# Pulmonary

### 1576 Clinicopathologic Analysis of Focal Adhesion Kinase (FAK) Expression in Primary Tumors of the Lung

*A Acurio, S Charoenthammaraksa, M Tetriakova, L Faoro, R Salgia, T Krausz, AN Husain.* Univ of Chicago, Chicago, IL.

**Background:** FAK is a cytoplasmic tyrosine kinase important in adhesion-dependent cell growth, proliferation, survival and motility. Upon integrin mediated survival signaling, FAK co-localizes at sites of contact with extracellular matrix, becomes activated via phosphorylation and promotes cell cycle progression. As cancer progresses, malignant cells acquire the ability to survive in the absence of cell adhesion. Preinvasive, invasive and metastatic lesions show higher expression levels of FAK compared to normal epithelium. In vivo models demonstrate that FAK phosphorylation is crucial in conferring increased metastatic potential of certain human cancers. The role of FAK expression and phosphorylation in primary lung tumors and in their metastatic capability has not yet been studied.

**Design:** Immunohistochemical analysis of native FAK and its phosphorylated form (pFAK) was performed (BD Labs/Biosource antibodies) on tissue microarrays (TMA) containing normal controls, primary lung cancers and corresponding metastases to lymph node and/or brain tissue. Quantitation of staining intensity was obtained with the Automated Cellular Imaging System (ACIS/Clarient). Average intensity of expression was calculated as a measure of integrated optical density (IOD). Statistical analysis was performed with SPSS program.

**Results:** FAK expression in lung cancer cells (primary or metastatic) is higher than in normal epithelium ($p<0.001$,t-test). There are significant differences of FAK expression among different histologic subtypes of lung cancer ($p=0.004$,ANOVA). Furthermore, high ratio of pFAK/FAK (pFAK ratio) correlates with poor clinical prognosis in all groups (p 0.031,Kaplan-Meier).

**Conclusions:** As part of the integrin mediated survival signal, FAK overexpression contributes to adhesion independent growth, proliferation and survival of cancer cells. Our study demonstrates FAK overexpression in primary tumors of the lung and their metastasis. Histologic subsets show different FAK expression patterns and high pFAK ratios correlate with worse clinical prognosis. No statistically significant difference between metastatic and primary lesions was found within any of the histologic subsets. These data supports the notion that overexpression of FAK influences tumor behavior, promotes malignancy and represents a potential target for lung cancer therapy.

FAK expression

| Histologic type | Number of cases | FAK IOD |
|---|---|---|
| Normal | 73 | 33 |
| Adenocarcinoma | 34 | 181 |
| Large cell | 40 | 163 |
| Squamous | 44 | 135 |
| Small cell | 44 | 43 |
| Metastasis | 38 | 59 |

### 1577 Germ Cell Tumors with Associated Vascular Neoplasia: Report of 14 Cases

*NP Agaram, TM Ulbright.* Indiana University-Perdue University Indianapolis, Indianapolis, IN.

**Background:** Germ cell tumors rarely develop sarcomas, most commonly rhabdomyosarcoma. Vascular neoplasia in a germ cell tumor is rare and may range from relatively benign appearing proliferations to frank angiosarcomas. In reviewing our experience, we attempt to define the spectrum of vascular neoplasia arising in germ cell tumors.

**Design:** We searched our pathology information system for germ cell tumors with associated vascular lesions. In addition, personal consultation files of one of us were searched for similar cases. The slides were reviewed to confirm the diagnosis and the medical records were reviewed for additional follow up information.

