GI restricted ROCK inhibitors show potential to treat fibrosis and stenosis associated with inflammatory bowel disease

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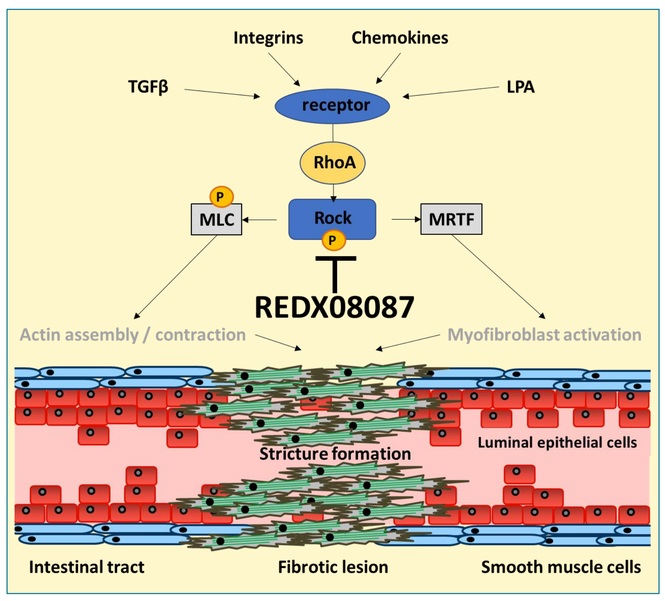
Intestinal fibrosis associated with Inflammatory Bowel Disease (IBD) is a significant issue

- In the U.S. 30-40% patients have stricture formation and obstruction to bowel wall fibrosis within 10 years of diagnosis of inflammatory bowel disease (IBD)[1]
- Surgical resections to remove fibrostenosis are required in 80% of cases but recurrence rates are up to 70%[2]
- Treatment with biologics has resulted in only a small decrease in the rate of surgical interventions[3]
- Even in the absence of inflammation, tissue damage and fibrosis continue to progress with increased accumulation and crosslinking of extracellular matrix (ECM)
- Preventing or reversing ECM deposition in IBD is a major therapeutic challenge

Key pathways in fibrosis

- The transition of quiescent mucosal fibroblasts into activated myofibroblasts occurs via multiple stimuli such as TGFβ, IL6 and mechanical stimuli (matrix stiffness)[4]
- Activated myofibroblasts produce ECM components such as collagen and remodelling enzymes e.g. matrix metalloproteinases (MMPs) and pro-fibrotic cytokines including TGFβ and IL6[5]
- Myofibroblasts formed by epithelial to mesenchymal transition (EMT) of epithelial cells into myofibroblasts are also a source of effector cells contributing to fibrosis[6]



ROCK is involved in multiple aspects of fibrosis

- ROCK (Rho-associated coiled-coil-containing protein kinase) is a serine/threonine protein kinase with two ubiquitously expressed isoforms, ROCK1 and ROCK2
- ROCK activation leads to the formation of stress fibres through actin polymerisation, this results in the release of myocardin-related transcription factors (MRTFs) that translocate to the nucleus and activate genes involved in cell differentiation and cytoskeletal organisation
- The involvement of ROCKs in myofibroblast activation, cytoskeletal organization, EMT and autophagy has highlighted their potential for anti-fibrotic therapy[7]

GI restricted ROCK inhibitors could avoid the toxicity of systemic ROCK inhibition

- The therapeutic potential of systemic ROCK inhibitors have been limited due to the target-related smooth muscle relaxation of systemic vasculature leading to hypotension[8]
- Local delivery of a potent ROCK1/2 inhibitor to the intestinal lumen could avoid the issues associated with systemic exposure
- REDX08087 is a potent and pan-kinase selective ROCK1/2 inhibitor with suitable properties for local delivery to the GI mucosa
- REDX08087 reaches high local GI concentrations but with limited systemic exposure due to rapid degradation in plasma

REDX GI restricted ROCK inhibitors are potent and selective

- Racemate REDX08087 (AMA0825) is an extremely potent inhibitor of both of the isolated ROCK kinase domains and downstream signalling in cellular assays
- Enantiomers REDX08397/ REDX08398 have very similar biological profiles

		(AMA0825) REDX08087	REDX08397	REDX08398
		Racemate	Enantiomer (A)	Enantiomer (B)
ROCK1†	IC50	0.2nM	0.1nM	0.1nM
ROCK2†	IC50	0.1nM	0.2nM	0.1nM
pMYPT1	IC50	22nM	24nM	18nM
α-SMA*	IC50	110nM	200nM	200nM

