Novel LOXL2 selective small molecule inhibitors reduce cross-link formation and alter matrix properties in an in vitro fibroblastic foci model of IPF

Mr. James Roberts (Synairgen research limited)
Mrs. Kerry Lunn (Synairgen research limited)
Dr. Mark Jones (Synairgen research limited)
Mrs. Rona Beegan (Synairgen research limited)
Dr. James Roberts (Synairgen research limited)
Dr. Ponniah Rangaswamy (Synairgen research limited)
Dr. Jonathan McQuilter (Cedars-Sinai Medical Center)
Mr. Alex Wilkinson (Pharmaxis Ltd)
Mrs. Elaine Gower (GlaxoSmithKline)
Dr. Lucy Cao (Pharmaxis Ltd)
Dr. Philip Monk (Synairgen research limited)

Introduction: The lung tissue of patients with idiopathic pulmonary fibrosis (IPF) is characterised by dense collections of myofibroblasts and extracellular matrix (ECM) termed ‘fibroblastic foci’. Using a novel in vitro model of fibroblastic foci (Jones et al., AJRCCM 191;2015:A4912) we have characterised the formation of lysyl-oxidase (LOX) mediated collagen cross-links and profiled the effects of the non-selective LOX inhibitor BAPN and LOXL2-selective inhibitors. LOXL2 is an attractive drug target as its expression is increased in IPF (Nat Med 2010;16;9:1009-1018) and increased serum LOXL2 is associated with more rapid disease progression (ERJ 2014;43:1430-1438).

Methods: Lung fibroblasts were obtained from IPF patients and seeded onto transwells under optimised conditions for mature collagen matrix deposition in the presence of BAPN or LOXL2-selective inhibitors. Following treatment with transforming growth factor β1 (TGF-β1) multicellular foci formed which were histochemically similar in organization to fibroblastic foci in vivo.

Results: The number of immature and mature LOX family-mediated collagen crosslinks increased over the 6 week duration of the model. Both BAPN and the LOXL2-selective inhibitors dose-dependently reduced cross-link formation. Histochemical analysis and second harmonic generation (SHG) imaging revealed that collagen fibrils were less organised in these cultures compared to controls.

Conclusions: We have shown that LOXL2 selective inhibitors can reduce cross-link formation in a fibroblastic foci model of IPF demonstrating their potential as a novel treatment for IPF. It is hypothesised that reducing collagen cross-linking will reduce tissue stiffness, tipping the balance in favour of matrix breakdown over deposition, beneficially altering the course of disease.
fluorescence-activated cell sorting strategy to fractionate enzymatically dissociated human lung tissue for analysis of the molecular phenotype and function of epithelial progenitor cells. Epithelial cells were isolated from distal lung tissue based upon their CD45- CD31- CD326+ surface phenotype and captured using a Fluidigm C1 microfluidic system for single-cell whole-transcriptome analysis. RNA-Seq analysis from normal versus IPF tissue revealed decreased expression of alveolar type II (AT2) cell markers in IPF relative to normal, yet simultaneously induced expression of proximal "basal" epithelial cell markers including TP63, KRT5, and KRT14. Similar analysis performed using epithelial cells isolated from normal bronchial airway tissue identified distinct basal cell subsets that preliminary data suggests are functionally different in their capacity for proliferation and differentiation in vitro. Ongoing studies are investigating the role of these unique subsets in the pathogenesis of interstitial lung diseases.

P111
Cationic conductances in human pulmonary fibroblasts
Dr. Luke Janssen (McMaster University)
Dr. Mozibur Rahman (McMaster University)
Dr. Subhendu Mukherjee (McMaster University)

Patch-clamp studies were performed in human pulmonary fibroblasts (HPF) at rest and during stimulation with TGFβ, hyposmotic media (to simulate stretch), and heat. We obtained evidence for several distinct cationic conductances:

1. a large K+ current sensitive to TEA (10 mM) or paxillin (1 μM); there was little evidence for voltage-dependent, 4-aminopyridine-sensitive K+ currents;
2. a voltage-dependent Ca2+-current sensitive to nifedipine, verapamil or mibefradil (all 1 μM);
3. a large conductance activated by the TRPV4-selective agonists 4α- phosphoryl-12,13-didecanoate or GS1016790A;
4. a large ohmic conductance which is gradually activated by hyposmotic media, having electrophysiological characteristics of a TRP current, and is immediately blocked by gadolinium (3 mM);
5. two distinct ohmic conductances which are activated by heat and both are immediately blocked by gadolinium (3 mM); one activates at ~30–35 °C (TRPV3 or TRPV4) and the other at ~40–45 °C (TRPV1);
6. a large ohmic conductance which is activated spontaneously at periodic intervals of 1–3 minutes, and which may underlie the periodic oscillations which we have found to be induced by growth factors (PDGF, TGFβ) or ATP.

