Redx Pharma
(LON:REDX)
Focused on taking targeted oncology and fibrosis medicines into the clinic

ROCK2 inhibitors for the treatment of NASH
Agenda

• Executive Summary
• ROCK2, a central node in fibrosis pathology
• ROCK2 inhibitors – *in vitro* selectivity and fibrosis assays
• CV safety study with tool compound REDX10178
• REDX10178 and REDX10616 – Suitable ROCK2 selective probes for *in vivo* studies
## Redx pipeline
Highly selective targeted products for cancer & fibrosis

<table>
<thead>
<tr>
<th>Target/Product</th>
<th>Primary Focus</th>
<th>Research</th>
<th>Preclinical (CTA/IND enabling)</th>
<th>Clinical (Phase 1)</th>
<th>Milestone Date</th>
</tr>
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<tbody>
<tr>
<td>RXC004 Porcupine</td>
<td>Combination with PD-1/PD-L1 in solid tumour (colorectal cancer)</td>
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<td>Phase 1 safety completion – H1 20</td>
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<td>Porcupine (RXC006)</td>
<td>Idiopathic pulmonary fibrosis (IPF)</td>
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<td>Preclinical 2019 Clinic ready 2020</td>
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<tr>
<td>Anti-fibrotics</td>
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<td></td>
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<td>Preclinical development candidate H2 19 Clinic ready H2 20</td>
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<tr>
<td>ROCK2 selective</td>
<td>Non-alcoholic Steatohepatitis (NASH)</td>
<td></td>
<td></td>
<td></td>
<td>Preclinical development candidate H2 19 Clinic ready H2 20</td>
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<tr>
<td>GI-targeted ROCK</td>
<td>Crohn’s Related Fibrosis</td>
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<td>Preclinical development candidate H2 19 Clinic ready H2 20</td>
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<tr>
<td>Research</td>
<td>Validated targets</td>
<td>Oncology and Fibrosis</td>
<td></td>
<td></td>
<td>Lead Optimisation with development candidate in 2020</td>
</tr>
</tbody>
</table>
Anti-fibrotic pillar: Selective ROCK2 inhibitor programme

ROCK2 target

• ROCK is central to cellular pathways associated with aberrant wound healing and fibrosis.
• ROCK pathway inhibition decreases fibrosis \textit{in vivo} and fibrotic markers \textit{in vitro}.
• ROCK2 is upregulated in diseases associated with acute and chronic inflammation, for example where damage is caused by high glucose and high fat diets.
• ROCK2 haplotype KO mice are protected from bleomycin-induced fibrosis.
• ROCK2 inhibition decreases disease severity in patients with IPF.

Programme highlights

• Highly selective ROCK2 compounds identified against ROCK1 and across kinase panel.
• Potent compounds in biochemical, mechanistic and disease-relevant phenotypic cellular assays.
• Orally available compounds with good PK.
• Demonstrated \textit{in vivo} efficacy in independent models of fibrosis.
• Composition of matter patent filed.
ROCK2 is central to disease processes driving fibrosis pathology

- ↑ROCK2 activity in diabetic endothelium
- ROCK2+/- protected against loss of insulin sensitivity (HFD) & diabetes induced hypertension
- ↑ROCK2 in liver fibrosis models
- ↑ROCK2 signalling drives HSC activation
- ROCK2 inhibition = ↓ liver fibrosis

- ↑ROCK2 in patients with PAH
- ROCK2 conditional KO = ↓ hypertension, hypertrophy & atherosclerosis

- ROCK inhibitors = ↓ fibrosis in lung, liver, kidney and heart fibrosis models
- ROCK2+/- protected lung fibrosis
- ROCK2 inhibitor = ↓ fibrosis in cGVHD

- ↑ROCK2 in acute inflammation
- ROCK inhibitors = ↓ pro-inflammatory response

**ROCK1 & ROCK2 control endothelial vascular tone. Targeting only ROCK2 does not induce hypotension.**
ROCK2 inhibitors for the treatment of NASH | NASH Summit | 22-25 April, 2019
In vitro selectivity and fibrosis assays
Redx ROCK2 inhibitors are potent and selective in biochemical assays

ROCK2 compounds have greater than 100 fold selectivity over ROCK1 in biochemical assay and are highly selective against a panel of 468 kinases and 44 other receptor targets

<table>
<thead>
<tr>
<th>Assay / IC₅₀ (µM)</th>
<th>REDX10178</th>
<th>REDX10616</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biochemical Activity ROCK2</td>
<td>0.002</td>
<td>0.004</td>
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<tr>
<td>Biochemical Activity ROCK1</td>
<td>0.9</td>
<td>1.0</td>
</tr>
<tr>
<td>CEREP SafetyScreen44™ at 10 µM</td>
<td>1 target &gt; 50% inhibition</td>
<td>1 target &gt; 50% inhibition</td>
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</tbody>
</table>

Waterfall plot analysis of kinase panel of 468 kinases for REDX10178 at 1 µM.