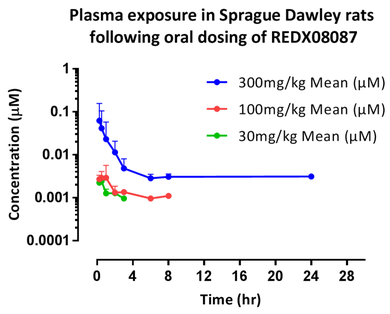
† isolated enzyme assay truncated kinase domain
* Human lung fibroblasts

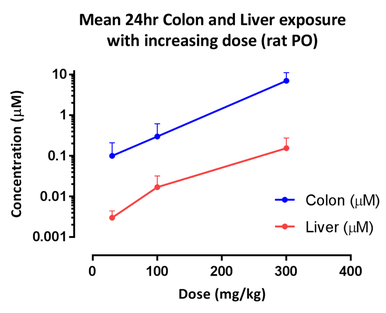
- Racemate REDX08087 has excellent selectivity in kinase and secondary target panels

Selectivity REDX08087 (100nM)		
Kinase	% Activity	IC_{50} (nM)
DMPK2	11	NT
LATS2	45	NT
PKCδ	8	5
PKCε	16	20
PKCθ	69	100
PRK1	9	NT
PRK2	18	NT
ROCKI	2	NT
ROCKII	0	< 0.05

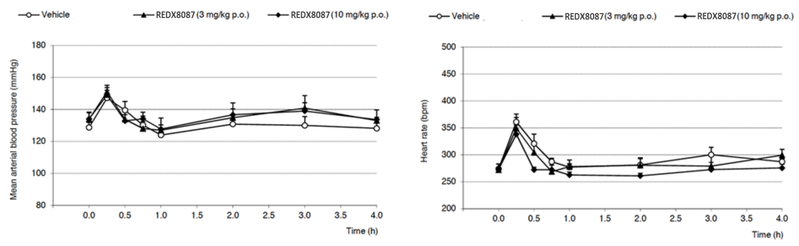
GI restricted ROCK inhibitors have limited systemic exposure

- Limited systemic exposure in rat pharmacokinetic (PK) studies at doses significantly above therapeutic levels
- Significant drug concentrations remain in colon tissue, even 24hr after dosing



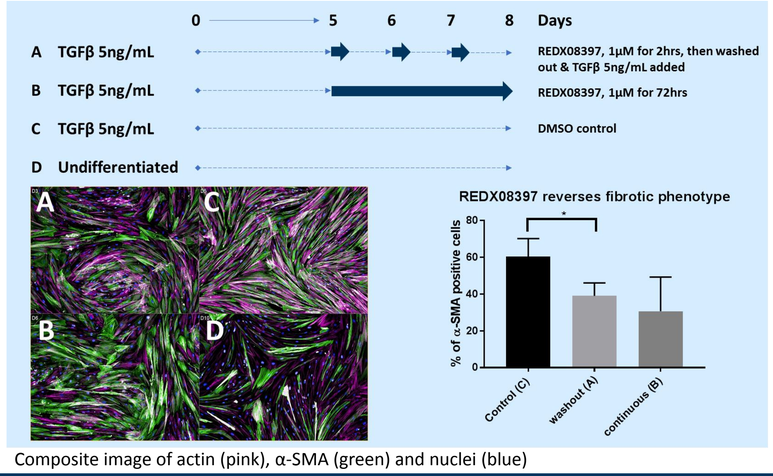


- No evidence of heart rate or blood pressure changes with REDX08087 in spontaneously hypertensive rats (SHR)



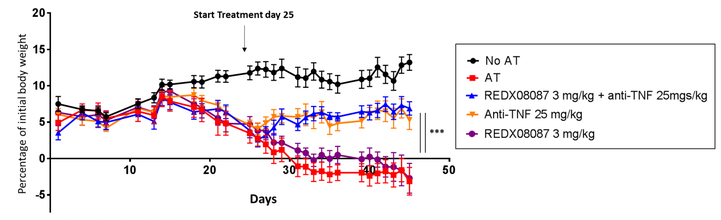
GI restricted ROCK inhibitors can reverse a fibrotic phenotype in human intestinal fibroblasts

- Significant suppression of the number of activated fibroblasts expressing α-SMA after intermittent treatment with REDX08397
- Suggests that ROCK inhibition can induce reversal of the pro-fibrotic myofibroblast phenotype