These various Ca2+-conductances can account for the elevation of intracellular [Ca2+] seen in response to growth factor, stretch and matrix stiffness. The K+-conductance can modulate the contribution to both (enhancing TRPV currents, but opposing the voltage-dependent Ca2+-influx).

P112
Discovery of a Novel, High Affinity, Small Molecule αvβ6 Inhibitor for the Treatment of Idiopathic Pulmonary Fibrosis
Dr. Rob Slack (GlaxoSmithKline)
Dr. Alison John (University of Nottingham)
Dr. Ellen Forty (University College London)
Dr. Paul Mercer (University College London)
Mrs. Rebecca Graves (GlaxoSmithKline)
Dr. Tao Pun (GlaxoSmithKline)

Mr. Giovanni Vitulli (GlaxoSmithKline)
Mrs. Elaine Gower (GlaxoSmithKline)
Mrs. Valerie Morrison (GlaxoSmithKline)
Dr. Steve Ludbrook (GlaxoSmithKline)
Dr. Carmel Nanthakumar (GlaxoSmithKline)
Dr. Niall Anderson (GlaxoSmithKline)
Dr. Pan Procopiou (GlaxoSmithKline)
Dr. John Pritchard (GlaxoSmithKline)
Dr. David Budd (GlaxoSmithKline)
Prof. David Flint (University of Strathclyde)
Prof. Susan Pyne (University of Strathclyde)
Dr. Jane Denyer (GlaxoSmithKline)
Prof. John Marshall (Queen Mary, University of London)
Prof. Andrew Fisher (Newcastle University)
Prof. Rachel Chambers (University College London)
Dr. Gisl Jenkins (University of Nottingham)
Dr. Pauline Lucy (GlaxoSmithKline)
Prof. Simon Macdonald (GlaxoSmithKline)
Prof. Richard Marshall (GlaxoSmithKline)

Fibrosis is the formation of scar tissue due to injury or long-term inflammation and is a leading cause of morbidity and mortality in disorders that include IPF. The αvβ6 integrin has been identified as playing a key role in the activation of TGFβ that is hypothesized to be pivotal in the development of IPF. Therefore, a drug discovery programme to identify small molecule αvβ6 selective RGD-mimetics was initiated.

As part of a medicinal chemistry programme GSK3008348 was identified and profiled in a range of pre-clinical in vitro and in vivo systems. It was shown to bind to the αvβ6 with high affinity and fast association followed by slow dissociation kinetics in all systems investigated, including IPF human lung tissue. In primary human lung epithelial cells GSK3008348 induced rapid internalisation of αvβ6 (minutes) followed by a slow return of the integrin to the cell surface (hours). The slow return of αvβ6 to the cell surface was inhibited by a lysosomal degradation inhibitor suggesting the sustained duration of action observed post-internalisation by GSK3008348 was a consequence of αvβ6 degradation.

GSK3008348 was shown to engage with αvβ6 and inhibit the activation of TGFβ with a prolonged duration of action using in vivo mouse bleomycin lung fibrosis models measuring αvβ6 engagement and TGFβ activation/signalling.

In summary, GSK3008348 has been shown to display the desirable pharmacological characteristics required for targeting a prolonged inhibition of TGFβ activation in the IPF lung via blockade of the αvβ6 integrin and is currently in Phase I trials for IPF.

P113
Reduced exosomes expression in BAL fluid from fibrotic interstitial lung disease due to blockade of autophagy in alveolar macrophages
Dr. Feng Li (MRC Centre for Inflammation Research, Queen’s Medical Centre for Inflammation Research, Queen’s Medical Research Institute, University of Edinburgh, EH16 4TJ)
Dr. Ailang Zhang (MRC Centre for Inflammation Research, Queen’s Medical Research Institute, University of Edinburgh, EH16 4TJ)
Dr. Ross Mills (MRC Centre for Inflammation Research, Queen’s Medical Research Institute, University of Edinburgh, EH16 4TJ)
Dr. John Marwick (University of Edinburgh)
Dr. Lisa Nicol (MRC Centre for Inflammation Research, Queen’s Medical Research Institute, University of Edinburgh, EH16 4TJ)
Dr. John Pound (MRC Centre for Inflammation Research, Queen’s Medical Research Institute, University of Edinburgh, EH16 4TJ)
Dr. Alison MacKinnon (MRC Centre for Inflammation Research, Queen’s Medical Research Institute, University of Edinburgh, EH16 4TJ)
Prof. Rachel Chambers (University College London)
Prof. William Macnee (MRC Centre for Inflammation Research, Queen’s Medical Research Institute, University of Edinburgh, EH16 4TJ)