ROCK2 selective inhibition is an exciting innovative approach to treat fibrotic diseases. Redx are advancing towards the clinic.
Rho-associated coiled-coil containing kinases (ROCKs)

- 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
- ROCKs facilitate RhoA-induced stress fiber formation and focal adhesion assembly
- ROCKs are expressed in both invertebrates and vertebrates
- RhoA/ROCK signalling is closely involved in regulating cell morphology, growth, migration, and apoptosis
- Activated GTP-bound RhoA can further activate ROCK to phosphorylate several substrates
  - Substrates involved in autophagy, cell survival & apoptosis, vesicle dynamics, cytoskeleton regulation, cell growth & regeneration, cell shape & motility

Two isoforms of ROCK, ROCK1 (ROCKβ) and ROCK2 (ROCKα)

Selective ROCK isoform inhibition is challenging because of high degree of sequence homology
- 65% amino acid sequences in common
- 92% homology within their kinase domains

ROCK1 and ROCK2 widely expressed in tissues of embryos and adults

ROCK1 and ROCK2 are ubiquitously expressed in different tissues.
- ROCK1 mRNA is highly expressed in the lungs, liver, spleen, kidneys, and testis
- ROCK2 mRNA is enhanced in the brain, heart, lung and skeletal muscle
- Both isoforms expressed in endothelial cells (ECs)
  - ROCK1 mainly distributed in the plasma membrane
  - ROCK2 localized in cytoplasm

Simultaneous inhibition of ROCK1 and ROCK2 induce hypotension

ROCK2 is overexpressed in key diseases

Koch et al. Pharmacol Ther. 2018 Sep;189:1-21
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

ROCK2 inhibition could target many diseases, highlighted by clinical validation across multiple organs in cGvHD

ROCK2 selective inhibitors for the treatment of fibrosis
Selective ROCK2 inhibitors can inhibit fibrosis without inducing hypotension associated with ROCK1/2 inhibitors

- ROCK1/2 inhibitors deliver an anti-fibrotic effect in preclinical studies but induce hypotension, limiting further clinical development
- Selective ROCK2 inhibitors can inhibit fibrosis without inducing hypotension
- No cardiovascular events highlighted from PhI or PhII clinical trials with selective ROCK2i KD025

**Hypotension is induced by a ROCK1/2 inhibitor in rats**

**Redx ROCK2i has no impact on mean blood pressure in rats**

*Effect of a single oral treatment of azaindole 1 (0, 3, 10 mg/kg) on mean arterial blood pressure in normotensive rats. N=6, data are % change from baseline. British Journal of Pharmacology (2007) 152, 1070–1080.*
Redx ROCK2 inhibitors are potent and have greater than 100-fold selectivity over ROCK1 in biochemical assays

![Graphs showing percentage inhibition of ROCK1 and ROCK2 for different inhibitors]

Note: data are all from n≥2

<table>
<thead>
<tr>
<th>Assay</th>
<th>REDX10178 IC$_{50}$ (µM)</th>
<th>REDX10616 IC$_{50}$ (µM)</th>
<th>REDX10843 IC$_{50}$ (µM)</th>
<th>REDX10842 IC$_{50}$ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biochemical Activity ROCK2 [ATP 20 µM]</td>
<td>0.002</td>
<td>0.004</td>
<td>0.017</td>
<td>0.006</td>
</tr>
<tr>
<td>Biochemical Activity ROCK1 [ATP 20 µM]</td>
<td>0.2</td>
<td>3.0</td>
<td>2.5</td>
<td>2.2</td>
</tr>
<tr>
<td>Fold selectivity ROCK2/ROCK1</td>
<td>90-fold</td>
<td>730-fold</td>
<td>150-fold</td>
<td>&gt;300-fold</td>
</tr>
</tbody>
</table>
Redx ROCK2 inhibitors are potent and highly selective in cellular mechanistic assays

- MCF7 cell line expresses both ROCK1 and ROCK2 isoforms (parental line)
- ROCK1 or ROCK2 stably knocked down using shRNA to develop cell lines selective for each ROCK isoform
- ROCK inhibition in cells analysed by the inhibition of pMYPT1, downstream of ROCK signalling

<table>
<thead>
<tr>
<th>Assay</th>
<th>REDX10178 IC₅₀ (µM)</th>
<th>REDX10616 IC₅₀ (µM)</th>
<th>REDX10843 IC₅₀ (µM)</th>
<th>REDX10842 IC₅₀ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellular activity ROCK2</td>
<td>0.9</td>
<td>1.0</td>
<td>2.4</td>
<td>1.3</td>
</tr>
<tr>
<td>Cellular activity ROCK1</td>
<td>20</td>
<td>&gt; 30</td>
<td>26</td>
<td>&gt; 30</td>
</tr>
</tbody>
</table>
Redx ROCK2 inhibitors reduce pro-fibrotic and pro-inflammatory activity of kidney mesangial cells cultured in high glucose

