Background Lung injury in cystic fibrosis (CF) is caused by recurrent airway infection and inflammation partially due to the massive infiltration of neutrophils in airways. The processes regulating neutrophil migration across the bronchial and the alveolar epithelia are poorly understood especially in CF. The aim of this study is to analyse the adhesion molecules expressed by neutrophils and epithelial cells during the neutrophil trans-epithelial migration through the bronchial epithelium. We have already shown that ICAM-2, previously thought to be present only on endothelial cells, is also expressed on the bronchial epithelium and plays a key role in T cell migration[1].
Objectives We investigated whether ICAM-2 regulates neutrophil trans-epithelial migration through the bronchial barrier.
Methods We have used human bronchial epithelial cell lines and primary human bronchial epithelial cells (HBECs) from non CF and CF patients, at baseline and on TNF- exposure for 24h.
Results We have shown a constitutive expression of ICAM-2 at the basal side of the primary HBECs grown at air-liquid interface for 21 days. A significant 4-fold increase in ICAM-2 mRNA expression was observed 24h after TNF- treatment in non CF cell line and primary HBECs. Moreover, from confocal microscopy and immunoblots, we have found that ICAM-2 protein expression is statistically up-regulated 24h after TNF- treatment. We have performed the same experiments in non CF and CF paraffin embedded lung sections and we demonstrated a significant increase in ICAM-2 expression in CF. It has previously been pointed out that in CF cells there is actin disorganisation and disruption of the tight junctions leading to an increase in the neutrophil migration[2]. Our preliminary data showed that interaction neutrophil-epithelium provokes an actin remodelling that we can avoid using an ICAM-2 blocking antibody prior the contact with neutrophils.
Conclusions ICAM-2 mRNA and protein levels are higher in CF lung sections and in non CF cells treated with TNF- than in controls. Understanding the interactions neutrophil-epithelium in CF could prevent neutrophil accumulation in airways and attenuate lung injury.

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S104 TARGETING THE BACTERIAL CYTOSKELETON OF CF PATHOGENS FOR ANTIMICROBIAL DEVELOPMENT–A CAUTIONARY TALE?

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Background *Burkholderia cepacia* complex (BCC) bacteria are opportunistic pathogens that cause severe lung infections in cystic fibrosis (CF). Treatment of BCC infections is difficult due to the inherent multidrug resistance of BCC. There is a pressing need to find new bacterial targets for antimicrobials. We have previously shown that the novel compound Q22, which is related to A22 and inhibits the bacterial cytoskeletal protein MreB, inhibits growth of BCC bacteria.
Aims We aimed to further analyse the phenotypic effects of Q22 treatment on BCC virulence traits to assess its feasibility as an antimicrobial.
Methods BCC bacteria were grown in the presence of Q22 and a broad phenotypic analysis was performed, including resistance to H_2O_2 induced oxidative stress, changes in inflammatory potential of cell surface components and *in vivo* drug toxicity studies. The influence of Q22 treatment on inflammatory potential was measured by monitoring the cytokine responses of BCC whole cell lysates, purified lipopolysaccharide and purified peptidoglycan extracted from bacterial cultures grown in the presence or absence of Q22 in differentiated THP-1 cells. Compound Q22 was also assessed for toxicity in both zebrafish and mouse infection models.
Results BCC bacteria grown in the presence of Q22 displayed varying levels of resistance to H_2O_2 induced oxidative stress with some strains showing increased resistance upon Q22 treatment. An increased response in pro inflammatory activity elected by whole Q22 treated bacterial lysate was observed for cytokines TNFa and IL-1b but this was variable between strains. Further dissection of this response is under investigation. Despite minimal toxicity previously shown *in vitro* with primary CF cell lines, *in vivo* studies demonstrated Q22 toxicity in both zebrafish and mouse infection models.
Conclusions In the case of BCC bacteria destabilisation of the bacterial cytoskeleton using compounds such as Q22 can lead to unexpected increases of *in vitro* virulence-related traits. These changes appear to vary depending on strain and species. Future development of antimicrobials targeting the BCC bacterial cytoskeleton may be hampered if such effects translate into the *in vivo* environment of CF infection.

