**9060** POSTER

**Phase I Trial of Pemetrexed and Cisplatin Combination Chemotherapy With Concurrent Thoracic Radiotherapy in Japanese Patients With Locally Advanced Non-Small-Cell Lung Cancer**

I. Sekine[1], K. Kubota[1], S. Niho[2], M. Sumi[3], K. Nihei[4], R. Sekiguchi[5], J. Funai[6], S. Enatsu[7], Y. Ohe[2], T. Tamura[1]. *[1]National Cancer Center Hospital, Internal Medicine and Thoracic Oncology, Tokyo, [2]National Cancer Center Hospital East, Thoracic Oncology, Chiba, [3]National Cancer Center Hospital, Radiation Oncology, Tokyo, [4]National Cancer Center Hospital East, Radiation Oncology, Chiba, [5]Eli Lilly Japan, Statistical Science, Kobe, [6]Eli Lilly Japan, Science Communications, Kobe, [7]Eli Lilly Japan, Medical Science, Kobe, Japan*

**Background:** Pemetrexed (PEM) was found to have a radiosensitizing potential when evaluated *in-vitro* in combination with platinum-containing compounds and radiation. The purpose of this study was to determine the recommended dose (RD) of PEM and cisplatin (CDDP) combination chemotherapy with concurrent thoracic radiotherapy in Japanese non-small-cell lung cancer (NSCLC) patients (pts).
**Materials and Methods:** Eligible pts were histologically or cytologically confirmed non-squamous NSCLC with unresectable IIIA or IIIB disease. Written informed consent was obtained from all patients. The study treatment consisted of 2 phases: a chemo-radiation therapy phase (CRT) and a consolidation phase (CONS). In CRT, the first 6 pts received the level 1 dose (L1D), PEM 500 mg/m$^2$ plus CDDP 75 mg/m$^2$, on day 1 of a 21-day cycle for 3 cycles. Thoracic radiotherapy was given concurrently at a total dose of 60 Gy. If the dose limiting toxicity (DLT) occurred in <2 pts at L1D, the radiation dose was escalated to a total dose of 66 Gy (L2D). In CONS, PEM 500 mg/m$^2$ was administered in 3 cycles beginning 4 to 6 weeks after CRT. DLT was defined as any grade 3/4 hematological or non-hematological toxicity which resulted in delay or omission of study treatment, or any other grade $\geqslant$3 therapy-related adverse event except nausea or vomiting.
This study was sponsored by Eli Lilly Japan K.K.
**Results:** Eighteen pts (14 male, 4 female), median age 61 years were enrolled. In CRT, 1 patient experienced 2 DLTs (Grade 3 anorexia and diarrhea) at L1D, however no DLT was seen in the 6 pts at L2D. Therefore, L2D was determined as RD and an additional 6 pts were treated L2D. All 18 pts completed CRT. Twelve pts (5 at L1D and 7 pts at L2D) completed the CONS phase. No deaths occurred during the study. Common grade $\geqslant$3 toxicities were lymphocytopenia, neutropenia and leukopenia with frequencies of 89, 67 and 67%, respectively. At L1D, 2 pts with radiation pneumonitis (grade 3 and 2, 1 each) and 2 pts with radiation oesophagitis (grade 2 and 1, 1 each) were observed. There were 11 pts with radiation pneumonitis (6 grade 2 and 5 grade 1) and 11 pts with radiation oesophagitis (4 grade 2 and 7 grade 1) at L2D. Of 18 pts, 15 achieved partial response; 2 had stable disease and 1 progressive disease.
**Conclusions:** Combination chemotherapy of pemetrexed 500 mg/m$^2$ plus cisplatin 75 mg/m$^2$ with concurrent thoracic radiotherapy at a total dose of 66 Gy was well-tolerated; further development is warranted.

**9061** POSTER

**E-cadherin Expression in Lung Cancer and Its Clinical Importance**

N. Tsoukalas[1], T. Oikonomaki[2], M. Tolia[2], A. Tzovaras[3], E. Tsiambas[4], A. Karameris[4], I. Sfiniadakis[5], E. Manolis[2], S. Theocharis[2], C. Kittas[2]. *[1]401 General Army Hospital, Medical Oncology, Athens, [2]National and Kapodistrian University of Athens, Medical School, Athens, [3]Aghios Savvas Anticancer Hospital, Medical Oncology, Athens, [4]417 NIMTS, Pathology, Athens, [5]NNA, Pathology, Athens, Greece*