**Results:** Seventeen cases from 14 patients were identified from 2000 -2008. All were males and the age range was 24 – 46 years, with a median of 29 years. Eleven (79%) were primary mediastinal germ cell tumors and 3 (21%) were primary testicular germ cell tumors. After review, 7 cases (41%) were diagnosed as angiosarcoma, 9 (64%) as atypical vascular proliferation and 1 (5%) as prominent vascular proliferation. Angiosarcoma was the only sarcomatous differentiation diagnosed in all cases except for 1 case that had other sarcomatous elements. Histologically, many cases demonstrated a myxoid angioblastic mesenchyme wherein neoplastic endothelium appeared to derive from atypical stromal cells and gradually coalesced into recognizable vessels. Follow up from 1 month to 5 years was available in 12 patients. 8/12 (67%) developed metastasis, confirmed as angiosarcoma in 3cases (all of which had an initial diagnosis of atypical vascular proliferation). Two patients with died of postoperative complications and 2 patients are disease free.

**Conclusions:** Vascular neoplasia in germ cell tumors occurs predominantly in the mediastinum. They typically range from dysplastic-appearing vessels in myxomatous stroma to angiosarcoma. Germ cell tumors with angiosarcoma have increased rates of metastases and a poor prognosis.

### 1578 Is TTF-1 a Predictor of EGFR Mutation in Primary Adenocarcinoma of the Lung?

*MB Alikhan, V Reddy, J Coon, P Bonomi, L Buckingham, P Gattuso.* Rush University Medical Center, Chicago, IL.

**Background:** A recent abstract suggested that expression of thyroid transcription factor-1 (TTF-1) in primary lung adenocarcinomas is a strong predictor of epithelial growth factor receptor (EGFR) mutation and response to tyrosine kinase inhibitor (TKI) treatment. We undertook a retrospective study of 24 patients with Stage IV primary lung adenocarcinoma treated with a TKI, gefitinib, to assess if the expression of TTF-1 is a strong indicator of EGFR mutation and patients' outcome.

**Design:** Paraffin embedded material from 24 patients with primary lung adenocarcinoma, who were treated with TKI in a compassionate use setting, were stained with standard immunoperoxidase technique for the expression of TTF-1. EGFR mutation status for exons 19 and 21, covering 90% of mutations, was determined previously (Buckingham L, et al. *J Thoracic Oncol*. 2:414-422, 2007). The results of TTF-1 were compared to EGFR mutations and response to treatment.

**Results:** There were 13 female and 11 male subjects. The age range was between 43 and 85 years of age with a mean of 64. All patients were clinical cancer stage IV when treatment with TKI started. The overall expression of TTF-1 was 15 out of the 24 cases (63%). 4 patients had EGFR mutations (17%), all in exon 19. The patient population was divided into three groups: Group A had EGFR mutation and TTF-1 expression (4 patients); Group B had TTF-1 expression but no EGFR expression (11 patients); Group C had neither TTF-1 nor EGFR expression (9 patients). Group A included two patients who showed partial clinical response, one with stable disease, and one with progression on TKI. All patients died between 25 days and 39 months after beginning treatment, with a mean survival of 9 months. Group B patients included one with partial response, 6 with stable disease, and 4 with progression on TKI. All patients died between 2 and 64 months after starting treatment, with a mean survival of 28 months. Group C included one patient with partial response, 5 with stable disease, and 3 patients with progression on TKI. All patients died between one and 34 months with a mean survival of 10.5 months.

**Conclusions:** 1) TTF-1 expression in our study was not a significant predictor of EGFR mutations since only 4/15 TTF-1 positive tumors (27%) showed EGFR mutation. 2) Despite the small size of the study population, it appears that patients with TTF-1 positive stage IV adenocarcinoma of the lung without concomitant EGFR mutations (Group B) may still benefit from TKI treatment (mean survival of 28 months), whereas Group C may not.

### 1579 T Cell Receptor (TCR) Vβ Repertoire Clonality Screening in Lung Transplant Bronchoalveolar Lavage (BAL) Specimens Using a Simple One Step PCR Method

*RW Allan, SM Shunkwiler, ZC Kou, CS Dadisman, IM Bovio, MA Baz.* Univ. Florida, Gainesville, FL; BioMed Immunotech, Tampa, FL.