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S105 INNATE B1 CELLS ARE A NOVEL SOURCE OF IL-17 IN CHRONIC PULMONARY PSEUDOMONAS AERUGINOSA INFECTION

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Introduction and Objectives *Pseudomonas aeruginosa* (PA) is an important respiratory pathogen resulting in damaging neutrophilic responses. The cytokine IL-17 is important in orchestrating such inflammation. Cells producing IL-17, such as Th17 cells, have been shown to be important in host defense in chronic pulmonary PA infection. We set out to determine the origin and role of IL-17 in a model of chronic pulmonary PA infection.
Methods Experimental chronic pulmonary infection in mice was produced by intra-tracheal instillation of mucoid PA strains embedded in agar-beads; sterile beads were utilised as controls. Thoracic lymph nodes (LNs), splenocytes and peritoneal B1a cells were restimulated with PA followed by cytokine assay and immunostaining to define responding cell subsets. PA-specific immunoglobulins were measured in sera and culture supernatants. Intrapulmonary B cells were identified via immunohistochemistry. Mice genetically engineered to lack B cells (MT strain) were utilised to examine the effect on pathogenesis in the absence of B cell responses.
Results Chronic PA infection developed in 43% (SD: 25%) of infected animals at 14-days.

Following infection, the pulmonary LN B cell compartment expanded, with a large B1 population ($B220^+$ $CD19^+$ $CD43^+$ IgM hi IgD lo and predominantly $CD5^+$) that expressed

intracellular IL-17A. Infected animals also developed peribronchial B220$^+$ cellular foci.

In mediastinal LNs following infection, PA-specific responses were dominated by B220$^+$ CD19$^+$ CD43$^+$ CD23$^-$B5$^+$ cells expressing and producing IL-17A and IL-22 as well as PA-specific IgM but not IgG. This PA-specific B1 response was not seen in the thoracic lymph nodes of sterile-bead treated animals. In splenocytes, there was a pre-existing B cell response to PA with identical features. Peritoneal B1a cells isolated from untreated controls also produced IL-17A, IL-22 and anti-PA IgM following infection, confirming the existence of pre-existing B1 cells that can respond to PA. In MT animals, chronic colonisation rates, bacterial burden and neutrophilic inflammation did not differ from WT littermates. However, classical PA-specific Th17 responses dominated following infection in MT animals, suggesting alternative compensatory IL-17 sources acting in the absence of B cells.

Conclusions In chronic pulmonary PA infection, innate-like B1 cells migrate to the site of infection and are a novel source of pro-inflammatory IL-17 cytokines.

Lung cancer: reasons to be cheerful

S106 "REASONS TO BE CHEERFUL"–DATA FROM YEAR 8 OF THE NATIONAL LUNG CANCER AUDIT

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Introduction The National Lung Cancer Audit, now in its 8th year, is run jointly by the Royal College of Physicians and The Information Centre for health and social care, and is commissioned by the Healthcare Quality Improvement Partnership (HQIP). Over this period, the audit has collected rich data of increasing quality and has charted improving standards of care for patients, as well as persistent variation across organisations which in most cases is independent of case-mix.

Methods Although several other countries also submit data to the audit, this abstract presents provisional results for England only for patients first seen in 2012.

Results 31,003 patient records were submitted with the improvement in recording of stage being the most noteworthy change in data quality. Full details are given in **Table 1**. Spirometry data is available for 63% of Stage I-II/PS 0–1 NSCLC patients. The histological confirmation rate has risen slightly after a dip in the previous year, and the proportion of patients with non-subtyped NSCLC continues to fall. There have been small but incremental rises in the anti-cancer treatment rate, the resection rate in histologically-confirmed NSCLC, and the proportions of patients having the input of specialist nurses and having the nurse present at the time of diagnosis. Increases are also noted in the proportion having CT scan before bronchoscopy (90%) and having chemotherapy for locally advanced NSCLC with good PS (57%).

