ROCK2 selective inhibitors for the treatment of fibrosis

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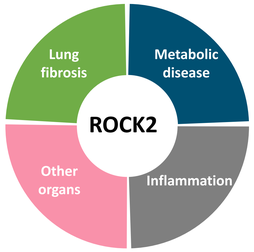
INTRODUCTION

ROCK2 is a nodal point in cell signalling pathways associated with fibrotic diseases

- ROCK2 inhibitor PoC in human IPF trial
- Clinical response in lung scores in cGvHD
- ROCK2+/- protected lung fibrosis



- ROCK2 inhibitor = efficacy across multiple organs in cGVHD clinical trial
- ROCK2 inhibitors = ↓ fibrosis in skin (SSc) model
- ROCK2 conditional KO = ↓ hypertension, hypertrophy & atherosclerosis

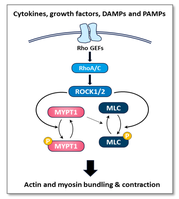
- ↑ROCK2 in liver fibrosis and diabetic kidney models
- ROCK2 inhibition = ↓ liver fibrosis
- ROCK2 inhibition = ↓ kidney fibrosis
- ROCK2 haplotype KO = ↓ fibrosis in UUO model

- ↑ROCK2 in acute and chronic inflammation
- ROCK2 inhibitors shown to be anti-inflammatory *in vivo*
- ROCK2 inhibition protects from inflammatory damage in IBD models

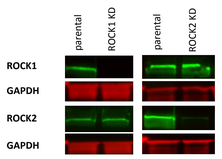
RESULTS

REDX10843 is a potent and highly selective ROCK2 inhibitor with a suitable ADMET profile

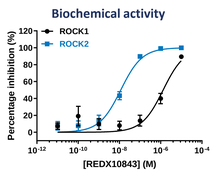
ROCK cellular signalling



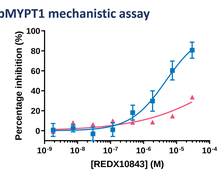
ROCK expression in MCF7 cell lines



Biochemical activity



pMYPT1 mechanistic assay



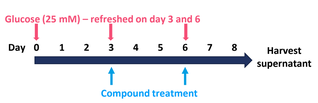
Assay	REDX10843 IC_{50} (μM)
Biochemical Activity ROCK2	0.017
Biochemical Activity ROCK1	2.5
Cellular activity ROCK2[a]	2.4
Cellular activity ROCK1[b]	26

- REDX10843 is highly selective across a panel of 468 kinases, and in the CEREP safety panel with no significant off target activities observed.
- No activity in cardiotoxicity assays.
- No genotoxicity or mutagenicity observed in *in vitro* tests.

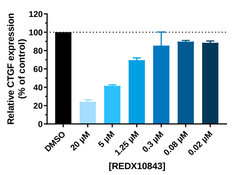
*Note: data are all from n≥2; (a) ELISA MCF7 ROCK1 KD pMYPT1 T853 (ROCK2 selective); (b) ROCK2 KD pMYPT1 T853 (ROCK1 selective); *Expect low activity for ROCK2 selective compounds.*

ROCK2 inhibitors prevent the release of pro-inflammatory and pro-fibrotic factors in kidney mesangial cells grown in high glucose

Assay schematic



CTGF release from kidney mesangial cells



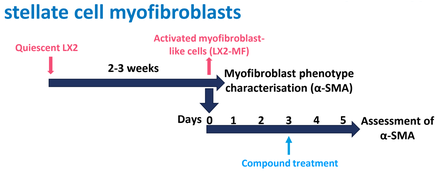
Assay	REDX10843 IC_{50} (μM)
Secreted TIMP-1	2.8
Secreted PDGF-BB	1.4
Secreted MCP-1	2.1
Secreted CTGF	1.5

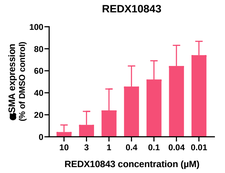
Summary of analysis of culture supernatant from cells cultured with REDX10843. Supernatant analysed by western blot (CTGF) or ELISA. Data are from n≥3.

- Protein expression of secreted factors detected in the culture media
- High glucose stimulates a profibrotic phenotype in kidney mesangial cells
- Cells secrete growth factors and cytokines into the supernatant e.g. CTGF, PDGF-BB, TIMP-1 and MCP-1
- REDX10843 reduces pro-fibrotic and pro-inflammatory activity of kidney mesangial cells

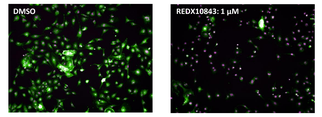
RESULTS

REDX10843 reverse the myofibroblast phenotype of activated human hepatic stellate cell myofibroblasts





- HSC cell line (LX2) cultured for 2-3 weeks on plastic to induce differentiation into myofibroblasts (LX2-MF)
- No exogenous stimuli: cells are activated by matrix stiffness and autocrine factors
- LX2-MF plated for assay and allowed 48 -72 h to recover and re-organise stress fibres

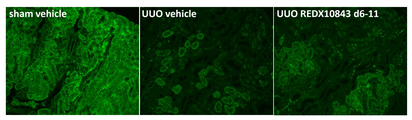


Expression of α-SMA detected by immunocytochemistry — Green: αSMA; purple: nuclei (DRAQ5)

