Effects of RXC007, a highly potent and selective ROCK2 inhibitor, in *ex-vivo* and *in vivo* models of pulmonary fibrosis

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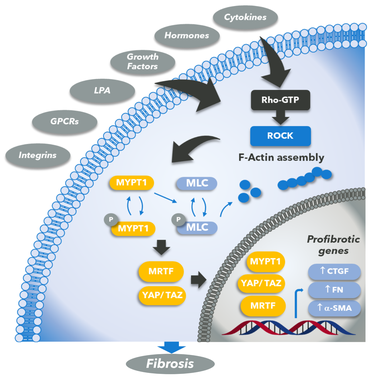
INTRODUCTION

ROCK2 is an Attractive Fibrosis Target

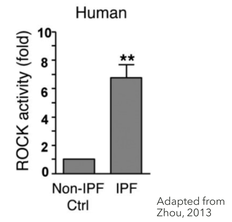


- Rho-associated Coiled-coil Containing Kinases (ROCKs) are members of the AGC (protein kinase A, G and C, PKA/PKG/PKC) family of serine/threonine kinase family, originally identified as downstream effectors of RhoA
- ROCK sits at a nodal point in cell signalling pathways associated with fibrosis
- Systemic inhibition of ROCK1/2 results in hypotension, this is not seen with ROCK2 selective inhibition
- Validated target with belumosudil approved by FDA for cGvHD
- RXC007 is a potent and highly selective oral ROCK2 Inhibitor
- RXC007 lead indication: Idiopathic Pulmonary Fibrosis (IPF) - a disease with high unmet need
- Potential for disease modifying efficacy across multiple fibrotic conditions

Rationale for Targeting ROCK2 in IPF

Increased Expression and Activation of ROCK2 and pMYPT1 in IPF patients

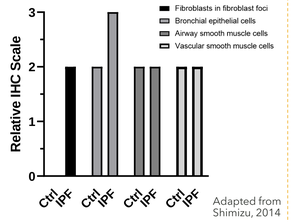
RhoA/ROCK signalling pathway is enhanced in human IPF patients



Adapted from Zhou, 2013

- (Myo)fibroblasts isolated from lungs of patients with IPF (n = 8), non-IPF control subjects (n = 6)
- ROCK activity assays using a colorimetric approach
  Data are mean ± SD

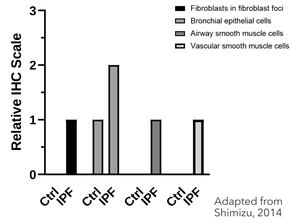
Strong increase of ROCK2 expression in IPF bronchial epithelial cells and fibroblasts



Adapted from Shimizu, 2014

- Intensity of immunostaining evaluated on a scale of 0 to 3
- N=6 per group; NF: not found

Moderate increase of p-MYPT-1 expression in IPF bronchial epithelial cells



Adapted from Shimizu, 2014

- Intensity of immunostaining evaluated on a scale of 0 to 3
- N=6 per group; NF: not found

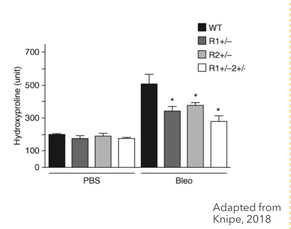
ROCK2+/- knockout mice are protected from bleomycin-induced pulmonary fibrosis

RhoA/ROCK signalling pathway is enhanced in bleomycin-induced mice

Adapted from Zhou, 2013

- (Myo)fibroblasts isolated from lungs of bleomycin-treated mice (n = 8), and saline-treated mice (n = 8)
- ROCK activity assays using a colorimetric approach
  Data are mean ± SD

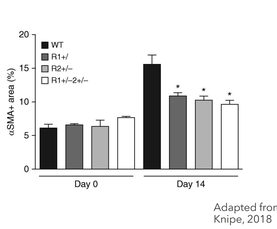
Genetic reduction in either or both ROCK isoforms affords protection from bleomycin-induced pulmonary fibrosis



Adapted from Knipe, 2018

- Significant reduction in hydroxyproline content (lung collagen) at day 14 post-bleomycin

Both ROCK isoforms contribute to fibroblast activation induced by bleomycin injury

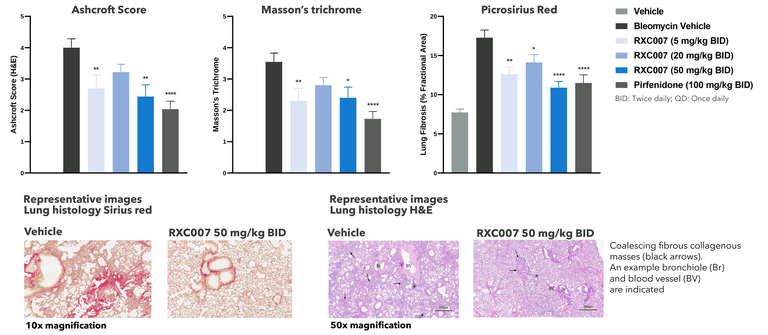


Adapted from Knipe, 2018

- Significant reduction in α-SMA staining at Day 14 post-bleomycin

RESULTS

RXC007 Reduces Fibrosis and Collagen Deposition in a Therapeutic Murine Bleomycin-induced Lung Fibrosis Model

