RXC008: First- In-Class Gastrointestinal-Targeted Potent Pan-ROCK Inhibitor for Treatment of Fibrostenotic Crohn's Disease

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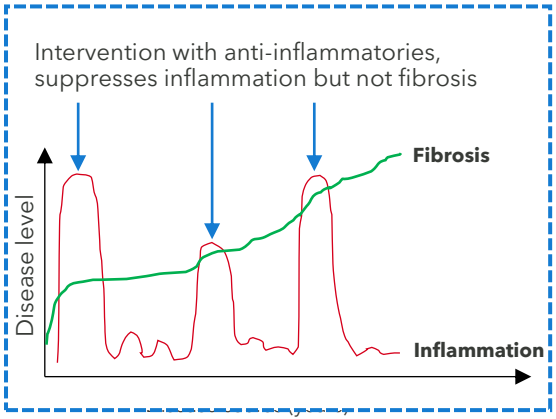
Disclosure of Conflicts of Interest

Elaine Kilgour is an employee of Redx Pharma LTD

RXC008: Potential First-in-Class Treatment in Fibrostenotic Crohn's Disease – An Area of High Unmet Clinical Need

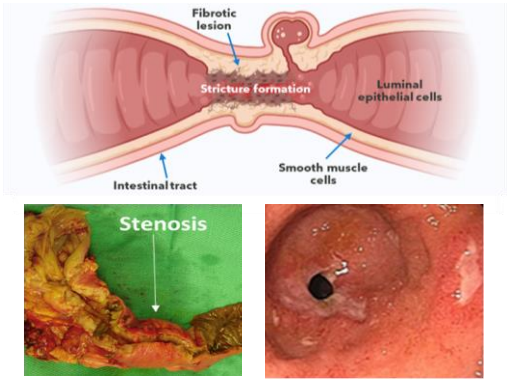
"The ultimate goal remains the development of selective anti-fibrotic therapies for patients with fibrostenosing Crohn's disease" – STAR Consortium, July 2024

Clinical progression in Crohn's



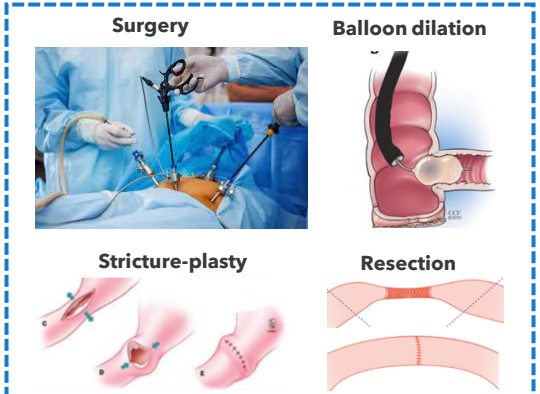
1.7 million(1) patients globally affected by Crohn's disease

Fibrotic stricture formation



Within 10 years of diagnosis, **>50% of patients**(2) develop fibrostenosis and strictures