- Protein expression of secreted 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

### Assay

<table>
<thead>
<tr>
<th>Assay</th>
<th>REDX10178 IC(_{50}) (µM)</th>
<th>REDX10616 IC(_{50}) (µM)</th>
<th>REDX10843 IC(_{50}) (µM)</th>
<th>REDX10842 IC(_{50}) (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenotypic activity TIMP-1 Mouse mesangial cells</td>
<td>0.2</td>
<td>0.9</td>
<td>2.8</td>
<td>0.9</td>
</tr>
<tr>
<td>Phenotypic activity PDGF-BB Mouse mesangial cells</td>
<td>0.2</td>
<td>0.4</td>
<td>1.4</td>
<td>0.8</td>
</tr>
<tr>
<td>Phenotypic activity MCP-1 Mouse mesangial cells</td>
<td>0.3</td>
<td>1.3</td>
<td>2.1</td>
<td>0.7</td>
</tr>
<tr>
<td>Phenotypic activity CTGF Mouse mesangial cells</td>
<td>0.4</td>
<td>0.4</td>
<td>1.5</td>
<td>ND</td>
</tr>
</tbody>
</table>

Glucose (25 mM) – refreshed on day 3 and 6

Day 0 1 2 3 4 5 6 7 8

Harvest supernatant

Compound treatment

Phenotypic activity TIMP-1 Mouse mesangial cells

Phenotypic activity PDGF-BB Mouse mesangial cells

Phenotypic activity MCP-1 Mouse mesangial cells

Phenotypic activity CTGF Mouse mesangial cells

5 Day 0 2 4 6 8
Selective ROCK2 inhibitors reverse the myofibroblast phenotype of activated human hepatic stellate cell myofibroblasts

- ROCK signalling central to the mechanosensing of the ECM tension
- 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
  - Phenotype and activation status confirmed by expression of α-SMA
- Selective ROCK2 inhibitors suppress α-SMA in the LX2-MF cells – suggesting a reversal of the myofibroblast like phenotype
- No toxicity was observed with compounds (up to 10 µM)
REDX10842 is highly selective when tested against 468 kinases at 10 µM

- 20 targets inhibited with > 50% of control by 10 µM of REDX10842
- Kinases with IC$_{50}$ values < 3 µM assayed at $K_m$ [ATP] marked with circles
- ROCK2, the most potently inhibited kinase, is denoted with a blue circle
- IC$_{50}$ values of kinases inhibited > 50% by 10 µM of REDX10842 with IC$_{50}$ < 30 µM are reported in the table
REDX10842 is highly selective when tested in a CEREP SafetyScreen panel at 10 µM

- 7 targets inhibited with more than 25%
- Follow up IC$_{50}$: PDE3A IC$_{50}$ 23 µM

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDE3A (h)</td>
<td>60</td>
</tr>
<tr>
<td>dopamine transporter (h) (agonist radioligand)</td>
<td>51</td>
</tr>
<tr>
<td>5-HT transporter (h) (agonist radioligand)</td>
<td>51</td>
</tr>
<tr>
<td>COX2(h)</td>
<td>49</td>
</tr>
<tr>
<td>delta (DOP) (h) (agonist radioligand)</td>
<td>30</td>
</tr>
<tr>
<td>CCK1 (CCKA) (h) (agonist radioligand)</td>
<td>27</td>
</tr>
<tr>
<td>A2A (h) (agonist radioligand)</td>
<td>25</td>
</tr>
</tbody>
</table>
Effect of ROCK2 selective inhibitors in a murine bleomycin-induced IPF model

Murine bleomycin-induced IPF model
- REDX10843 dosed therapeutically in the murine bleomycin induced lung fibrosis model at 50 mg/kg BID
- Pirfenidone used as positive control and dosed at 100 mg/kg BID
- Oropharyngeal administration of 1.5 U/kg bleomycin on day 1, compound dosing initiated from day 7-21

Murine bleomycin-induced IPF model
- REDX10842 dosed therapeutically in the murine bleomycin induced lung fibrosis model at 5, 20 and 50 mg/kg BID
- Pirfenidone used as positive control and dosed at 100 mg/kg BID
- Oropharyngeal administration of 1.5 U/kg bleomycin on day 1, compound dosing initiated from day 7-21
REDX10843 reduces fibrosis and collagen deposition in the lung in murine bleomycin-induced IPF model

**Ashcroft score**

- No Bleomycin
- Vehicle BID
- REDX10843 50 mg/kg BID
- Pirfenidone 100 mg/kg BID

**Masson’s trichrome score**

- No Bleomycin
- Vehicle BID
- REDX10843 50 mg/kg BID
- Pirfenidone 100 mg/kg BID

**BALF Collagen**

- No Bleomycin
- Vehicle BID
- REDX10843 50 mg/kg BID
- Pirfenidone 100 mg/kg BID

ROCK2 selective inhibitors for the treatment of fibrosis
REDX10843 suppresses pro-fibrotic and pro-inflammatory gene expression in lung and plasma in murine bleomycin-induced IPF model.