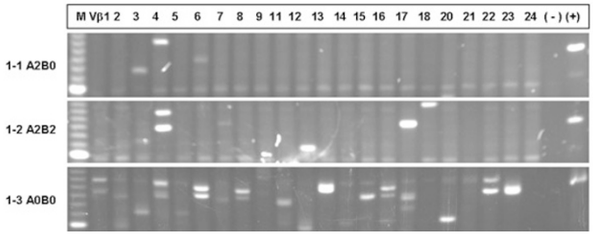
**Background:** Clinical management of lung transplant patients requires accurate, timely diagnosis and treatment of rejection. Transbronchial biopsies are performed for the monitoring of rejection though the method is invasive and is associated with complications. BAL is routinely performed at bronchoscopy on transplant patients. BAL fluid contains abundant graft infiltrating lymphocytes; therefore this may be monitored for the development of allograft reactive T cells. We sought to determine if a simple, clinically applicable assay, SuperTCRExpress$^{tm}$ TCR clonality detection [S-TCREx] (BioMed Immunotech, FL), could be used to diagnose rejection by identifying restricted TCR Vβ usage in BAL lymphocytes. S-TCREx uses a one step-PCR and a simple agarose gel based assay to determine clonality of the 22 individual Vb families.

**Design:** Five lung transplant patients were selected at three time points with differing degrees of rejection at transbronchial biopsy. A total of 15 BAL samples were retrieved from a frozen bank (5 patients x 3 time points) and analyzed using the S-TCREx method and visualized on 4% high resolution agarose gels. For each sample the individual Vβ family usage was scored as: no amplified signal, clonal (1 discreet band), oligoclonal (>1 discreet band), or polyclonal (ill defined band).

**Results:** S-TCREx yielded amplified bands from frozen banked BAL samples in all samples. The total number of amplified Vβ families ranged from 3 to 20, in 3/5 patients cellular rejection was associated with a restricted Vβ repertoire (<10 amplified families). In all samples there was a range of clonal (5%-67%), oligoclonal (17-75%) and polyclonal (0-70%) Vβ families between patients (Fig 1).

**Conclusions:** Restricted Vβ repertoire and clonal/oligoclonal T cell expansion within Vβ families are common in transplant BAL samples. At at cutoff of ≥50% of the amplified Vβ families showing clonal bands, there was an association with clinically significant acute cellular rejection (A2) ($p=0.01$, Fisher exact test, Sensitivity 75%, Specificity 100%) using ROC analysis.

| PT- BX | REJECTION | CLONAL Vβ (%) | OLIGOCLONAL Vβ (%) | POLYCLONAL Vβ (%) |
|---|---|---|---|---|
| 1-1 | A2B0 | 2/3 (67%) | 1/3 (33%) | 0/3 (0%) |
| 1-2 | A2B2 | 3/6 (50%) | 1/6 (17%) | 2/6 (33%) |
| 1-3 | A0B0 | 2/20 (10%) | 15/20(75%) | 3/20 (15%) |
| 2-1 | A2B0 | 3/20 (15%) | 7/20 (35%) | 10/20 (50%) |
| 2-2 | A2B0 | 4/19 (21%) | 5/19 (26%) | 10/19 (53%) |
| 2-3 | A0B1 | 4/17 (24%) | 10/17 (59%) | 3/17 (18%) |
| 3-1 | A2B0 | 9/17 (53%) | 8/17 (47%) | 0/17 (0%) |
| 3-2 | A2B0 | 11/17 (65%) | 3/17 (18%) | 3/17 (18%) |
| 3-3 | A0B0 | 5/16 (31%) | 6/16 (38%) | 5/16 (31%) |
| 4-1 | A2B0 | 6/8 (75%) | 2/8 (25%) | 0/8 (0%) |
| 4-2 | A0B0 | 3/10 (30%) | 7/10 (70%) | 0/10 (0%) |
| 4-3 | A1B0 | 6/16 (38%) | 9/16 (56%) | 1/16 (6%) |
| 5-1 | A2B0 | 5/10 (50%) | 4/10 (40%) | 1/10 (10%) |
| 5-2 | A1B0 | 4/13 (31%) | 6/13 (46%) | 3/13 (23%) |
| 5-3 | A1B0 | 1/20 (5%) | 5/20 (25%) | 14/20 (70%) |