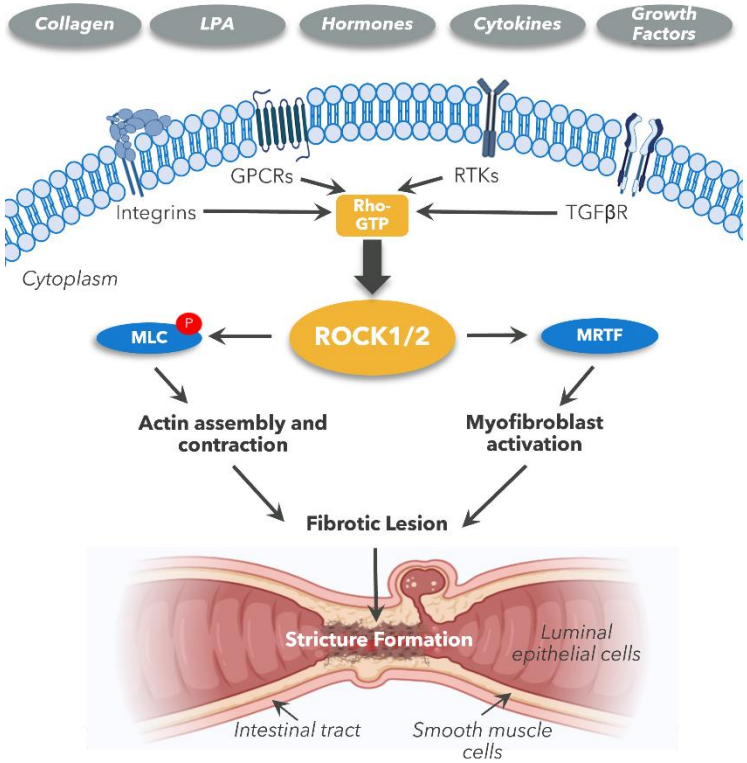
Surgical interventions



No approved therapies for underlying fibrosis; only treatment options are debilitating surgical intervention

(1) Clarivate, Crohn's disease landscape & forecast pg 39, Published Sep 2022; (2) Chan et al, 2018

RXC008: GI-targeted pan-ROCK Inhibitor Targets a Fibrotic Pathway Nodal Point without Systemic Breakthrough



Why target ROCK?

- ROCK (Rho-associated coiled-coil kinases 1 and 2) is a nodal point in the fibrotic signalling pathway
- ROCK1/2 inhibition has demonstrated robust anti-fibrotic activity in preclinical models
- Inhibiting ROCK 1 &2 systemically is known to result in hypotension

Why RXC008?

- Targets both ROCK1/2
- RXC008 designed to be retained in the GI tract via high efflux and low permeability, rapidly metabolised by paraoxonase enzymes in the plasma
- RXC008 gut restriction prevents systemic exposure and avoids hypotension

GI: Gastrointestinal; GITR: ROCK: Rho-associated protein kinase: Gastrointestinal targeted ROCK; MRTF: myocardin-related transcription factor; MLC: myosin light chain

RXC008 is a Potent and Selective pan-ROCK Inhibitor

RXC008 potently and selectively inhibits ROCK1/2

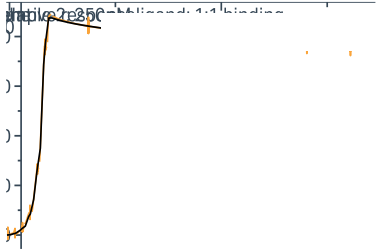
Biochemical assay
(ADP-Glo, 20 µM ATP)

	RXC008 (nM)
ROCK1 IC_{50}	1.6
ROCK2 IC_{50}	1.3

Note. RXC008 hits the tight binding limit of both ROCK1 and ROCK2 Biochem Assays

RXC008 binds to ROCK1/2 with high affinity

Surface Plasmon Resonance
(representative ROCK1 sensorgram shown)



ROCK 1 (n=3): KD = 3.5 pM

ROCK 2 (n=5): KD = 63.8 pM

RXC008 potently inhibits ROCK1/2 in cells

MCF7 cells

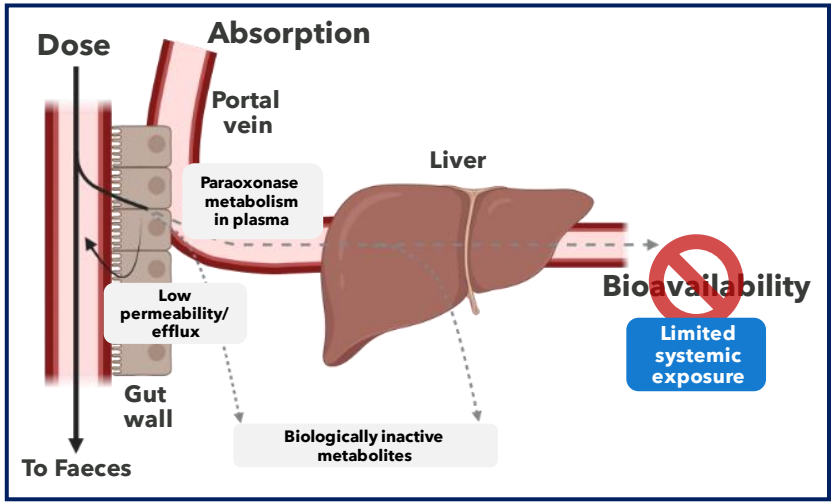
	RXC008 (nM)
ROCK1 pMYPT1 IC_{50}	2.0
ROCK2 pMYPT1 IC_{50}	2.3
ROCK1 + ROCK2 pMYPT1 IC_{50}	4.6

In a Kinome screen RXC008 tested at 100nM (>1000 x ROCK KD by SPR) inhibits only 9 kinases >50% (excluding ROCK1 and 2). Follow-up showed 15-fold to >50-fold cellular selectivity.