**PD lung gene expression**

- **FN1**
- **COL1A1**
- **MMP2**
- **TIMP-1**
- **CTGF**
- **TGFβ**
- **IL-6**
- **MCP-1**

**Reduction from vehicle (%)**

<table>
<thead>
<tr>
<th>Gene</th>
<th>REDX10843 50 mg/kg BID</th>
<th>Pirfenidone 100 mg/kg BID</th>
</tr>
</thead>
<tbody>
<tr>
<td>FN1</td>
<td>*</td>
<td>***</td>
</tr>
<tr>
<td>COL1A1</td>
<td>*</td>
<td>**</td>
</tr>
<tr>
<td>MMP2</td>
<td></td>
<td>***</td>
</tr>
<tr>
<td>TIMP-1</td>
<td>**</td>
<td>***</td>
</tr>
<tr>
<td>CTGF</td>
<td>*</td>
<td>***</td>
</tr>
<tr>
<td>TGFβ</td>
<td></td>
<td>**</td>
</tr>
<tr>
<td>IL-6</td>
<td>*</td>
<td>***</td>
</tr>
<tr>
<td>MCP-1</td>
<td>*</td>
<td>**</td>
</tr>
</tbody>
</table>

**Plasma PD biomarkers**

- **ICAM-1**
- **WISP1**
- **S100A9**
- **TIMP-1**
- **MMP2**
- **MMP3**
- **PDGF-BB**
- **HGF**

**Reduction from vehicle (%)**

<table>
<thead>
<tr>
<th>Plasma biomarker</th>
<th>REDX10843 50 mg/kg BID</th>
<th>Pirfenidone 100 mg/kg BID</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICAM-1</td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>WISP1</td>
<td>*</td>
<td>**</td>
</tr>
<tr>
<td>S100A9</td>
<td>*</td>
<td>**</td>
</tr>
<tr>
<td>TIMP-1</td>
<td></td>
<td>**</td>
</tr>
<tr>
<td>MMP2</td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>MMP3</td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>PDGF-BB</td>
<td>*</td>
<td>**</td>
</tr>
<tr>
<td>HGF</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

ROCK2 selective inhibitors for the treatment of fibrosis
REDX10842 reduces fibrosis and collagen deposition in the lung in murine bleomycin-induced IPF model

**Ashcroft score**

- No bleomycin: Higher score, indicating more fibrosis.
- Vehicle: Intermediate score.
- REDX10842 50 mg/kg BID: Lower score, indicating reduced fibrosis.

**Masson’s Trichrome**

- No bleomycin: Higher score, indicating more collagen deposition.
- Vehicle: Intermediate score.
- REDX10842 50 mg/kg BID: Lower score, indicating reduced collagen deposition.

Statistical significance:
- **p=0.07**
- **p=0.08**
REDX10842 suppresses pro-fibrotic and pro-inflammatory gene expression in lung, BAL and plasma in murine bleomycin-induced IPF model

ROCK2 selective inhibitors for the treatment of fibrosis
Effect of ROCK2 selective inhibitors in a murine unilateral ureteral obstruction (UUO) model

Murine unilateral ureteral obstruction (UUO) model

- REDX10843 dosed therapeutically in the unilateral ureteral obstruction (UUO) murine model at 50 mg/kg BID
- Surgery performed on day 0, compound dosing from day 6-11
**REDX10843** reduces kidney tubular damage and atrophy in murine UUO model

- Tubular autofluorescence loss correlates with the break down of energy supply and is a damage marker for tubuli.
- Masson’s trichrome measure indicates tubular atrophy
- Enhanced tubular cell survival and reduced damage as measured by auto-fluorescence
- REDX10843 significantly reduced tubular damage

![Graphs showing Masson’s trichrome and tubular damage](image)

**Sham vehicle**

**UUO vehicle**

**UUO REDX10843 d6-11**
REDX10843 modulates collagen deposition, macrophage infiltration and markers of tissue injury, inflammation and fibrosis in murine UUO model

