were conducted to determine the correlation between *in vitro* CS sensitivity and different clinical parameters.

Results There was no difference in baseline or LPS-induced cytokine release from PBMCs between the two groups. The inhibition of TNF- α release by DEX was significantly diminished in children with asthma compared to healthy controls at 10^{-8} M concentration ($p = 0.018$) but no differences were noticed at 10^{-6} M concentration, or on LPS-induced IL-8 production. A significant inverse correlation between % inhibition of TNF- α and body mass index (BMI) at 10^{-8} M ($r = -0.84$, $p = 0.02$) and 10^{-6} M DEX ($r = -0.82$, $p = 0.02$) was found.

Conclusions Our results show the existence of an impaired CS sensitivity in PBMCs from children with severe asthma, suggesting that these cells can be used for mechanistic investigations. Interestingly, we observed a negative correlation between CS sensitivity and BMI, a novel *in vitro* finding which supports the association between overweight/obese asthmatic children and a decreased clinical response to CS therapy. Together, these results merit further studies with a larger sample size.

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S132 SPUTUM AND BRONCHIAL BIOPSY EXPRESSION OF 8-EXO-7, 8-DIHYDRO-2'-DEOXYGUANOSINE (8-OXODG) IN ASTHMA IS RELATED TO NEUTROPHILIC INFLAMMATION AND POOR ASTHMA CONTROL

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10.1136/thoraxjnl-2014-206260.138

Introduction and objectives Oxidative stress has been implicated in the pathogenesis of asthma. Validated sputum biomarkers are required to assess this and its relationship to other clinical variables.

We sought to compare sputum and bronchial 8-oxodG expression in asthma and health; assess the sputum repeatability; and explore its relationship with induced sputum inflammatory cells counts and exacerbations.

Methods Asthmatics and healthy controls were recruited from a single centre and underwent clinical characterisation including sputum induction (asthma $n = 58$, health $n = 27$) and bronchial biopsy (asthma $n = 16$, health $n = 10$).

Sputum and epithelial 8-oxodG expression was measured by ELISA and Immunohistochemistry respectively. Sputum asthmatics were assessed at a repeat stable visit at 6 months.

Results Between health and asthma, there were no significant differences in the median (IQR) sputum 8-oxodG levels [12 (16) ngml⁻¹ vs. 11 (15) ngml⁻¹, $p = 0.36$] or the mean (SEM) percentage area of epithelium reaching threshold intensity for 8-oxodG [2.0 (0.7)% vs. 4.4 (1.0) %, $p = 0.12$].

Asthma sputum 8-oxodG correlated with the sputum total cell count ($r_s = 0.53$, $p < 0.01$), sputum neutrophils ($r_s = 0.27$, $p = 0.04$), sputum macrophages ($r_s = -0.31$, $p = 0.02$) and serum IgE ($r_s = -0.27$, $p = 0.04$). Epithelial 8-oxodG correlated to the number of exacerbations in the previous year ($r_s = 0.70$, $p < 0.01$) and the ACQ 6 ($r_s = -0.52$, $p = 0.04$).

Abstract S132 Figure 1 Scatter plots of A) Sputum 8-oxodG in health vs. asthma; B) Epithelial 8-oxodG in health and asthma; C) Sputum 8-oxodG vs. sputum neutrophils and D) Epithelial 8-oxodG vs. the number of exacerbations in the previous year.