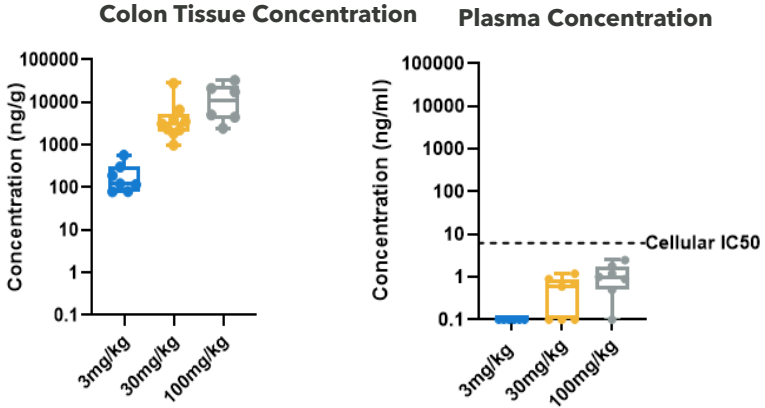
RXC008: Designed to be GI-Restricted – Resulting in Virtually No Systemic Exposure Avoiding the Risk of Hypotension

Multiple Mechanisms Drive Gut Restriction

- Low permeability / high efflux
- Rapidly metabolised by plasma paraoxonases
- Rapidly cleared by liver
- Plasma half-life RXC008 < 10 minutes across species but >2h in human intestinal prep



RXC008 is GI-restricted at efficacious doses in Adoptive T cell transfer Murine Crohn's Model



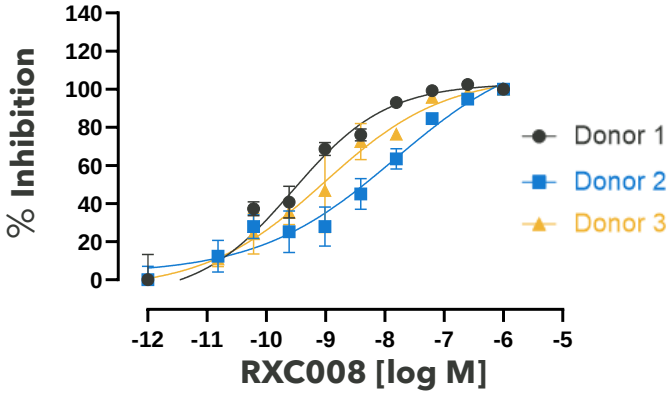
- RXC008 detected in GI tissue with minimal, not pharmacologically relevant, breakthrough to plasma

- PPB Corrected Cellular IC50 from mouse SV40 MES cells

GI: Gastrointestinal

RXC008 Inhibits ROCK1/2 and Downstream Fibrotic Pathways in Human Disease Relevant Cells

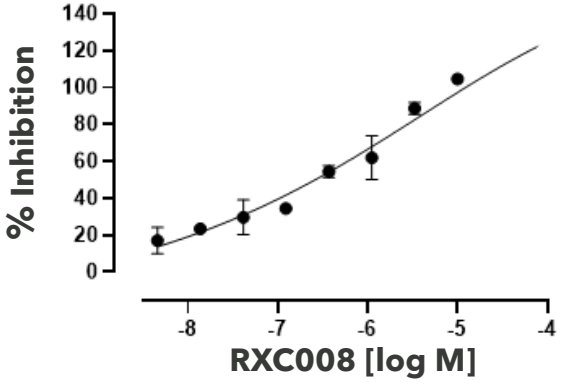
RXC008 inhibits phosphorylation of MYPT1 in Stenotic Fibroblasts

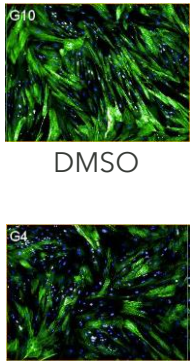


IC50 = 5.4 nM ± 8 nM

Phosphorylation of MYPT1 was measured by ELISA in Fibroblasts isolated from resection tissue from Fibrostenotic Patients.