**Background:** Cadherins are considered to be the most important adhesion molecules necessary for the maintenance of normal tissue architecture. E-cadherin is a basic factor in the connection adhesions between epithelial cells. Reduced expression of these molecules correlates well with increased invasiveness, metastases, and poor prognosis in neoplasms.
**Material and Methods:** Patients with clinically operable primary lung cancer were included in the present study. The tissue samples of the tumours (paraffin cubes) were analysed using the tissue microarrays method. This was followed by immunohistochemistry study of E-cadherin and Ki-67 cell proliferation factor. The image analysis and processing were accomplished using special software. Finally, a database was created with all the clinical and histological data of the patients.
**Results:** In total, 108 patients (81 men and 27 women) with a mean age of 62 years were assessed. The histology types were: 44% adenocarcinoma, 31% squamous cell carcinoma, 9% large cell carcinoma and 16% other types. Associations between variables were analyzed by the application of Univariate Analysis Of Variance with SPSS v15.0 software (SPSS Inc., Chicago, IL, v.15.0). Two tailed p values $\leqslant$ 0.05 were considered to be statistically significant. Statistical significance was identified correlating E-cadherin lower-expression to grade III of the tumours (p-value 0.011), to stage IV (p-value 0.045) and in a positive correlation between E-cadherin and Ki-67 (p-value 0.040). In contrast, protein expression was not strongly associated to tumour size, to histological type, to patient age or to gender.
**Conclusions:** The most important conclusions of this study are that there is a low-expression of E-cadherin protein mainly in grade III and also in stage IV lung cancers and that there is a positive correlation between E-cadherin and Ki-67 expression.

**9062** POSTER

**Development of a Novel RT-PCR Assay for the Detection of EML4-ALK Fusion Genes in FFPE Speciments**

G. Nasioulas[1], E. Papadopoulou[1], E. Razis[2], P. Kosmidis[2], E. Briasoulis[3], S. Murray[1]. *[1]GENEKOR, Molecular Oncology, Glyka Nera, [2]Hygeia Hospital, Oncology Unit, Halandri-Athens, [3]University Hospital of Ioannina, Oncology Unit, Ioannina, Greece*

**Background:** Echinoderm microtubule-associated protein-like 4-anaplastic lymphoma kinase (EML4-ALK) is a fusion-type protein tyrosine kinase identified recently in a subset of human lung carcinomas and seems to be a promising candidate for a therapeutic target as well as for a diagnostic molecular marker in NSCLC.
Indeed, ALK kinase inhibitors (Crizotinib (PF0234–1066, Pfizer) have already been developed and have been reported to be efficient only in patients positive for the EML4-ALK fusion.
To date, several *EML4-ALK* variants (1, 2, 3a, 3b, 4, 5a, 5b, 6, 7, "4" and "5") have been identified in lung cancer samples. A variety of methods have been used for the detection of these fusions, including immunohistochemistry, fluorescent in situ hybridization, and reverse transcriptase polymerase chain reaction (RT-PCR), which is the only method that can distinguish between different variants.
Existing RT-PCR methods though, are designed to amplify large cDNA fragments and are inadequate for the analysis of formalin-fixed paraffin-embedded (FFPE) tissues which produce cDNA fragments of limited size. Thus, we designed an RT-PCR assay that can detect all published *EML4-ALK* variants and is suitable for use with this commonly available material.
**Methods:** The study included FFPE speciments from NSCLC patients without EGFR and K-RAS mutations. Detection of all EML4-ALK fusions was achieved using a multiplex reverse transcription-PCR (RT-PCR). For this reason specific primers that enhance specifically EML4-ALK transcripts 1, 2, 3a, 3b, 4, 5a, 5b, 6, 7, "4" and "5" were deisigned. Synthetic DNA fragments for each variant were cloned using the pCR2.1 cloning vector and used as positive controls. DNA sequencing analysis was performed to confirm the specificity of the obtained PCR products.
The sensitivity of the method was calculated by adding to 1 $\mu$g RNA serial dilutions of the synthetic DNA fragments. It was found that up to 22 copies of the translocation can be detected per $\mu$g of RNA.
**Results:** None of the 26 FFPE speciments tested so far, was positive for the EML4-ALK fusion. The study is in process and we plan to test 100 FFPE speciments.
**Conclusions:** We designed a robust multiplex RT-PCR assay that permits the sensitive detection of all published EML4-ALK variants and is suitable for use with commonly available materials such as FFPE speciments and spuntum samples.