### 1580 S100P Expression in 449 Non-Small Cell Lung Carcinomas (NSCLCs): A Potential Diagnostic and Prognostic Marker

*TC Allen, PT Cagle, LR Chirieac, S Dacic, Q Zhai.* The University of Texas Health Science Center at Tyler, Tyler, TX; The Methodist Hospital, Houston, TX; Brigham and Women's Hospital and Harvard Medical School, Boston, MA; University of Pittsburg School of Medicine, Pittsburg, PA.

**Background:** S100P, a member of the S100 family of calcium-binding proteins, is involved in cell growth and differentiation, cell cycle regulation, and metabolic control. It has been shown to be upregulated in malignant neoplasms, including lung adenocarcinoma (AD). NSCLC has an overall poor prognosis, even in surgically-resectable patients, and better diagnostic and prognostic markers are needed. We studied S100P expression in 449 NSCLCs.

**Design:** Tissue microarrays from three institutions containing 3 punches each of 449 NSCLCs collected over the last three decades were stained with S100P (1:100 AbKam, UK). Immunopositivity in tumor cells was graded as 0 = no staining, 1 = weak, 2 = moderate, and 3 = strong.

**Results:** Of the 449 cases (254 male; 195 female), 312 AD, of which 45 were bronchioloalveolar carcinomas with no or minimal invasion (AD-BAC), 80 squamous cell carcinomas (SCC), 57 large cell carcinomas (LCC)), 175 (39%) showed nuclear immunopositivity: 137/312 AD (44%); 23/80 SCC (29%); 15/57 LCC (26%). Staining showed 54 (39%) weak (34 AD, 13 SCC, 7 LCC); 36 moderate (28 AD, 4 SCC, 4 LCC); 85 strong (75 AD, 6 SCC, 4 LCC). Moderate/strong staining: 103/312 (33%) AD, 10/80 (12%) SCC, 8/57 (14%) LCC. 25/45 (55%) AD-BAC were negative/weak; 20/45 (45%) moderate/strong. Of negative/weak AD, 25/209 (12%) were AD-BAC; of moderate/strong AD, 20/85 (16% were AD-BAC.

**Conclusions:** AD were more likely to show moderate/strong S100P staining than other NSCLCs, and almost half of AD-BAC showed moderate/strong staining. Recent studies have shown that increased percentage of bronchioloalveolar component predicts a better prognosis in lung adenocarcinomas (Castro et al. Ann Diagn Pathol. 2001;5:274-84). S100P may potentially serve as a diagnostic and prognostic marker of AD-BAC. Further studies may help determine if S100P expression can help differentiate atypical adenomatous hyperplasia from AD-BAC.

### 1581 Glypican-3 Preferentially Stains Lung Squamous Cell Carcinomas: A Microarray Study of 479 Non-Small Cell Lung Carcinomas (NSCLCs)

*TC Allen, PT Cagle, S Dacic, LR Chirieac, Q Zhai.* The University of Texas Health Science Center at Tyler, Tyler, TX; The Methodist Hospital, Houston, TX; University of Pittsburg School of Medicine, Pittsburg, PA; Brigham and Women's Hospital and Harvard Medical School, Boston, MA.

**Background:** Glypican-3, a membrane-bound heparan sulfate proteoglycan whose expression is linked to malignancies through the existence of both mutations and aberrant protein expression, has been identified in hepatocellular carcinoma, chromophobe renal cell carcinoma, liposarcoma, testicular germ cell tumor, and lung squamous cell carcinoma. Extensive studies of Glypican-3 in lung cancer have not been performed. NSCLC prognosis is poor, even in surgically-resectable patients, and improved diagnostic and prognostic markers are needed. We studied Glypican-3 expression in 479 NSCLCs.

