vs. high grade dysplasia (HGD- severe dysplasia (SD) and carcinoma-in-situ). A lesion was considered to have progressed/regressed if it crossed between groups (LGD, HGD, invasive cancer).

**Results** A total of 117 separate lesions that were biopsied on more than one occasion were identified of which 61 were HGD and 56 LGD. Of the low grade lesions 54/56 (96%) regressed or remained static, 1 (2%) progressed to CIS and 1 (2%) to invasive carcinoma both of these lesions progressed from moderate dysplasia. Of the high grade lesions there were 13 SD and 48 CIS, overall 35/61 (57%) of HGD progressed to invasive cancer 9/61 (15%) regressed and 17/61 (28%) remained static. There was a trend toward higher progression to cancer (62% vs 56%) and lower rates of regression (8% vs. 17%) for SD versus CIS in the HGD cohort although the numbers are too small to be statistically significant (see fig. 1). In the HGD group median time to invasion was 9.5 months (range 3–49), static lesions were documented to have remained as such for a median of 17 months (range 4–60).

**Conclusions** In our cohort we see very few lesions following the traditional stepwise progression and LGD remains relatively indolent. There is a significant proportion of HGD that progresses to invasive cancer and further studies are required to test the role of endobronchial intervention to prevent progression and to determine the most efficacious modality of treatment.

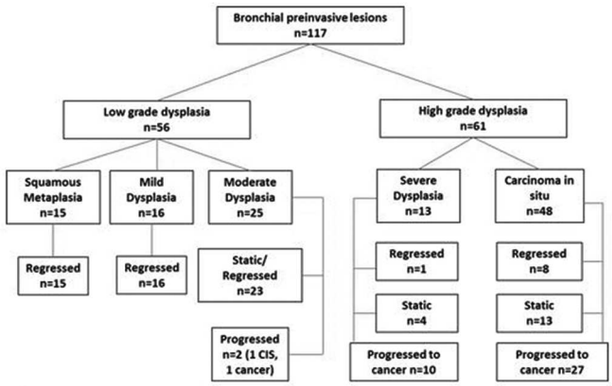


Figure 1. Flow diagram showing progressive vs regressive lesions stratified according to initial histology.

**Abstract S129 Figure 1.**

## S130 ROLE OF CADM1 IN SQUAMOUS CELL CARCINOMA PROGRESSION

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**Introduction** Lung cancer is the second most common cancer in the UK with about 42,000 people being diagnosed in 2010 alone (Office of National Statistics, 2012). With a tendency to form invasive metastases coupled with its frequent late stage diagnosis, lung cancer attributes to the largest cause of cancer related mortality worldwide. Despite advances in treatment and care, the five-year mortality rate remains at 90%. There is a desperate need to improve patient survival, which can be achieved partly through improved screening techniques and more importantly by expanding our understanding of the molecular changes associated with lung cancer development and progression.

We investigate the role of a tumour suppressor gene, first identified in lung cancer, tumour suppressor in lung cancer 1 (TSLC1) or cell adhesion molecule 1 (Cadm1) in regulating squamous cell carcinoma (SqCC) growth and metastases.

**Methods** Cadm1 expression levels were examined using q-PCR analysis on human pre-invasive airway and normal lung tissue collected as part of an on-going UCL/CRUK longitudinal-tracking study (Lung-Surveillance and Lung-SEARCH trials). Cadm1 was introduced into an established SqCC cell line (A431) and *in vitro* functional assays performed to investigate its effect on tumour growth, progression and invasion. Pre-clinical mice models were used to study the effect of Cadm1 expression in tumour growth and metastatic potential.

**Results** q-PCR analyses demonstrated that loss of Cadm1 expression is a frequent early event in pre-invasive human airway compared to normal tissue ($p = 0.001$). Functional assays using A431, with Cadm1 reintroduced, showed Cadm1 expression levels directly associated with a significant decrease in cell proliferation ($p = 0.001$) over 10 days and significant reduction in invasion ($p = 0.001$) over 72 hours compared to control A431 cells without Cadm1. Pre-clinical xenograft tumourigenecity experiments in mice showed that Cadm1 expression significantly inhibited tumour growth ($p = 0.01$) together with a significant reduction in the number of metastases observed ($p = 0.01$) when compared with the control group.

**Conclusion** These data suggest that restoration of Cadm1 expression in human squamous cell carcinomas play an important role in regulation of tumour growth and metastasis. Understanding the mechanism through which Cadm1 expression is able to modulate cancer progression maybe therapeutically beneficial.

## S131 IRON CHELATION REDUCES LUNG CANCER PROLIFERATION IN VITRO

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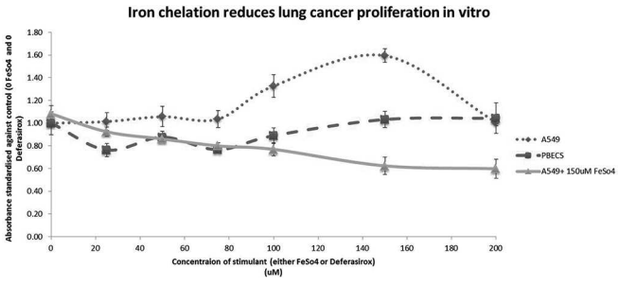
**Introduction** There is growing evidence that iron plays an important role within the lung cancer, the leading cause of cancer-related mortality worldwide. As a result of this, iron homeostasis has potential as a new avenue for targeting and treatment of lung cancer. In this study, the effect of iron loading on cellular proliferation and iron homeostasis gene expression was investigated. In addition, the effect of the chelator deferasirox on cellular iron levels and proliferation rates was studied.