RXC008 inhibits Myofibroblast Differentiation in Intestinal Fibroblasts





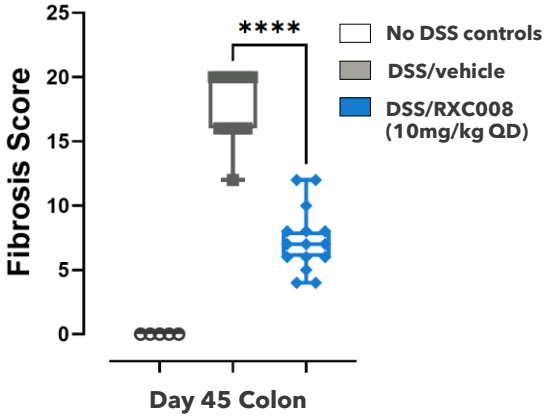
IC50 = 560 nM

Myofibroblast Differentiation was measured by alpha-SMA immunofluorescence in Human Intestinal Fibroblasts.

ELISA: Enzyme-linked immunosorbent assay; ROCK: Rho-associated protein kinase

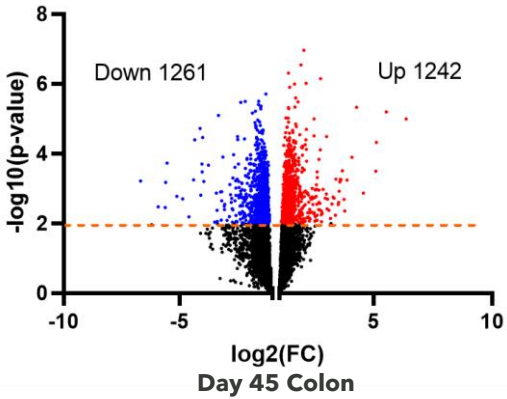
RXC008 is Efficacious and Induces Gene Expression Changes in 9 week DSS Colitis Model. Anti-Fibrotic Efficacy can be Monitored by Non Invasive MRI Imaging

RXC008 decreases fibrosis (measured by IHC)



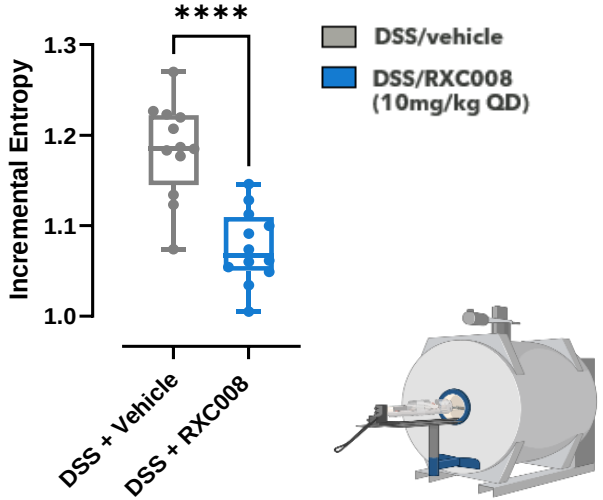
RXC008 reduces fibrosis (IHC score) in the colon when dosed once daily in a 9 week DSS model of colitis.

RXC008 alters expression of ~2500 genes



RXC008 alters profibrotic gene expression in the colon, e.g. COL1A1 and TGFβ, when dosed once daily in a 9 week DSS model of colitis.