Waterfall plot analysis of kinase panel of 422 kinases for REDX10616 at 10 µM.
Redx ROCK2 inhibitors are potent and selective in cellular mechanistic assays

ROCK2 compounds are potent and selective in cellular mechanistic assays

**ROCK cellular signalling**

A: ROCK expression in MCF7 cell lines

- Stable knockdown of ROCK1 or ROCK2 in MCF7 cells using lentiviral shRNA.

B: pMYPT1 mechanistic assay

- ROCK1 KD (IC₅₀ 0.9 µM)
- ROCK2 KD (IC₅₀ 20 µM)
- ROCK1 KD (IC₅₀ 1 µM)
- ROCK2 KD (IC₅₀ >30 µM)
Redx ROCK2 inhibitors are potent in high glucose kidney phenotypic assay

Redx ROCK2 inhibitors reduce pro-fibrotic and pro-inflammatory activity of kidney mesangial cells cultured in high glucose

**Assay schematic**

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

<table>
<thead>
<tr>
<th>Assay / IC$_{50}$ (µM)</th>
<th>REDX10178</th>
<th>REDX10616</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCF7 ROCK1 KD pMYPT1</td>
<td>0.9</td>
<td>1.0</td>
</tr>
<tr>
<td>Mesangial cells - CTGF</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Mesangial cells – TIMP-1</td>
<td>0.2</td>
<td>0.9</td>
</tr>
<tr>
<td>Mesangial cells – PDGF-BB</td>
<td>0.2</td>
<td>0.4</td>
</tr>
<tr>
<td>Mesangial cells – MCP-1</td>
<td>0.3</td>
<td>1.3</td>
</tr>
<tr>
<td>Mesangial cells – MMP-2</td>
<td>1.2</td>
<td>2.2</td>
</tr>
</tbody>
</table>

**CTGF release from kidney mesangial cells**

- Protein expression of secreted CTGF, PDGF-BB, TIMP-1, MCP-1 and MMP-2 detected in the culture media.
ROCK2 inhibitors reduce markers of fibrosis in liver in vitro models

Primary human hepatic stellate cells (HSC)

- TGFB (5 ng/mL)
- Day 0 1 2 3 4 5 6
- Compound treatment 4 h
- Activation confirmed with aSMA expression
- Harvest cells for qPCR

Gene expression analysis

A: TGFB

B: ACTA2

C: Fibronectin

D: Bcl-2

E: Col1a1

- DMSO only
- REDX10178 1 µM

Primary human hepatocytes

- Primary hepatocytes were incubated with oleic acid ± compound.
- Cells were incubated for 48 h to allow lipid accumulation.
- Lipids were stained with Nile red probe and measured by flow cytometry.

Hepatocyte lipid accumulation

  - Gene expression of αSMA, collagen 1, TGFB, fibronectin and Bcl2 inhibited by selective ROCK2 inhibitor.
- Redx ROCK2 inhibitors reduce lipid accumulation in primary human hepatocytes in vitro.
Hepatic stellate cells are activated by stiff matrix and drive liver fibrosis

Differentiated and activated hepatic stellate cells are a key driver of fibrosis in the liver as the activated myofibroblast cells secrete profibrotic cytokines and generate extracellular matrix (ECM).

Sensing matrix stiffness is a major driver of HSC activation and is self-perpetuating in a feed-forward mechanism.

ROCK signaling is central to the mechanosensing of ECM and the generation of actin and myosin stress fibres that promote the contractile HSC-myofibroblast phenotype that drives fibrosis.
**In vitro** liver fibrosis assay – HSC activated to myofibroblast phenotype

**Experiment time course**

- HSC cell line (LX2) cultured for 2-3 weeks on plastic to induce differentiation into myofibroblasts (LX2-MF);
  - Phenotype and activation status confirmed by expression of αSMA.
- LX2-MF are plated for assay and allowed 48 -72 h to recover and re-organise stress fibres.
- Redx ROCK2 inhibitors are dosed for 48 h and expression of αSMA (with DRAQ5 as nuclear stain) is detected by immunocytochemistry.
- No exogenous stimuli; cells are activated by matrix stiffness and autocrine factors, mimicking *in vivo* conditions.
Rock2 inhibitors reduce markers of fibrosis in human liver in vitro models

Human hepatic stellate cell myofibroblasts (LX2-MF)

A: Expression of αSMA

B: Mean expression of αSMA in LX2 myofibroblasts

- Selective ROCK2 inhibitors reverse myofibroblast phenotype of activated human hepatic stellate cell myofibroblasts.
  - Redx ROCK2 inhibitors, dosed for 48 h, reduce the expression of αSMA in these cells, indicating suppression of the myofibroblast phenotype.
  - No toxicity was observed with compounds (up to 10 µM).

