive immunomodulatory effects in the KPC tumours, with increased CD4+ and CD8+ T cell infiltrate into the tumor cortex, a reduction in immunosuppressive Tregs at the tumor border and altering of macrophage polarization. In addition to impacts upon both the immune and stromal TME, combining RXC007 with anti-PD1 or FOLFIRINOX led to increased survival in the immunocompetent, chemotherapy and immunotherapy non-responsive KPC model.

Furthermore, in a high-ECM partially chemo-responsive patient derived orthotopic model of PDAC ROCK2 inhibition with RXC007 when combined with SoC chemotherapy (G/A) led to a dose dependent increase in survival when compared to chemotherapy alone.

The data presented here provides a rationale for clinical investigation of zelasudil in combination with standard of care chemotherapy or immunotherapy for the treatment of pancreatic cancer.

References

1. Rath *et al.* (2017), *EMBO Molecular Medicine, 9(2).*; **2. Vennin *et al.* (2017)**, *Science Translational Medicine, 9(384).* **3. Hingorani et al. (2005),** *Cancer Cell, 7(5).*

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