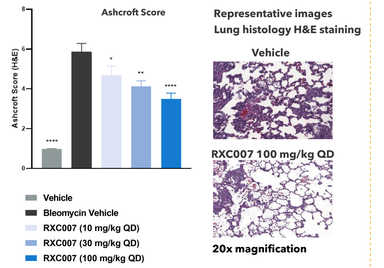


- 21-Day therapeutic murine bleomycin-induced lung fibrosis model (dosing from day 7, p.o)
- Significantly reduced fibrosis (Ashcroft score) and collagen deposition (Masson's trichrome) and picrosirius red in the lung

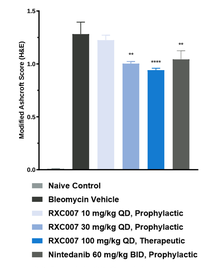
Note: Pirfenidone used only as a positive control and dosed in different vehicle to RXC007

RXC007 Reduces Fibrosis and Collagen Deposition in Rat Bleomycin-induced Lung Fibrosis Models

10-Day prophylactic rat BLM-induced lung fibrosis model (dosing from day 1)



28-Day prophylactic & therapeutic rat BLM-induced lung fibrosis model (dosing from day 1 or 7)

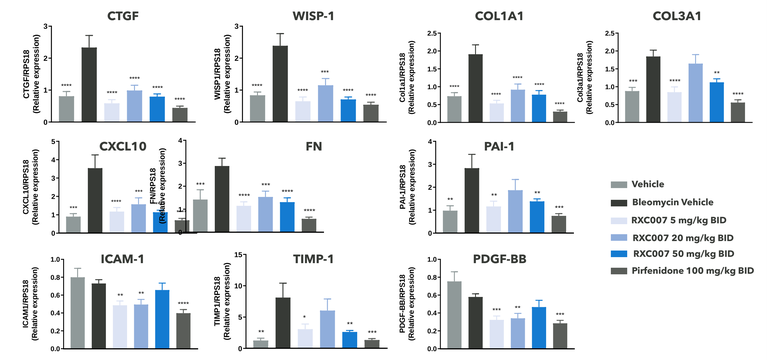


BID: Twice daily; QD: Once daily
Dosing p.o

- Significantly attenuated fibrosis and collagen deposition in the lung (Ashcroft score)

Note: Nintedanib used only as a positive control and dosed in different vehicle to RXC007

RXC007 Suppresses the Expression of a Number of Genes Strongly Associated with Fibrosis and Known Pro-fibrotic Drivers in IPF



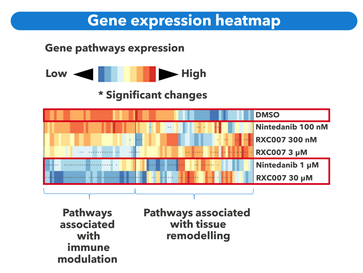
- Analysis of lung tissue in the 21-day mouse bleomycin model by qPCR showed that numerous genes strongly associated with fibrosis and known pro-fibrotic drivers in IPF were strongly suppressed by RXC007 at all tested doses
- Connective Tissue Growth Factor (CTGF), Fibronectin (FN), WISP-1, Collagen 1a1 and 3a1, CXCL10, PAI-1 and PDGFβ were all significantly attenuated following treatment of RXC007

Note: Pirfenidone used only as a positive control and dosed in different vehicle to RXC007

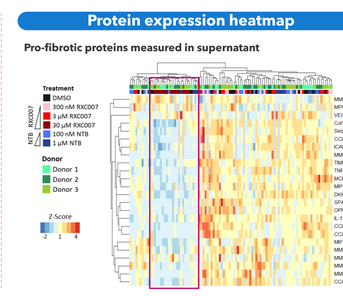
RXC007 Modulates Pro-fibrotic Gene and Protein Expression in Human IPF *Ex-vivo* Lung Tissue

Response of diseased lung tissue to treatment of RXC007 or nintedanib evaluated in Precision-Cut Lung Slices (PCLS) from patients with pulmonary fibrosis

Gene expression heatmap



Protein expression heatmap



- Analysis of gene expression pathways grouped by KEGG†
- Significant and comparable modulation of pathways associated with immune regulation and tissue remodeling observed with RXC007 and nintedanib.

†Kyoto Encyclopedia of Genes & Genomes

- Significant effect of RXC007 at 30 μM dose with 20 analytes showing significant downregulation including COL1A1, TIMP1, ICAM-1, metalloproteins MMP3, MMP7, MMP8, MMP12, PAI-1/Serpin1, Osteopontin (OPN), MCP-1

SUMMARY

- RXC007 is a highly potent, selective and orally active ROCK2 inhibitor demonstrating strong preclinical efficacy in rodent models of lung fibrosis and human fibrotic lung tissue
- The preclinical data supports clinical development plan in IPF
- To date no clinically significant adverse events reported in Phase 1 healthy volunteers and observed PK profile in line with prediction from preclinical data. Profile supports further development.
- Phase 2a in IPF expected to commence 2022

References: *Knipe RS, Probst CK, Lagares D, et al. The Rho Kinase Isoforms ROCK1 and ROCK2 Each Contribute to the Development of Experimental Pulmonary Fibrosis. Am J Respir Cell Mol Biol. 2018;58(4):471-481. doi:10.1165/rcmb.2017-0075OC*
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