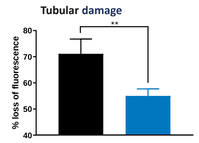
- REDX10843 dosed for 48 h and expression of α-SMA (with DRAQ5 as nuclear stain) is detected by immunocytochemistry
- REDX10843 suppresses α-SMA in the LX-2 cells – suggesting a reversal of the myofibroblast like phenotype
- No toxicity was observed with compounds (up to 10 μM)

REDX10843 reduces kidney tubular damage and fibrosis in UUO model

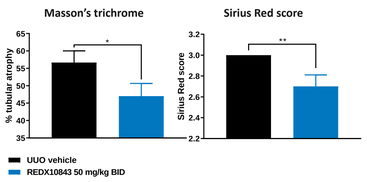
Protection from tubular damage and atrophy



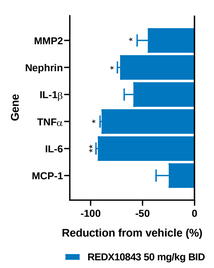
Tubular damage



Reduced collagen deposition

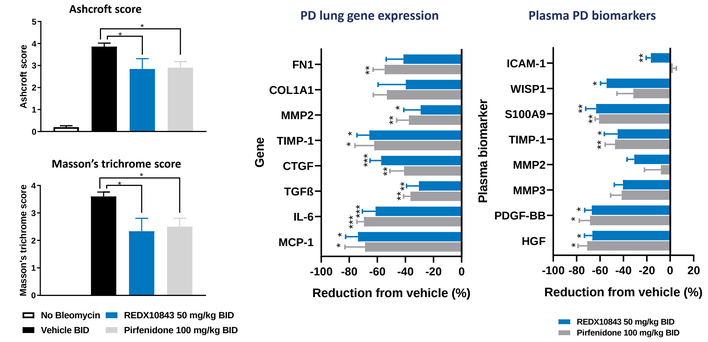


PD gene expression in kidney



- Enhanced tubular cell survival and reduced damage as measured by auto-fluorescence
- Reduced collagen deposition as measured by Masson's trichrome & Sirius red
- Reduction in gene expression markers of tissue injury, inflammation and fibrosis

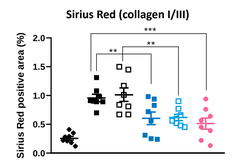
REDX10843 suppresses fibrosis in murine bleomycin-induced IPF model

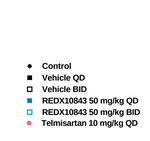


- Significantly reduced fibrosis (Ashcroft score) and collagen deposition (Masson's trichrome) in the lung
- Highly significant reduction in pro-fibrotic and pro-inflammatory gene expression in the lung
- Reduced plasma and BAL expression of PD biomarkers

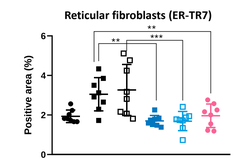
REDX10843 suppresses fibrosis in murine STAM NASH liver model

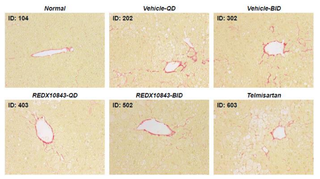
Reduced collagen deposition in the liver

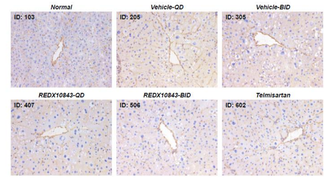




Reduced profibrotic fibroblasts in the liver







- Reticular fibroblasts produce and deposit collagen III in the liver, positive cells determined by automated quantification
- Significant reduction of pro-fibrotic fibroblasts in the liver when REDX10843 dosed QD or BID
- Significant reductions in both liver collagen quantity and bridging fibrosis with QD and BID dosing

SUMMARY

- Redx have developed a series of compounds that are potent ROCK2 inhibitors in biochemical & cellular in vitro assays and highly selective against ROCK1 and a panel of kinases and other receptor targets.
- Demonstration that physiologically relevant markers of fibrosis pathways can be modulated *in vitro* in disease relevant phenotypic assays.
- No safety concerns highlighted from early *in vitro* assessment (hERG, CEREP, AMES, micronucleus, CYP inhibition, CYP TDI, reaction phenotyping).
- Robust preclinical efficacy demonstrated with REDX10843, a lead from the series, in murine liver, kidney and lung fibrosis models

References: *1. Soliman et al, 2016; 2. Xie et al, 2006; 3. Waddingham et al, 2015; 4. Cicek et al, 2013; 5. Shimizu et al, 2013; 6. Yao et al, 2013; 7. Okamoto et al, 2013; 8. Zhou et al 2012; 9. Hu et al. 2018; 10. Luo et al, 2012; 11 Zhang et al 2016; 12. Trebicka et al, 2007; 13. Wang et al, 2018; 14. Kolavennu et al. 2008; 15. Baba et al. 2014; 16. Sun et al. 2006; 17. Nozaki et al 2015; 18. Zhou et al 2013; 19. Ho et al. 2012; 20. Knipe et al 2015; 21.* Kast *et al, 2017; 22. Flynn et al, 2016. 23.* Guisot *et al,* 3rd NASH Summit, 2019