<table>
<thead>
<tr>
<th>Assay / IC₅₀ (µM)</th>
<th>REDX10178</th>
<th>REDX10616</th>
</tr>
</thead>
<tbody>
<tr>
<td>LX2-MF αSMA assay</td>
<td>ND</td>
<td>0.2</td>
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</table>
CV safety study with tool compound REDX10178
Analysis of cardiac safety in telemetered Han Wistar rats

Example of hypotension induced by a pan-ROCK inhibitor

Study design

- REDX10178 at 100 mg/kg in normotensive rats.
- Systemic exposure over cellular MOA assay (pMYPT1) IC$_{50}$ for ~10 h (free concentration over IC$_{50}$ pMYPT1 of 1-1.6).
- N=6 Han Wistar telemetered rats – crossover design.
- Animals dosed PO with vehicle or REDX10178 and monitored over 24 h.
- Analysis of HR, BP, activity and temperature.

Example of hypotension induced by a pan-ROCK inhibitor

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.
REDX10178 has no impact on cardiac parameters in telemetered rats

The pleiotropic effects of ROCK inhibition have previously raised concerns about on-target adverse effects such as hypotension. With REDX10178, Δ 5 mmHg (4%) mean blood pressure and 10-20 bpm (4-8%) in heart rate were observed which are not biologically significant. These data are consistent with clinical selective ROCK2 inhibitor KD025, that has shown no CV effects in clinical trials.

Data are plotted LS mean ±SEM n=6 animals. Statistical effect of treatment analysed by one way ANOVA with Fisher’s LSD post test, compared to vehicle treated animals, *p<0.05.

References:
In vivo efficacy studies
REDX10178 and REDX10616 are suitable probe for *in vivo* studies

<table>
<thead>
<tr>
<th>Mouse PK parameters</th>
<th>REDX10178</th>
<th></th>
<th>REDX10616</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Route</td>
<td>IV</td>
<td>PO</td>
<td>PO</td>
<td>IV</td>
</tr>
<tr>
<td>Dose (mg/kg)</td>
<td>1</td>
<td>10</td>
<td>30</td>
<td>2</td>
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<tr>
<td>$C_{max}$ (µM)</td>
<td>1.9</td>
<td>4.6</td>
<td>13</td>
<td>5.2</td>
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<tr>
<td>Plasma protein binding % unbound (%Fu)</td>
<td>5.7</td>
<td>5.7</td>
<td>5.7</td>
<td>8.8</td>
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<tr>
<td>Unbound $C_{max}$ (µM)</td>
<td>0.01</td>
<td>0.03</td>
<td>0.08</td>
<td>0.05</td>
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<tr>
<td>$T_{max}$ (h)</td>
<td>-</td>
<td>0.5</td>
<td>2.0</td>
<td>-</td>
</tr>
<tr>
<td>$t_{1/2}$ (h)</td>
<td>1.7</td>
<td>4.0</td>
<td>3.5</td>
<td>3.6</td>
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<tr>
<td>$V_{dss}$ (L/kg)</td>
<td>0.8</td>
<td>-</td>
<td>-</td>
<td>1.2</td>
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<tr>
<td>CL (mL/min/kg)</td>
<td>5.3</td>
<td>-</td>
<td>-</td>
<td>14</td>
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<tr>
<td>% LBF</td>
<td>3.0</td>
<td>-</td>
<td>-</td>
<td>20</td>
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<tr>
<td>AUC$^{0-t}$ (h.µM)</td>
<td>6.3</td>
<td>26</td>
<td>74</td>
<td>5.1</td>
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<tr>
<td>Unbound AUC$^{0-t}$ (h.µM)</td>
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<td>0.2</td>
<td>0.4</td>
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<tr>
<td>Bioavailability (%)</td>
<td>-</td>
<td>55</td>
<td>39</td>
<td>-</td>
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</table>
Redx ROCK2 inhibitors suppresses fibrosis pathways

**Acute kidney injury model**

- Cisplatin induces acute toxicity in the kidney and induces an upregulation of ROCK2, with no change in ROCK1 expression.
- Toxicity is typically induced by an inflammatory phase with leukocyte infiltration followed by tissue remodelling - acutely engaging the signalling pathways active in chronic fibrosis.
- Cisplatin injury has been reported to induce apoptosis in podocytes and damage to kidneys.
- We have used this acute model to look at fibrosis pathway engagement and PK/PD relationships.