**9063** POSTER

**Pilot Results for the Detection of Minimal Residual Disease in Lung Cancer**

A. Benedikova[1], J. Srovnal[1], J. Klein[2], P. Skalicky[2], M. Szkorupa[2], L. Radova[1], M. Hajduch[1]. *[1]Faculty Hospital Olomouc, Institute of Molecular and Translational Medicine, Olomouc, [2]Faculty Hospital Olomouc, Department of Surgery, Olomouc, Czech Republic*

**Background:** Lung cancer is one of the leading causes of cancer death and has become an increasingly urgent worldwide health problem. The term minimal residual disease (MRD) in solid tumours refers to the presence of tumour cells in the body of a patient who has undergone surgery without further clinical signs of the disease. These isolated tumour cells are considered to be the precursors of micrometastases. Testing MRD lung cancer patients can eliminate complicated surgical procedures in patients with systemic molecular dissemination of the disease and can improve the prognosis.
**Material and Methods:** The basic principle for the detection of MRD by methods based on quantitative reverse-transcriptase polymerase chain reaction (QRT-PCR) is the detection of biomarkers of epithelial tumour cells in compartments of mesenchymal origin. We validated the QRT-PCR method for 4 selected biomarkers for MRD detection in lung cancer − carcinoembryonic antigen (CEA), epidermal growth factor receptor (EGFR),

lung-specific X protein (LUNX) and hepatocyte growth factor receptor (c-met). In total 51 patients were enrolled in the study.
**Results:** Expression of selected biomarkers in tumours of different histogenetic origin was analysed. Expression of LUNX is highly specific for tumours of lung origin, as well as in EGFR and CEA. C-met does not show this specificity.
We found an increasing trend of CEA expression in bone marrow with higher clinical disease stage of patients ($p < 0.11$). A higher CEA ($p = 0.10$) and c-met ($p < 0.02$) expression rate in the pulmonary blood of patients with histologically proven lymph node metastases was detected than in patients with negative lymph nodes. An increasing trend of CEA expression in bone marrow ($p < 0.025$) was found with higher grade of tumour.
The percentage representation of MRD positive samples in individual patient groups divided by clinical stage was further analyzed. Increasing positivity of CEA in bone marrow and c-met in systemic blood was found with higher clinical disease stage. Marker LUNX showed significantly higher positivity in the pulmonary blood of patients with a higher clinical disease stage.
**Conclusion:** The pilot results show that LUNX is a good candidate marker for the MRD detection in lung cancer in blood as well as in bone marrow. Scanning MRD in lung cancer is a potential prognostic marker. Its significance requires further study.
**Acknowledgement:** This work was financially supported by MSMT CZ1.07/2.3.00/09.0089, IGA MZ CR NS 10285−3, CZ.1.05/2.1.00/01.0030.

**9064** POSTER

**Timeliness of Referral of Patients With Abnormal Chest X-Ray Suggestive of Lung Cancer**

K. Eremeishvili[1], R. Nagendran[2], G. Constantinescu[2], M. Gulati[2], T.C. Stokes[1]. [1]*Queen Elizabeth Hospital, Respiratory, London,* [2]*Queen Elizabeth Hospital, Radiology, London, United Kingdom*

**Background:** 'All patients with chest radiograph report suggestive of lung cancer should be seen by respiratory physician within 14 days' (Nice 2004). Requesting clinician is responsible to review chest x-ray (CXR) reports and refer suspected patients for investigation.
Our aim was to determine:
– if the number of delayed referrals following an abnormal x-ray in patients diagnosed with lung cancer in 2010 has been reduced;
– if earlier unreported or undetected radiographic abnormalities existed that might have led to earlier referral;
– To compare our results to the results of studies performed in 2005, 2008 and 2009.
**Material and Methods:** The CXR of lung cancer patients diagnosed in 2010 have been inspected retrospectively and patients considered "delayed" if a CXR prior to the diagnostic radiograph showed suspicious lesion. "Delayed" patients were further categorised according to whether the x-ray was reported abnormal but no action was taken (Delayed action), reported normal (Undetected lesion) or not reported (Unreported) and compared to the same patient groups in studies performed in 2005, 2008 and 2009.
**Results:** Chest x-ray of 140 patients diagnosed with lung cancer in 2010 were reviewed. 78/140 had no previous x-ray at Queen Elizabeth Hospital. 42/140 patients' previous x-ray were not suggestive of lung cancer. Our colleague radiologists, who were otherwise uninvolved in the study, were asked to review 20 anonymised CXR that could have been suggestive of lung cancer. Of these 16 patients had abnormalities on previous CXR that were either Delayed action (3) Unreported (3) or lesions Undetected (10). The table below shows 2010 study results compared to results of 2005, 2008, 2009 studies:

| Year | 2005 | 2008 | 2009 | 2010 |
|---|---|---|---|---|
| Total patients | 169 | 149 | 232 | 140 |
| Delayed action | 18 (10.6%) | 5 (3.4%) | 5 (2.2%) | 3 (2.1%) |
| Undetected lesion | 23 (13.6%) | 11 (7.4%) | 12 (5.2%) | 10 (7.1%) |
| CXR unreported | 0 (0.0%) | 4 (2.7%) | 9 (3.9%) | 3 (2.1%) |
| Total | **41 (24.2%)** | **20 (13.4%)** | **26 (11.2%)** | **16 (11.4%)** |