**Methods** Cellular proliferation was assessed by the BrdU assay and cellular iron levels were assessed using the ferrozine assay. Manipulation of *IREB2* gene expression was achieved using short interfering RNA (siRNA) and subsequent expression of this and other iron homeostasis genes was assessed using real time PCR. All experiments were carried out on both the A549 adenocarcinoma and QG56 squamous cell carcinoma cell lines in triplicate. Primary bronchial epithelia cells (PBEC) were used as reference of normal behaviour.

**Results** A dose of 150uM of iron was seen to cause a significant increase in proliferation in both the A549 (50% increase) and QG56 (40% increase) cell lines ($P = 0.002$ and 0.03 respectively) whilst no change was seen in the PBECs. A corresponding increase in cellular iron was also seen.When the cancer cell lines were treated with deferasirox, cellular iron loading decreased by roughly 25% in each cell line ($P = 0.001$ and 0.01 respectively)

and cellular proliferation decreased below levels seen in unstimulated cells. Deferasirox was also seen to effect unstimulated cancer cells, reducing their proliferation by 50% (P = 0.02 and 0.03 respectively).

**Conclusion** Iron exposure was shown to have a significant effect on cellular proliferation within lung cancer cell lines, although the underlying mechanism is not yet fully understood. This iron mediated cellular proliferation could be reversed using the chelator deferasirox. Down-regulated expression of *IREB2* may cause the cancer cell lines to exhibit similar behaviour to the PBECs when stimulated with iron. These finding show that iron may provide a potential new target and deferasirox a potential new therapeutic agent for lung cancer.




**Abstract S131 Figure 1. The dotted line shows that the increasing concentration of FeSO4 has a statistically significant effect at l00 M (M = 1.33, SD = 0.19 P = 0.04), however, l50 M shows an even more significant increase in proliferation (M = 1.59, SD = 0.12, P = 0.002). A dose of 200 M of FeSO4 shows a return to base line and no significant difference in cellular proliferation. The solid line shows that deferasirox causes a decrease in proliferation when applied to cells after incubation with 150 M of FeSO4. This is statistically significant at 50 (M = 0.86, SD = 0.03, P = 0.04), 150 (M = 0.62, SD = 0.08, P = 0.01) and 200 M (M = 0.60, SD = 0.08, P = 0.0004) of deferasirox and the greater the dose of deferasirox, the greater the decrease in proliferation. The dashed line indicates the effects of FeSO4 incubation on PBECS. There is no statistical significance seen in proliferation rates for any concentration of FeSo4.**

## S132 LINEAGE TRACING IN HUMANS REVEALS STOCHASTIC HOMEOSTASIS OF AIRWAY EPITHELIUM RESULTING FROM NEUTRAL COMPETITION OF BASAL CELL PROGENITORS

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In recent years, the development of lineage tracing approaches has provided quantitative new insights into tissue homeostasis in mice. However, the relevance of these discoveries to human epithelial homeostasis and alterations in disease is not known. We demonstrate that the statistical analysis of pathologically neutral somatic mitochondrial mutations that are accumulated over time can provide access to clonal fate behaviour at single cell resolution in human, providing a direct means to explore mechanisms of cell fate and tissue maintenance. Employing this approach, we define the progenitor cell population and the cellular hierarchy of the major human airways. By applying a novel quantitative approach to lineage tracing data, we conclude that, in normal homeostasis, the lining of human lung epithelium is maintained by an equipotent progenitor cell population of basal cells, in which the chance loss of cells due to commitment is perfectly compensated by the duplication of neighbouring cells, leading to neutral drift dynamics of the clone population. Further, we show that in airways of smokers, this process is accelerated leading to intensified clonal consolidation and a fertile background for tumorigenesis. This study provides the benchmark for the use of somatic mutations to quantitatively explore patterns of homeostatic growth in human tissues, and a platform to explore factors leading to homeostatic dysregulation and disease.

# Outcomes post critical care

## S133 OBSERVATIONAL COHORT STUDY OF OUTCOME OF PATIENTS REFERRED TO A REGIONAL WEANING CENTRE

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**Introduction** Data on outcome of the patients referred to weaning and rehabilitation centres are limited. In this observational cohort study, we report the outcomes of patients referred to a specialist complex home ventilation, weaning and rehabilitation centre.

**Methods** Data from the LFRU database from February 2005 to February 2011 were analysed. The primary diagnosis causing prolonged mechanical ventilation (MV) were classified into five groups: (1) neuromuscular and chest wall disease (NMD-CWD); (2) chronic obstructive pulmonary disease (COPD); (3) post-surgical patients; (4) obesity related respiratory failure (ORRF); and (5) other causes. The principal outcomes measured were weaning success, hospital mortality, 1-year and 2-year survival following discharge.

**Results** A total of 369 patients were referred over the 6 year period. Of these, 194 (52.6%) were admitted. The commonest outcome was total liberation from all forms of MV (45%). The remainder were shown to (1) require nocturnal non-invasive ventilation (NIV) (22%); (2) require nocturnal and intermittent daytime NIV (1%); (3) require long-term tracheostomy ventilation (24%); and (4) died in hospital (8%). Post-surgical and COPD patients had the highest rate of total liberation from mechanical ventilation at 60% and 54%, respectively. The median time from admission to tracheostomy decannulation was 18 days (9–33). NMD-CWD patients had the lowest hospital mortality (7%), whereas COPD patients had the highest hospital mortality (29%). The overall survival at 12 and 24 months was 60% and 50%, respectively. 25% of the COPD patients were alive and 59% of the NMD-CWD patients were alive at 24 months (Figure 1).

**Conclusions** The majority of patients with weaning failure were successfully liberated from mechanical ventilation. The weaning