Spearman's rank correlation coefficients are also given. Comparisons were made using t tests and Mann Whitney U tests for parametric and non-parametric data respectively

The upper 95th confidence interval of sputum 8-oxodG and epithelium 8-oxodG reaching threshold in healthy controls was used to split asthma patients into 8-oxodG^{high} and 8-oxodG^{low} groups. The sputum 8-oxodG^{high} group ($n = 13$) had significantly higher sputum total cells $8.08 [8.41] \times 10^6 \text{ g}^{-1}$ vs. $2.25 [2.91] \times 10^6 \text{ g}^{-1}$, $p < 0.01$, higher sputum neutrophils $82.25 [32.75]\%$ vs. $55.50 [29.75]\%$, $p < 0.01$ and lower serum IgE $30 [76.50] \text{ KUL}^{-1}$ vs. $157 [212.90] \text{ KUL}^{-1}$, $p < 0.01$. The epithelial 8-oxodG^{high} group ($n = 8$) had significantly more exacerbations $3.9 (0.3)$ vs. $0.5 (0.3)$ $p < 0.01$ and a lower ACQ 6 score $1.4 (0.3)$ vs. $2.4 (0.3)$ $p = 0.04$.

In the asthmatic group, the intra-class correlation coefficient of sputum 8-oxodG between the 2 visits was 0.51 ($p < 0.01$).

Conclusions 8-oxodG expression in sputum and bronchial biopsies was not different between asthma and health, although we did identify an 8-oxodG^{high} group in asthma. Interestingly, expression in asthma was associated with neutrophilic inflammation and poor asthma control.

S133 β_2 -ADRENERGIC RECEPTOR GLY16ARG POLYMORPHISM IS NOT ASSOCIATED WITH IMPAIRED ASTHMA CONTROL IN CORTICOSTEROID TREATED ADULT ASTHMATICS

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10.1136/thoraxjnl-2014-206260.139

Introduction The Arg-16 β_2 -adrenergic receptor allele is associated with increased exacerbations in asthmatic children exposed to combination therapy with long-acting beta-agonists (LABA) and inhaled corticosteroids (ICS).¹ We evaluated whether the Gly16Arg polymorphism is associated with impaired asthma control in ICS treated adult asthmatics and whether this was influenced by concomitant LABA use.

Spoken sessions

Abstract S133 Table 1 Demographics and clinical characteristics according to β_2 -adrenergic receptor genotype

	Gly-Gly n = 105	Arg-Gly n = 88	Arg-Arg n = 3	p value
Age (years)	46 (43–49)	46 (43–49)	45 (39–52)	0.97
FEV ₁ (% predicted)	86 (82–90)	85 (81–89)	88 (82–94)	0.72
FEF _{25–75} (% predicted)	59 (53–65)	57 (51–63)	58 (49–66)	0.85
FEV ₁ /FVC	0.72 (0.70–0.75)	0.71 (0.68–0.74)	0.74 (0.70–0.77)	0.49
ICS Dose (BDP equivalent, µg/day)	800 (800–1500)	800 (680–2000)	800 (400–1250)	0.65
LABA (%)	76	74	60	0.21
ACQ-6	1.78 (1.53–2.03)	1.53 (1.28–1.78)	1.93 (1.36–2.50)	0.23

Data presented as mean (95% CI) except for ICS dose which is presented as median (IQR)

Methods In this cross-sectional study, demographics, medication and spirometry were prospectively recorded from patients attending a secondary care asthma clinic who were also genotyped and completed the Asthma Control Questionnaire (ACQ-6).

Results A total of 223 patients prescribed ICS were included in the analysis. Overall mean age was 46 years, FEV₁ 86%, median ICS dose 800 µg/day and 73% were prescribed LABA. There were no differences in terms of spirometry and ACQ-6 between the three genotypes (Table 1). In patients who were prescribed LABA there was no difference in ACQ-6 comparing patients with no Arg copies (n = 80, ACQ-6 1.82) versus those with one or two Arg copies (n = 83, ACQ-6 1.70). Moreover salbutamol reliever use was no different.

Conclusion Gly16Arg polymorphism was not associated with impaired asthma control in ICS treated adult asthmatics irrespective of LABA exposure.

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Basic mechanisms of IPF

S134 KINASE SELECTIVITY PROFILES OF NINTEDANIB AND IMATINIB

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10.1136/thoraxjnl-2014-206260.140

Introduction Tyrosine kinase inhibition has shown inconsistent success in the treatment of idiopathic pulmonary fibrosis (IPF). While a study of imatinib showed no impact on survival or lung function in a placebo-controlled study, two recently announced placebo-controlled phase 3 trials of nintedanib demonstrated statistically significant impact on forced vital capacity. Comparing the kinase target profiles could inform future target selection for drugs in IPF.

