immunohistochemistry, expression of the most promising candidates was examined using a tissue microarray (TMA).

Results The two most promising candidate genes were identified as DNA Polymerase β, a DNA repair enzyme and NKX2.2, a neuroendocrine transcription factor. Specific inhibition of DNA Polymerase β with Pamoic Acid reduced the numbers of cells surviving treatment with Etoposide (p=0.029) and increased the amount of DNA damage in the cells (p<0.001). Stable overexpression of NKX2.2 significantly increased cell survival in response to Etoposide in two different SCLC cell lines. In keeping with this, we found that absence of nuclear staining for NKX2.2 in the TMA was an independent predictor of improved outcome in chemotherapy treated SCLC patients (HR 0.52, 95% CI 0.33 to 0.82, p=0.005).

Conclusion Using a biologically plausible model of in vivo acquired resistance to Etopoide we have identified two novel Etoposide resistance factors—DNA Polymerase β and NKX2.2. Our *in vitro* data, in conjunction with the TMA results, provide justification for further prospective work to confirm the roles of these molecules in chemotherapy resistance in SCLC.

Novel disease mechanisms in pulmonary arterial hypertension

S95 | APPLICATION OF PROTEOMICS TO EXPLORE THE PATHOBIOLOGY OF PULMONARY ARTERIAL HYPERTENSION

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Introduction and Objective Pulmonary arterial hypertension (PAH) is a poorly understood debilitating disorder which is characterised by increased resistance to the pulmonary blood flow caused by the remodelling of the pulmonary arteries. The objective of this study was to identify differentially expressed proteins in lungs of patients with PAH that may further help us understand the pathobiology of this disease.

Methods Label-free liquid chromatography tandem mass spectrometry was used to generate protein expression profiles from the lung homogenates of 8 PAH patients and 8 Controls. Progenesis-MS software was then used to compare the relative levels of detected proteins.

Results After application of stringent criteria to confirm proteins identities and to detect differences, 27 proteins were found to be differentially expressed from a total of 363 proteins. Proteins that had increased expression in PAH lungs were mainly involved in cell growth, proliferation and cell metabolism. Examples of affected proteins included chloride intracellular channel 4 (CLIC4) and periostin which were both up-regulated and these findings were confirmed by immunoblotting. CLIC4 is known to play a vital role in angiogenesis and has also been implicated in transforming growth factor-β, vascular endothelial growth factor and bone morphogenetic protein receptor two signalling pathways. Periostin is an extracellular matrix protein considered to be involved in vascular injury responses and regulation of smooth muscle cell proliferation. Immunohistochemistry studies showed predominant localisation of CLIC4 in the endothelium of remodelled pulmonary arteries and in plexiform lesions while periostin was prominent in the airways and in the neointimal layer of the remodelled pulmonary arteries.

Conclusions Many proteins that may be involved in the pathogenesis of PAH have been discovered using label free proteomics. Examples include CLIC4 and periostin, which may be involved in the remodelling of vessels in PAH.

S96 | NOVEL BIOMARKERS IN IDIOPATHIC PULMONARY ARTERIAL HYPERTENSION

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Background Circulatingosteopontin levels predict survival in right heart failure and lung tissue expression is increased in hypoxic animal models of pulmonary hypertension. Growth differentiation factor (GDF)-15 and red cell distribution width (RDW) are potential biomarkers in idiopathic pulmonary arterial hypertension (IPAH).

Objectives We assessed the prognostic value of these novel biomarkers, along with the more established N-terminal pro-brain natriuretic peptide (NT-proBNP) and other clinical indices, in treatment-naïve and treated patients with IPAH.

Methods Plasmaosteopontin, GDF-15, and NT-proBNP levels and RDW were determined in 126 patients with IPAH, who were followed for 4.0±2.2 years.

Results All four biomarkers correlated with WHO functional class and 6 min walk distance(6MWD) and cut-offs of osteopontin (53.4 ng/ml, p<0.05), GDF-15 (800 pg/ml)and RDW (15.65%) predicted significant differences in survival in patients with IPAH (Abstract S96 Figure 1). Raised NT-proBNP levels also predicted significantly poorer survival (p<0.01). A temporal increase in serially-measured levels ofGDF-15, osteopontin or RDW indicated poorer survival in IPAH. Patients with relatively low NT-proBNP levels (below median) who might normally be considered low risk, displayed higher mortality rates when GDF-15 levels or RDW were raised. Cox regression survival modelling confirmed RDW as a significant predictor of mortality, independent of established markers including NT-proBNP, 6MWD and WHO class. GDF-15, RDW and NT-proBNP predicted significant (p<0.05) survival differences in patients with reduced exercise capacity (6MWD <380 m); median survival with low 6MWD and raised RDW (>15.65%) was <1.5 year vs 3.6 year for low 6MWD irrespective of the RDW value.
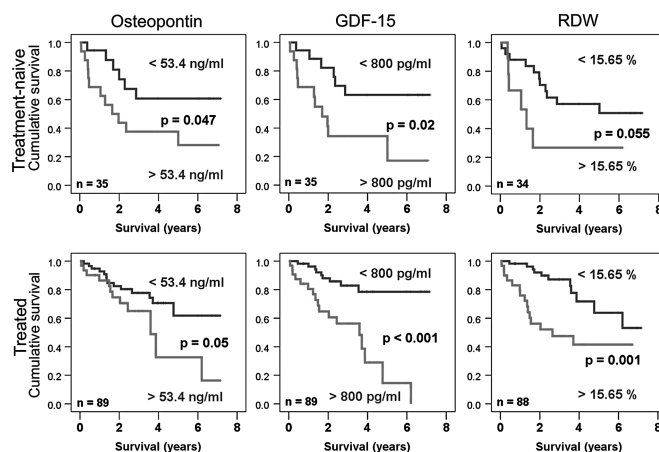
Abstract S96 Figure 1

Conclusions Osteopontin, GDF-15 and RDW all predicted survival in IPAH and were associated with poorer clinical status. Temporal changes in biomarkers appear to hold prognostic information. RDW and NT-proBNP may add the most value to 6MWD and WHO class.