Abstract S127 Table 1. Apoptosis induced by recombinant TRAIL and MSC-TRAIL on combination with Vorinostat

	rTRAIL	Vorinostat	rTRAIL and Vorinostat	MSCTRAIL not activated	MSCTRAIL Activated	MSCTRAIL not Activated + Vorinostat	MSCTRAIL Activated + Vorinostat
Ju77	7.71%	51.35%	80.77%	10.32%	48.73%	47.44%%	77.7%
CRL2081	56.75%	78.95%	96.6%	37.3%	57.63%	81.45%	90.93%
One58	13.41%	43.97%	79.27%	10.88%	53.8%	49.25%	77.8%

MPM cells are treated with recombinant Trail (100ng/ml) and Vorinostat (2.5 M). MSC are plated in 1:1 ratio with tumour cells. MSC are activated with doxycycline to induce TRAIL expression.

Introduction Malignant pleural mesothelioma (MPM) is a highly aggressive, incurable, chemoresistant tumour. Recent studies have shown that Mesenchymal stem cells (MSC) can home to and incorporate into the tumour stroma. Their tumour tropism can be used to deliver Tumour necrosis factor related apoptosis inducing ligand (TRAIL), a transmembrane protein that selectively induces apoptosis in transformed cells. However, not all tumours are sensitive to TRAIL. TRAIL works through triggering the extrinsic apoptotic pathway while conventional chemotherapeutic agents act by triggering the intrinsic apoptotic pathway. We hypothesised the crosstalk between these two pathways could be exploited by combining chemotherapy and MSC-TRAIL in MPM tumour cell lines.

Methods MSC were engineered to express TRAIL using a lentiviral plasmid vector. A Tetracycline (Tet)-inducible system was used as a backbone to control the expression of TRAIL. Apoptosis induced by recombinant TRAIL, MSC-TRAIL in MPM cell lines on combination with Vorinostat, a chemotherapeutic agent, was measured by Annexin-V/DAPI based flow cytometry.

Results The combination of recombinant TRAIL and Vorinostat act synergistically to induce apoptosis in MPM cell lines. Recombinant TRAIL and Vorinostat, as monotherapies induce 7.17% and 51.35% apoptosis in an MPM cell line JU77 respectively. In CRL2081 and ONE58 cell lines, recombinant TRAIL induces 56.75% and 13.41% apoptosis while Vorinostat leads to 78.95% and 43.97% apoptosis respectively. The combination of recombinant TRAIL and Vorinostat shows an increased amount of apoptosis in JU77, CRL2081 and ONE58 cell lines at 80.77%, 96.6% and 77.27% respectively (Table 1).

Similar synergistic affect was observed when TRAIL expressing MSCs were co-cultured with Vorinostat treated MPM cell lines. MSC-TRAIL induced apoptosis in JU77 (48.73%), CRL2081 (57.63%) and ONE58(53.8%). Combined treatment of Vorinostat and MSC-TRAIL significantly increased apoptosis to 77.7% in JU77, 90.93% in CRL2081 and 77.8% in ONE58 cells (Table 1).

Conclusion The combination of Vorinostat and recombinant TRAIL acts synergistically to induce apoptosis in malignant plural mesothelioma cells. Similar affect is observed with the combination of MSC-TRAIL and Vorinostat. This study indicates that Mesenchymal stem cells can be used as vectors for delivery of TRAIL and upon combination with Vorinostat, could be a potential therapy for malignant pleural mesothelioma.

S128 REDUCTION OF LUNG METASTASIS BY ENGINEERED MESENCHYMAL STEM CELLS EXPRESSING SECRETED SOLUBLE TRAIL

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10.1136/thoraxjnl-2013-204457.135

Bone marrow–derived mesenchymal stem cells (MSC) are promising tools for cancer therapy because they are able to home to and incorporate within tumour tissue. Tumour necrosis factor–related apoptosis-inducing ligand (TRAIL) is a pro-apoptotic protein that induces selective apoptosis of tumour cells, while sparing normal cells. Therefore it is expected that MSCs engineered to produce TRAIL would home to and kill cancer cells in a lung metastatic cancer model. Two lentiviral vectors were constructed to express the full-length (FL) TRAIL and a truncated soluble form (ILZ-sT), respectively. A secretion peptide and an isoleucine zipper (ILZ) peptide were added to the N-terminal of the soluble form to force its secreted expression and to enhance its trimerization. Human MSCs were transduced with viruses and both constructs produced soluble TRAIL into cell media that can rapidly induced apoptosis of cancer cells. However the ILZ-sT fusion construct expresses significantly higher level of soluble TRAIL, and causes better *in vitro* lung cancer cell (A549) killing than the FL one.

In coculture experiments both construct viruses transduced MSCs caused lung (A549), breast (MDAMB231), squamous (H357), and cervical (Hela) cancer cell apoptosis and death with similar efficiencies. A synergistic effect of cancer cell killing was observed for the combinational treatment of MSC-TRAIL cells with Saha, a histone deactylase inhibitor. When systemically delivered both MSC-FLT and MSC-ILZ-sT cells showed significant reduction of lung metastasis in a pulmonary metastasis murine model. Interestingly, ILZ-sT expressing cells demonstrated higher efficiency of metastasis reduction than FLT cells. These findings suggests that TRAIL expressing MSCs particularly ILZ-sT cells could be potentially developed as a therapy for lung metastasis diseases.

S129 THE NATURAL HISTORY OF BRONCHIAL PRE-INVASIVE DISEASE

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10.1136/thoraxjnl-2013-204457.136

Background Bronchial pre-invasive lesions represent the earliest stages of the stepwise progression of squamous carcinogenesis, they predominantly affect the large airways and are readily detectable using autofluoresence bronchoscopy (AFB) however very little is known about the natural history of these lesions and no randomised data exists to determine whether intervention before progression to invasion improves outcome.

Methods A total of 94 patients with bronchial dysplasia were enrolled into an on-going surveillance cohort at University College London Hospital running prospectively since 1999. Lesions were biopsied longitudinally and kept under regular surveillance with AFB and low dose annual CT scanning until resolution or progression to invasive disease occurred. Retrospective analysis of lesional destiny was undertaken to determine the proportions of progressive vs. regressive lesions that occur in low grade dysplasia (LGD- squamous metaplasia, mild and moderate dysplasia)

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.

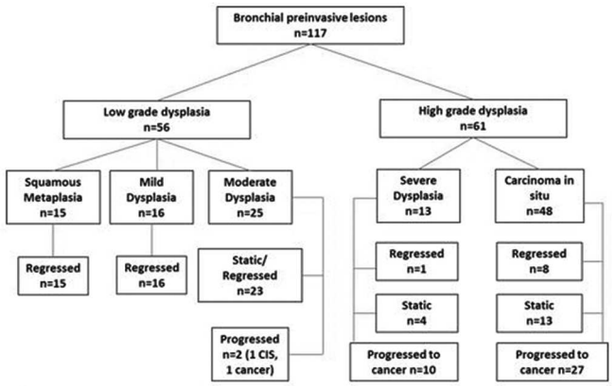

Figure 1. Flow diagram showing progressive vs regressive lesions stratified according to initial histology.

Abstract S129 Figure 1.

S130 ROLE OF CADM1 IN SQUAMOUS CELL CARCINOMA PROGRESSION

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10.1136/thoraxjnl-2013-204457.137

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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10.1136/thoraxjnl-2013-204457.138

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)