**Design:** Tissue microarrays from three institutions containing 3 punches each of 479 NSCLCs collected over the last three decades were stained with Glypican-3 (1:100 BioMosaics, Burlington, VT). Immunopositivity in tumor cells was graded as 0 = no staining, 1 = weak, 2 = moderate, and 3 = strong.

**Results:** Of the 479 cases (265 male; 214 female; 335 adenocarcinomas (AD), 81 squamous cell carcinomas (SCC), 63 large cell carcinomas (LCC)), 41 (9%) showed cytoplasmic immunopositivity; 30 (11%) male; 11 (5%) female. SCC = 28/81 (35%); AD = 5/335 (1%); LCC = 5/63 (8%). SCC = 24 male, 4 female; 14 weak, 11 moderate, 3 strong; AD = 3 male, 2 female; 2 weak, 4 moderate, 3 strong; LCC = 4 male, 1 female; 2 weak, 2 moderate, 1 strong. No correlation with age or smoking history was found.

**Conclusions:** Glypican-3 was immunopositive in approximately one third of SCC, whereas fewer than 2% of AD were positive. Glypican-3 expression in lung SCC suggests a role for it in early lung cancer detection, and its therapeutic manipulation could potentially be a basis for future NSCLC treatment.

### 1582 A Prognostically Significant Grading System for Lung Adenocarcinoma

*JA Barletta, LR Chirieac.* Brigham and Women's Hospital, Boston, MA.

**Background:** Grading is a standard component of the pathology report for many tumor types and typically incorporates evaluation of tumor architecture, cytologic atypia, and for some tumors, mitotic count. Although grading has been shown to predict outcome for many tumor types, a prognostically significant grading scheme has not been established for lung adenocarcinoma. The aim of this study was to evaluate the prognostic value of each histologic parameter of tumor grading and to develop a prognostically significant grading system for lung adenocarcinoma.

**Design:** We studied lung adenocarcinomas from 85 consecutive patients. Tumors were evaluated for the percentage of solid growth pattern, the degree of cytologic atypia, and the mitotic count. For each parameter we determined the optimal cut-off value with the strongest association with overall survival. Based on the results of this analysis, tumor architecture was scored as 1 = solid growth pattern ≤80% or 2 = solid growth pattern >80% and cytologic atypia was scored as 1 = tumors with uniform nuclei or 2 = tumors with bizarre, enlarged nuclei of varied sizes. Because the mitotic count was not predictive of outcome (p=0.11), it was not incorporated into the scoring system. A total grading score was computed as the sum of the architecture score and cytologic atypia score (score 2 = well-differentiated, score 3 = moderately-differentiated, score 4 = poorly-differentiated). We compared the total grading scores with overall survival.

**Results:** The percentage of solid growth pattern and the degree of cytologic atypia were both statistically significant predictors of outcome. Patients with tumors with ≤80% solid growth had a median overall survival of 67.1 months and those with tumors with >80% solid growth had a median overall survival of 20.1 months (p=0.002). The median overall survival was 69.4 months for patients with tumors with mild cytologic atypia and 32.1 months for patients with tumors with severe cytologic atypia (p=0.01). The total grading score was a significant predictor of overall survival: patients with well-differentiated tumors had a median survival of 69.4 months, patients with moderately-differentiated tumors had a median survival of 39.5 months, and those with poorly-differentiated tumors had a median survival of 9.9 months (p<0.0001).

**Conclusions:** We have described a prognostically significant grading system for lung adenocarcinoma that incorporates the percentage of solid growth pattern and the degree of cytologic atypia. Additional prospective studies are needed to validate this grading system.