We also investigated the time delay between a suspicious chest x-ray and the diagnosis of lung cancer being made. In 2008 and 2010 there was a large reduction in the number of patients having delay between 0−4 months. This reflects better pick up rates and more consistent referrals. But there are larger delays between 4−24 months explained by the following: if CXR lesion is initially missed there is no additional 'safety net' for the prompt diagnosis, so patients present at a later date with symptoms.
**Conclusion:** Significant improvement and adherence to referral guidelines have been seen since 2005. Immediate referral of suspicious CXR speeds diagnosis but only for patients who would have been diagnosed within 4 months implying that they have larger or more aggressive tumours which might become symptomatic sooner. 'Undetected lesion' group accounts for nearly 50% delays reflecting difficulty detecting small nodules which if not detected there is no other safety net to avoid further delay. More vigilance on the part of doctors requesting/reporting CXR will bring further improvement.

**9065** POSTER

**Psychological Adjustment in Patients With Lung Cancer**

K. Kritikos[1], P. Heras[1], D. Mendrinos[1], A. Hatzopoulos[1], G. Apostolidis[1], V. Salesiotou[1]. [1]*General Hospital of Nafplion, Internal Medicine, Athens, Greece*

**Background:** The aim of this pilot study was to identify predictors of adjustment and tests the efficacy of a psychological intervention with patients diagnosed with lung cancer on their adjustment over time.
**Material and Method:** All patients aged 43−77 years newly diagnosed with lung cancer who met the inclusion criteria and were awaiting surgery were assessed. The study assesses global and cancer specific stress, global and cancer specific coping and social support on depression, anxiety, positive and negative affect, body image and benefit finding in patients with first diagnosis of lung cancer.
**Results:** First wave of results report on the predictor of psychological adjustment pre- and post-surgery of 20 patients recently diagnosed and on the efficacy of the cognitive behavioral intervention on 7 patients who have been randomized to the intervention.
**Conclusion:** Results to date indicate that perceived stress is the strongest predictor of emotional adjustment at diagnosis and post-surgery. ANOVA results demonstrate the effectiveness of the intervention in reducing levels of lung cancer specific stress, distress and on increasing adaptive coping ang benefit finding.

## *Poster Presentations* (Mon, 26 Sep, 14:00−16:30)
## Lung Cancer − Metastatic

**9066** POSTER

**High ALK Gene Copy Number in Non-small Cell Advanced Lung Cancers**

K. Kalai[1], N. Auger[2], L. Lacroix[3], N. Dorvault[4], K. Olaussen[4], A. Valent[2], S. Rajpar[1], D. Planchard[1], B. Besse[1]. [1]*Institut Gustave Roussy, Medicine, Paris,* [2]*Institut Gustave Roussy, Cytogenetics, Paris,* [3]*Institut Gustave Roussy, Recherche Transitionnelle, Paris,* [4]*Institut Gustave Roussy, Biopathology, Paris, France*

**Introduction:** Increased ALK gene copy number has been described as a frequent event in non small cell lung cancers. We report a correlation between ALK status (copy number and rearrangement) and EGFR/KRAS mutational status among advanced non small cell lung cancer (NSCLC).
**Methods:** ALK status was evaluated by fluorescence in situ hybridization (FISH) in paraffin embedded specimens from advanced NSCLC patients. ALK scoring was performed following Cappuzzo criteria established for EGFR and HER2 in lung cancer. High gene copy number (GCN) was defined as the presence of $\geqslant 6$ copies of ALK per cell in $\geqslant 10\%$ of analyzed cells. FISH with CEP2 was performed to determine the ploïdy status in samples with high GCN.
All coding sequences of exon 18 to 21 of *EGFR*, and of exon 2 and 3 of *KRAS* were analyzed by Sanger direct sequencing performed after Polymerase Chain Reaction (PCR) amplification.
**Results:** Main characteristics of 76 tested pts were as follow: adenocarcinoma features in 50 cases (66%), median age (55, 24−79), 44 (58%) were male, 28 (37%) were never smokers. EML4-ALK translocation was present in 10 cases (13%) which were wild type for both EGFR and KRAS. Eight cases (11%) exhibited ALK high GCN and 46 (60%) GCN gains, whereas eight (11%) exhibited monosomy. FISH with CEP2 revealed a polysomy of chromosome 2 in ALK high GCN. EGFR was mutated in 7 cases, two having ALK high GCN. KRAS was mutated in six cases, five with over three ALK gain. Data survival will be reported.
**Comments:** Increased ALK GCN is mainly due to polysomy and is not exclusive from EGFR and KRAS wild type. Further preclinical studies in lung cancer models are ongoing to determine the predictive value of this event.