- REDX10843 reduced medulla collagen deposition, shown by a reduction in Sirius Red
- REDX10843 also reduced the macrophage infiltration score in the kidney medulla
- REDX10843 modulates gene expression markers of tissue injury, inflammation and fibrosis
Effect of ROCK2 selective inhibitors in a murine liver models

**Murine STAM NASH liver model**
- REDX10843 dosed therapeutically in the murine STAM NASH model at 50 mg/kg BID or 50 mg/kg QD
- Telmisartan used as positive control and dosed at 10 mg/kg QD
- STZ administration at day 2, HFD induced from week 4, compounds dosed weeks 6-9

**Murine CCl_4-induced liver model**
- REDX10842 dosed therapeutically in the murine CCl_4-induced liver model at 5, 20 and 50 mg/kg BID
- CCl_4 administered twice weekly IP
- Compounds dosed therapeutically from week 2 to 6
REDX10843 dosed QD or BID significantly reduces fibrosis area in a murine STAM NASH model

Fibrosis area (Sirius Red)

Vehicle QD  | Vehicle BID  | REDX10843 50 mg/kg QD  | REDX10843 50 mg/kg BID  | Telimistatan
--- | --- | --- | --- | ---
0.0  | 0.5  | 1.0  | 1.5  | 2.0

- **: Significantly different from control
- ***: Significantly different from Vehicle QD

**Notes:**
- ROCK2 selective inhibitors for the treatment of fibrosis
- REDX10843 50 mg/kg QD
- REDX10843 50 mg/kg BID
- Telimistatan

**Images:**
- Sirius Red positive area (%)
- Vehicle QD, Vehicle BID, REDX10843-QD, REDX10843-BID, Telimistatan
- Normal
REDX10843, dosed QD or BID, reduces profibrotic fibroblasts in a murine STAM NASH model
REDX10842 reduces fibrosis and collagen deposition in the liver in murine CCl₄-induced liver model

**Hydroxyproline**

- No CCl₄
- Vehicle BID
- REDX10842 5 mg/kg BID
- REDX10842 20 mg/kg BID
- REDX10842 50 mg/kg BID

**Ishak Score**

- No CCl₄
- Vehicle BID
- REDX10842 5 mg/kg BID
- REDX10842 20 mg/kg BID
- REDX10842 50 mg/kg BID

**Sirius Red (collagen I&III)**

- No CCl₄
- Vehicle BID
- REDX10842 5 mg/kg BID
- REDX10842 20 mg/kg BID
- REDX10842 50 mg/kg BID

*Significant difference compared to vehicle (p<0.05).
**High significance (p<0.01).
REDX10842 reduces pro-fibrotic and pro-inflammatory gene expression in the liver in murine CCl₄-induced liver model

PD liver gene expression

<table>
<thead>
<tr>
<th>Gene</th>
<th>Reduction from vehicle (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNFα</td>
<td></td>
</tr>
<tr>
<td>Bcl2</td>
<td></td>
</tr>
<tr>
<td>PDGF-BB</td>
<td></td>
</tr>
<tr>
<td>CXCL10</td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td></td>
</tr>
<tr>
<td>WISP-1</td>
<td></td>
</tr>
<tr>
<td>CTGF</td>
<td></td>
</tr>
</tbody>
</table>

PD plasma gene expression

<table>
<thead>
<tr>
<th>Gene</th>
<th>Reduction from vehicle (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT</td>
<td></td>
</tr>
<tr>
<td>S100A9</td>
<td></td>
</tr>
<tr>
<td>MMP8</td>
<td></td>
</tr>
<tr>
<td>MMP9</td>
<td></td>
</tr>
<tr>
<td>TIMP-1</td>
<td></td>
</tr>
</tbody>
</table>

ROCK2 selective inhibitors for the treatment of fibrosis
Selective inhibition of ROCK2 is an exciting approach to target fibrosis

Redx series has a good preclinical profile

Potent and highly selective ROCK2 inhibitors against ROCK1 and against a panel of kinases and other receptor targets

Redx ROCK2 inhibitors suppress pathways associated with fibrosis in *in vitro* kidney and liver models

Early PK/PD evidence of target engagement of physiologically relevant pathways for fibrosis

Robust preclinical efficacy demonstrated with REDX10843 and REDX10842 in murine liver, kidney and lung fibrosis models

No safety issues observed in preliminary *in vitro* studies (cardiotoxicity, genotoxicity, mutagenicity, CYP profile) and *in vivo* rodent toxicology studies (14-day and CV)

Redx plan to select an orally administered ROCK2 selective development candidate in H2 2019 and aim to enter clinical trials in 2021
Acknowledgments

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Thank You