Variation in practice still exists–for example, the resection rate in Stage I-II NSCLC varies from 35% to 62% across the cancer networks.

Our final presentation will contain further analyses of survival across the audit lifespan.

Conclusions The lung cancer community should be very proud of the quality of data that they provide to the audit, data which provides clear evidence of gradually improving standards of care. Demonstrating that these improved diagnostic pathways and increased treatment rates translate into longer survival has so far proven elusive since short-term survival is heavily influenced by the large numbers of patients presenting with advanced incurable disease, but as the data matures it is hoped that longer-term survival will indeed increase.

Abstract S106 Table 1.

	2005	2006	2007	2008	2009	2010	2011	2012
Data Completeness								
Number of cases	10,920	16,922	20,639	25,757	30,158	30,329	31,429	31,003
PS	66%	77%	80%	87%	88%	84%	89%	91%
Staging	51%	55%	70%	77%	80%	82%	84%	94%
Treatment	66%	72%	79%	82%	89%	89%	91%	91%
Process and Outcomes								
HCR	68%	66%	65%	66.7%	69.5%	76.5%	73.8%	75.5%
NSCLC NOS rate	-	36%	32%	33.6%	30%	24%	19%	16%
Discussed at MDT?	79%	84.3%	86.8%	88.6%	93.2%	96.1%	95.9%	95.6%
Anti-cancer treatment?	45%	50%	52%	54%	58.9%	58.5%	60.5%	61.0%
Overall resection rate	9%	9.4%	10.3%	11.2%	13.9%	13.9%	15.3%	15.5%
NSCLC resection rate	13.8%	14.3%	15.2%	16%	19%	18.3%	21%	22%
SCLC chemotherapy rate	57.7%	61.7%	64.5%	63%	66%	65%	68%	68%
Seen by LCNS	-	-	-	50.9%	64.4%	75.5%	79.4%	81.9%
LCNS at diagnosis	-	-	-	28.5%	41%	51.9%	58.7%	61.2%

HCR = histo-cytological confirmation rate; LCNS = lung cancer specialist nurse; NOS = not otherwise specified

S107 TREATMENT AND OUTCOMES FOR LOCALLY ADVANCED (STAGE IIIA) LUNG CANCER; 4 YEAR EXPERIENCE FROM THE NATIONAL LUNG CANCER AUDIT

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Background Surgery for lung cancer patients with mediastinal lymph node involvement (N2 disease) remains controversial. In one study (Albain 2009), progression-free (but not overall) survival was higher for patients who received induction chemoradiotherapy followed by lobectomy but post operative mortality was high in pneumonectomy patients. We describe treatment and outcomes for patients with pre-treatment IIIA disease using data submitted from England to the National Lung Cancer Audit (NLCA) 2008–2011.

Methods Patients with pre-treatment staging of T1–3, N2, M0 were included. Small cell cancer, mesothelioma and carcinoid were excluded. The extent and histological nature of pre-treatment N2 disease is not recorded in the NLCA. Survival analyses were performed according to treatment received.

Results 6,775 of 98,403 (6.9%) patients met the inclusion criteria. 2,669 (39%) patients had either chemotherapy or radiotherapy recorded and 2,250 (33%) patients had no treatment recorded. 948 (14%) patients received chemotherapy and radiotherapy however radiotherapy treatment intent was recorded as curative in only 12%. 907 (13%) patients had surgery recorded as part of their treatment plan. Of these, 70% had post operative pathological nodal status recorded (25% N0, 14% N1, 30% N2). Median survival following surgery for the 271