Methods *In vitro* kinase selectivity data of nintedanib and imatinib were collected using the kinomescan platform (DiscoverX Inc). Binding data (% binding) for 451 human kinases (~80% of the human kinome) were initially collected at a single concentration (10 µM). For kinases that displayed significant binding, potencies (K_D) were measured in dose-response format.

Results At a common concentration of 100 nM, imatinib and nintedanib bound to 12 and 50 kinases, respectively. Maximal

drug concentrations (C_{max}) observed in patients were used to project therapeutically relevant kinase inhibition for both drugs. Using these criteria, nintedanib binds 44 kinases at drug levels seen in patients (K_D < C_{max} of 64 nM). Imatinib binds 34 kinases at drug levels seen in patients (K_D < C_{max} of 7500 nM). 14 kinases were bound by both compounds, including PDGFRA, PDGFRB and VEGFR2.

Conclusions Our results suggest that nintedanib and imatinib have partially overlapping inhibition profiles; the kinases that are targeted by both agents are unlikely to be responsible for efficacy differences. Further work is required to identify which of the remaining kinase target (s) are responsible for efficacy in IPF and could therefore represent targets for follow-up compounds.

S135 DOES CD248 HAVE A ROLE IN IPF?

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10.1136/thoraxjnl-2014-206260.141

Idiopathic pulmonary fibrosis (IPF) has a complex pathophysiology with epithelial-mesenchymal transition (EMT) thought to be important to the pathogenesis of fibrotic lesions. CD248 is a membrane bound receptor that has collagen and lectins as ligands and is a stromal cell marker, whose expression is up-regulated post-natally during tissue inflammation and remodelling. A role of CD248 is emerging in kidney fibrosis, but its function in the lung is unknown. We hypothesised that CD248 is a mesenchymal marker of IPF severity and that CD248 contributes to IPF pathogenesis.

Methods CD248 expression was investigated in 23 IPF patient lung samples using immunohistochemistry (IHC) and qualitatively scored. Expression was assessed in cultured normal human lung fibroblasts (NHLFs), A549 cells, IPF derived fibroblasts and normal human primary ATIIIs, treated with or without TGF-β1 and PDGF-BB, using flow cytometry and qRT-PCR. siRNA CD248 knock down (KD) on NHLF mesenchymal marker expression and proliferation was evaluated using qRT-PCR and BrdU assays.

Results IHC revealed strong CD248 expression by fibroblasts in both fibrotic areas and physiological structures of IPF lung tissue (pericytes and pleural tissue). Expression was greatest in samples from lung transplant explants. *In vitro*, CD248 protein levels were significantly greater in IPF derived lung fibroblasts vs NHLFs (p < 0.01). CD248 KD significantly reduced proliferation of control, PDGF-BB and/or TGF-β1 treated NHLFs (p < 0.001), but collagen and αSMA mRNA levels were unaffected (p > 0.05). The alveolar epithelium did not express CD248 on the protein level and minimal CD248 mRNA levels were detected in cultured A549 cells and ATIIIs, which remained unchanged during TGF-β1 induced EMT.

Summary CD248 expression is elevated in the lungs of IPF patients especially in severe disease. CD248 expression appears specific for fibroblasts compared to epithelial cells and does not change during EMT. CD248 KD reduced fibroblast proliferation, but not myofibroblast differentiation.

We conclude that CD248 over-expression is involved in the pathogenesis of IPF – and that it has potential as a marker of mesenchymal/fibroblast lineage. Given that CD248 ligands are collagen type I, IV and fibronectin, we hypothesise that CD248 signalling represents a novel matrix-fibroblast interaction that may be a potential therapeutic target in IPF.