RXC008 decreases entropy (measured by MRI)



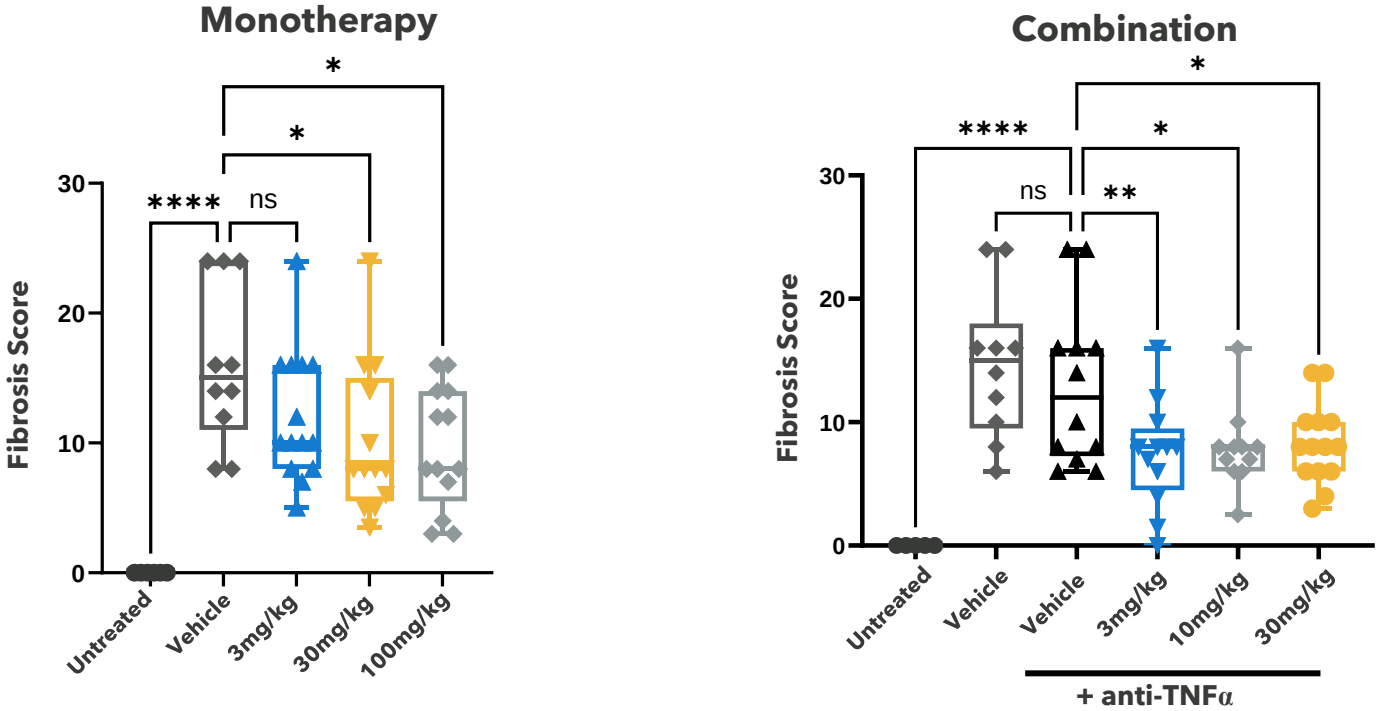
Non-invasive MRI imaging detects RXC008 antifibrotic effects in GI tract

1. De Kock I, et al. MRI texture analysis of T2-weighted images is preferred over magnetization transfer imaging for readily longitudinal quantification of gut fibrosis. Eur Radiol. 2023 Sep;33(9):5943-5952.

RXC008 in a 9 week DSS colitis model. Male C57BL/6J mice. 2.5% DSS in drinking water from Day 1-7 followed by 14 days of normal drinking water, cycle repeated 2 further times. RXC008 oral dosing daily from day 1-59. Terminal sampling and PD analysis at T = 24 h. Statistics shown: One-way ANOVA with Dunnett's multiple comparison test calculated relative to vehicle control.

RXC008 Demonstrates Efficacy as a Monotherapy and in Combination with Anti-TNFα in Adoptive T cell Transfer Crohn's Model

RXC008 reduces fibrosis (IHC score), anti-TNFα treatment alone has no effect on fibrosis in this model



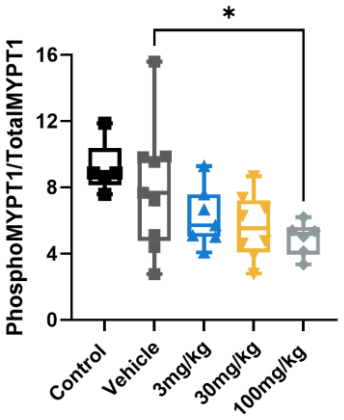
One-way ANOVA with Dunnett's multiple comparison test calculated relative to vehicle control.