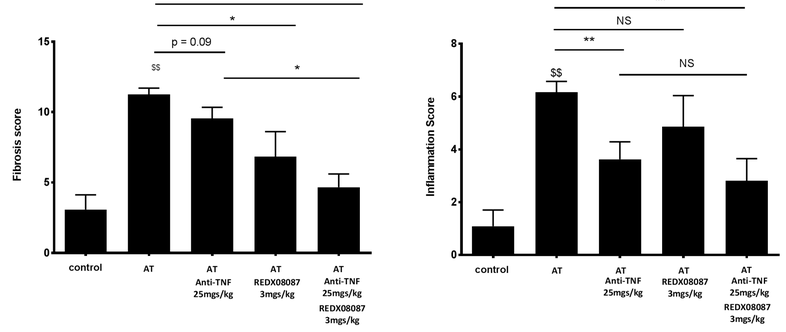


Composite image of actin (pink), α-SMA (green) and nuclei (blue)

GI restricted ROCK inhibitors can inhibit fibrosis in an adoptive T-cell transfer model of IBD but do not impact on inflammation



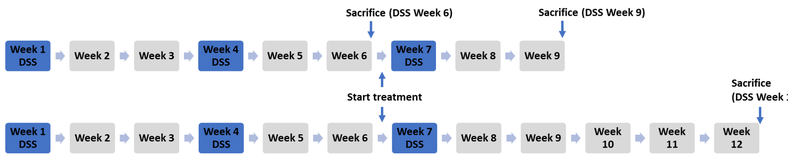
Design: CD4+CD25-CD62L+ naïve T-cells injected i.p. in CB-17 SCID mice on day 0 (adoptive transfer, AT). Mice developed symptoms of colitis at week 2 and beyond, at which point therapy was initiated. Mice were treated with 25 mg/kg anti-TNF i.p. QD or with a combination of anti-TNF i.p. and 3 mg/kg REDX08087 via oral gavage QD (n=10 in each group). Placebo-treated mice received 25 mg/kg IgG1 i.p. and vehicle p.o.



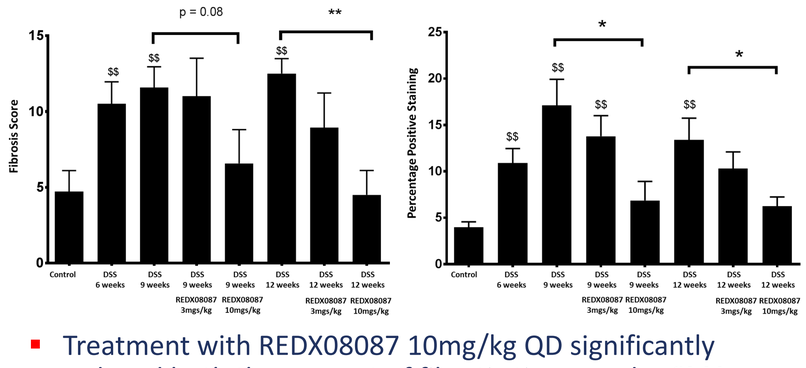
- Anti-TNF treatment improves inflammation scores and decreases the production of inflammatory markers IFNγ, MCP1, IL6 and IL1β but has no effect on fibrosis[9]
- In contrast, REDX08087 monotherapy has no effect on inflammation score but a reduction in histological fibrosis and the production of TGFβ and IL6 were observed
- Combination of REDX08087 and anti-TNF results in reduction of fibrosis to control levels

GI restricted ROCK inhibitors can reverse established fibrosis in a chronic DSS model of inflammatory bowel disease

- Fibrosis established in mice by treatment with 2 cycles of DSS, then subsequent treatment with REDX08087 for 3 or 6 weeks at either 3 or 10mg/kg QD



Design: Intestinal fibrosis induced by administering 2.5% DSS in drinking water for 1 wk, followed by 2 wks of normal drinking water, cycle repeated 3 times & groups of mice sacrificed at 6, 9, and 12 wks after first exposure to DSS. Fibrosis assessed by Masson's trichrome staining.



- Treatment with REDX08087 10mg/kg QD significantly reduced both the amount of fibrotic tissue and α-SMA protein density
- 3 or 6 weeks treatment with 10 mg/kg REDX08087 also significantly reduced colonic levels of IL6, TGF-β1, and MMP-2,-8, -9, and -12[9]

Conclusions

- REDX GI restricted ROCK inhibitors can potently suppress TGFβ activation of fibroblasts
- REDX GI restricted ROCK inhibitors also show the ability to suppress α-SMA in TGFβ activated human intestinal fibroblasts
- Fibrosis can be prevented from developing in an adoptive T-cell transfer model of inflammatory bowel disease
- Established fibrosis can be reversed in a DSS induced model of inflammatory bowel disease
- Further studies are ongoing to assess the therapeutic potential of GI restricted soft ROCK inhibitors to reduce stenosis formation in IBD

References

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