

allow distal alveolar imaging at micron resolution in an *ex vivo* ovine model of bacterial infection.

**Results** AMP-1 demonstrates bacterial binding affinity in a concentration dependent manner and labels a diverse panel of bacteria, including a panel consisting of >70% of ventilator-associated pneumonia causing organisms and the pathogenic fungi *Aspergillus fumigatus*. AMP-1 demonstrates significantly higher fluorescence over isomolar linear equivalents for *E. coli*, *K. pneumoniae*, *P. aeruginosa*, MSSA, *A. baumannii* and *S. pneumoniae* (all  $p < 0.01$ ), is selective for bacteria over mammalian cells and has improved chemical stability over the linear equivalent when incubated with bronchoalveolar lavage from patients with acute respiratory distress syndrome. Furthermore, AMP-1 can label *E. coli*, *K. pneumoniae*, *P. aeruginosa* and MSSA *in situ* in an *ex vivo* ovine model when instilled endobronchially and imaged with FCFM (pin vitro and remains selective for gram-negative bacteria over mammalian cells. In the *ex-vivo* model AMP-2 selectively labels the gram-negative bacterial segments (*P. aeruginosa*, *K. pneumoniae* and *E. coli*) over the gram-positive (MSSA, MRSA and *S. pneumoniae*) or control pulmonary segments (all  $p < 0.05$ ).

**Conclusions** A Smartprobe/FCFM strategy to immediately detect bacteria with gram selectivity in size relevant pre-clinical models is described, and are undergoing first-in-man translation.

#### T5 MICRORNA-140-5P REGULATES DISEASE PHENOTYPE IN EXPERIMENTAL PULMONARY ARTERIAL HYPERTENSION VIA SMURF1

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10.1136/thoraxjnl-2015-207770.5

**Introduction and objectives** Clinical therapies for the treatment of pulmonary arterial hypertension (PAH) target vasoconstriction. However, the proliferative pulmonary vascular remodelling that drives disease persists contributing to significant patient morbidity and mortality. MicroRNA (miR) are short non-coding RNA that mediate post-transcriptional regulation of mRNA targets. We hypothesise that dysregulation of miR leads to de-repression of cellular targets central to disease pathogenesis. We sought to identify dysregulated circulating miR in patients with PAH, determine their phenotypic effect using *in vitro* and *in vivo* models and identify key mechanistic regulators that may represent novel therapeutic targets.

**Methods** Two patient cohorts were used to identify and validate differential expression of miR in whole blood by microarray and single assay qPCR. Binding site and network analysis was used to identify key miR targets. Effect of miR on identified targets and disease phenotype was determined in pulmonary artery smooth muscle cells (PASMC) and in the monocrotaline (MCT) and Sugen5416 plus Hypoxia (SuHx) models of PAH.

**Results** Expression of miR-140-5p was reduced in whole blood samples from patients with PAH and experimental models of PAH. Network and pathway analysis identified key regulators of TGF $\beta$  and PDGF signalling as miR-140-5p targets. Transfection with miR-140-5p inhibitor resulted in increased proliferation and migration of PASMC and de-repression of key targets. Nebulised delivery of miR-140-5p mimic prevented the development of PAH in the MCT rat model and attenuated progression of established PAH in MCT and SuHx rat models. In experimental

models levels of SMURF1 protein correlated inversely with miR-140-5p. Direct regulation of SMURF1 by miR-140-5p was demonstrated *in vitro* by 3'UTR luciferase activity. Both miR-140-5p mimic and SMURF1 siRNA increased BMP response element activity identifying SMURF1 as a key negative regulator of BMP signalling in PASMC. Genetic ablation of SMURF1 in C57BL6 mice conferred allele dependent protection from SuHx induced PAH. Finally, whole blood mRNA and pulmonary vascular immunoreactivity of SMURF1 was increased in patients with PAH.

**Conclusions** These studies suggest that miR-140-5p and SMURF1 are key regulators of BMP signalling and disease pathology in PAH and highlight SMURF1 as a potential novel therapeutic target.

#### T6 MET TARGETED THERAPY IN LUNG ADENOCARCINOMA: DOES 'RESISTANT' EGFR MAKE A MET-RESPONSIVE DIMER?

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10.1136/thoraxjnl-2015-207770.6

**Introduction** Lung cancer has extremely poor survival with few effective treatments. Activating EGFR mutations (e.g. L858R), select for impressive EGFR tyrosine kinase inhibitor (TKI) responses but most develop resistance e.g. T790M mutations or MET amplification, which is thought to mediate EGFR-HER3 kinase switching. Preclinical studies suggest EGFR-MET TKI synergy and whilst phase III trials have failed, optimism remains for biomarker driven therapy.

**Hypothesis** EGFR-MET dimerisation determines MET TKI response.

#### Objectives

1. Explore MET TKI responsiveness in EGFR mutant lung adenocarcinoma.
2. Develop an EGFR-MET FLIM assay indicative of MET TKI responsiveness.

**Methods** An *in vitro* and murine xenograft model was derived from EGFR TKI resistant NCI-H1975 lung adenocarcinoma cells (L858R T790M). After shEGFR knockdown we re-expressed EGFR L858R or WT to represent three clinical scenarios: 1. H1975: 'EGFR-TKI Resistant' 2. H1975 L858R: 'EGFR Responders' or 3. H1975 WT (wildtype EGFR).

Cells and xenografts (gavage) were challenged with exquisitely selective MET TKI, SGX523. Response was assessed by BrdU proliferation assays *in vitro* alongside random migration assays mimicking tumour cell motility. Xenograft tumours (FFPE) were stained for Ki67/phosphohistoneH3 (proliferation) and Masson's trichrome/anti-sma (Collagen/stroma).

EGFR-MET interaction was assessed by co-immunoprecipitation and Forster resonance energy transfer (FRET) FLIM to quantify EGFR-MET dimers after SGX523 treatment.

**Results** SGX523 treatment significantly reduced H1975 xenograft tumour growth/weight. Proliferation was suppressed *in vitro* (BrdU %) and *in vivo* (phosphohistone-H3). Conversely in H1975 L858R, SGX523 reduced stroma (Masson's trichrome) and migration. EGFR-MET dimers were more common in H1975 than H1975 L858R/WT. This was associated with a