IHC: Immunohistochemistry

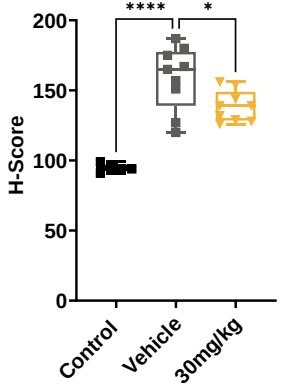
RXC008 Demonstrates Target Engagement in Adoptive T cell Transfer Crohn's Model

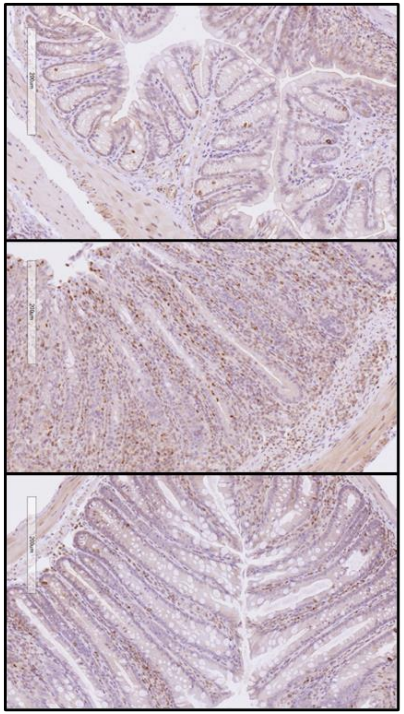
RXC008 inhibits the proximal target engagement marker pMYPT1

Adoptive Transfer pMYPT1 ELISA



pMYPT1 IHC Lamina Propria



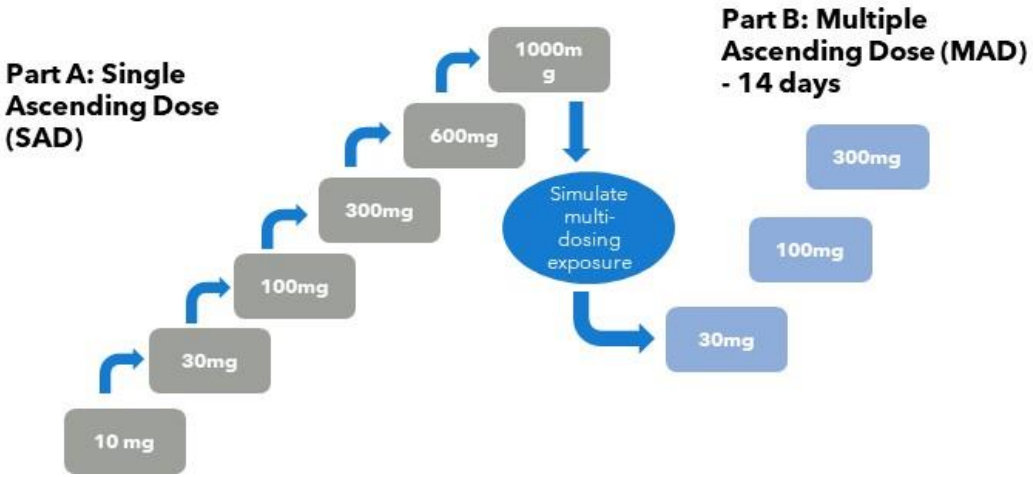


- pMYPT1 performed on colon tissue from the adoptive transfer study
- H scoring was performed blind by pathologist

One-way ANOVA with Dunnett's multiple comparison test calculated relative to vehicle control.

RXC008 is Currently Being Investigated in a Phase 1 Healthy Volunteer Study

RXC008 Phase 1 Dose Escalation In Healthy Volunteers, N = 59



Parts A and B in Healthy Volunteers

- Single Ascending Dose (SAD)
- Multiple Ascending Dose (MAD) cohorts with 14-day dosing
- Safety (no cardiovascular effects) & Tolerability
- PK (faeces, plasma and tissue in MAD cohorts)

Paired Biopsy Exploratory Analysis

- Tissue and plasma compound levels
- PD biomarker assessment

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