Data are plotted mean ± SEM n=4 animals. Analysed by t-test, *p<0.05.
Redx ROCK2 inhibitors suppress fibrosis pathways *in vivo* in a murine AKI model

Pro-inflammatory and pro-fibrotic gene expression change following REDX10178 treatment (BID)

**P value summary table**

<table>
<thead>
<tr>
<th>Gene of interest</th>
<th>10 mg/kg BID</th>
<th>30 mg/kg BID</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCP-1</td>
<td>*</td>
<td>***</td>
</tr>
<tr>
<td>TNFa</td>
<td>0.08</td>
<td>**</td>
</tr>
<tr>
<td>IL-1β</td>
<td>**</td>
<td>***</td>
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<tr>
<td>ICAM-1</td>
<td>0.4</td>
<td>**</td>
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<tr>
<td>IL-6</td>
<td>0.6</td>
<td>*</td>
</tr>
<tr>
<td>TGFβ</td>
<td>0.9</td>
<td>***</td>
</tr>
<tr>
<td>CTGF</td>
<td>0.4</td>
<td>**</td>
</tr>
<tr>
<td>TIMP-1</td>
<td>0.9</td>
<td>*</td>
</tr>
<tr>
<td>MMP-2</td>
<td>**</td>
<td>***</td>
</tr>
<tr>
<td>MMP-3</td>
<td>0.3</td>
<td>*</td>
</tr>
<tr>
<td>Collagen 1</td>
<td>0.3</td>
<td>*</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>0.1</td>
<td>***</td>
</tr>
<tr>
<td>Nephrin</td>
<td>0.1</td>
<td>**</td>
</tr>
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</table>

% change treated REDX10178 vs. vehicle
Redx ROCK2 inhibitors suppress fibrosis pathways *in vivo* in a murine AKI model

Pro-inflammatory and pro-fibrotic gene expression change following REDX10616 treatment (QD)

P value summary table

<table>
<thead>
<tr>
<th>Gene of interest</th>
<th>10 mg/kg QD</th>
<th>50 mg/kg QD</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCP-1</td>
<td>0.05</td>
<td>**</td>
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<tr>
<td>TNFa</td>
<td>0.09</td>
<td>**</td>
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<tr>
<td>IL-1β</td>
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<tr>
<td>ICAM-1</td>
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<tr>
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<td>TGFβ</td>
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<td>CTGF</td>
<td>0.9</td>
<td>0.2</td>
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<tr>
<td>TIMP-1</td>
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<tr>
<td>MMP-2</td>
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<td>MMP-3</td>
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<tr>
<td>Collagen 1</td>
<td>0.4</td>
<td>**</td>
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<tr>
<td>Fibronectin</td>
<td>0.09</td>
<td>***</td>
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<tr>
<td>Bcl2</td>
<td>0.2</td>
<td>**</td>
</tr>
<tr>
<td>Nephrin</td>
<td>0.2</td>
<td>*</td>
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</tbody>
</table>

**Note:** The % change treated REDX10616 vs. vehicle is shown for each gene of interest.
Redx ROCK2 inhibitor program summary

- ROCK2 inhibition is a promising approach
- Redx series shows 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.
  - Demonstrated CV safety in telemetered rats with REDX10178.
  - No safety issues observed in preliminary *in vitro* studies (hERG, CEREP, mini-AMES, micronucleus).
- Early PK/PD evidence of target engagement of physiologically relevant pathways for fibrosis.
  - Gene expression analysis in whole kidneys shows cisplatin-induced kidney damage is modulated with REDX10178 and REDX10616 in a dose-dependent manner.
  - Data suggest inhibition of ROCK2 protects the kidney from cisplatin damage (Jagged-1, Nephrin, Bcl-2).
  - Suppression of cytokine expression together with ICAM-1 (recruitment of leukocytes) with REDX10178 and REDX10616.
  - These pathways are also associated with chronic fibrosis providing some early evidence that REDX10178 and REDX10616 modulates physiologically relevant fibrosis pathways in this acute setting.
- Redx’s highly selective ROCK2 lead compound shows robust preclinical efficacy in murine liver, kidney and lung fibrosis models (undisclosed data)
- Candidate selection by mid 2019, and to enter IND enabling studies in 2019.
Acknowledgments

Core Team
• Stuart Best
• Philip MacFaul
• Emily Offer

Biology
• Sara Ceccarelli
• Rosie Knowles
• Rebecca Holland
• Kay Eckersley

DMPK
• Alison Hunter
• Amy Cooke
• Andrew Taylor
• Rebecca Taylor

Chemistry
• Andrew Belfield
• Matthew Box
• Chiara Colletto
• Charles Crossland
• Neil Hawkins
• Jean Marc Henry
• Steven Glossop
• Marcin Odachowski
• Sam Smith

Management
• Richard Armer
• Peter Bunyard
• Clifford Jones
Thank you for your attention