### 1583 Ki-67 Expression in Pulmonary Neuroendocrine Tumors

*JA Barletta, L Zellos, K Law, D Sugarbaker, S Rodig, LR Chirieac.* Brigham and Women's Hospital, Boston, MA.

**Background:** Mitotic count is an important component of the classification of pulmonary neuroendocrine tumors, but it can sometimes be difficult to evaluate. Immunohistochemical staining for Ki-67 is a robust and widely accepted method for evaluating proliferative activity in a variety of tumors; however, the association between Ki-67 and mitotic activity in pulmonary neuroendocrine tumors remains unclear. We evaluated the percentage of Ki-67 positive cells in pulmonary neuroendocrine tumors and investigated the association between the immunohistochemical expression of Ki-67 and the number of mitoses.

**Design:** We studied 92 pulmonary neuroendocrine tumors that included carcinoid tumorlets (CTs), typical carcinoid tumors (TCs), atypical carcinoid tumors (ACs), and large cell neuroendocrine carcinomas (LCNECs). All H&E slides (median 9, range 2-19) for cases were evaluated for mitotic count/10 hpf and the presence of necrosis. Ki-67 expression by immunohistochemistry (IHC) was assessed by a pathologist on a tissue microarray constructed from representative sections of each tumor. For each case the percentage of Ki-67 positive cells in the area with the highest percentage of positive cells was recorded. Expression of Ki-67 was also evaluated using immunofluorescence by digital image analysis (AQUA) of whole mount histopathologic sections (157 fields assessed for 13 cases).

**Results:** The mean mitotic count/10 hpf was 0.3 for TCs, 3.7 for ACs, and 21.0 for LCNECs. The mean Ki-67 expression was 1.1% for TCs, 3.6% for ACs, and 18.5% for LCNECs (p=0.007). Ki-67 expression by IHC was highly correlated with the mitotic count (Pearson r =0.77, p<0.0001). Moreover, immunohistochemical assessment of Ki-67 expression by a pathologist was highly correlated with Ki-67 expression assessed by quantitative AQUA image analysis (Pearson r=0.8, p<0.0001).

**Conclusions:** We found that there are significant differences in Ki-67 expression by IHC between the diagnostic categories of pulmonary neuroendocrine tumors. In addition, we demonstrated that immunohistochemical expression of Ki-67 assessed by a pathologist correlates with Ki-67 expression evaluated by quantitative AQUA image analysis and is highly correlated with the mitotic count in pulmonary neuroendocrine tumors.

### 1584 Survival in Lung Adenocarcinoma and Mesothelioma Is Influenced by Ig-Like Transcript 3 Expressing Macrophages

*NT Beaubier, CC Chang, S Alexander, N Suciu-Foca, AC Borczuk.* Columbia University Medical Center, New York, NY.

**Background:** Ig-like transcript 3 (ILT3) is a membrane bound receptor on antigen presenting cells (APC) that can sometimes be detected in soluble form. ILT3 induces CD8+ T suppressor cells, which in turn may be responsible for an ineffective immune response to neoplastic cells. Published reports have linked ILT3 expression to a diminished immune response to melanoma and gastrointestinal tract tumors. However, the relationship of ILT3 to survival in lung adenocarcinoma (AdCa) and malignant mesothelioma (MM) has not previously been studied.

**Design:** Tissue microarrays of clinically annotated lung AdCa (n = 167) and MM (n = 68) were immunostained with antibody to ILT3 and graded from 0 to 3 in the tumor cells, non-tumor stromal cells, and cells with dendritic morphology (dendritic pattern). Association with survival was calculated using Cox regression analysis and Kaplan-Meier curves with log rank statistic (SPSS version 16.0). Correlation with lymph node metastasis in lung tumors was also performed.

**Results:** Kaplan-Meier survival analysis showed that in lung AdCa the cases with the ILT3 positive dendritic pattern had a median survival of 1903 days while the cases without an ILT3 dendritic pattern had a significantly longer (p = 0.005) survival of 3200 days. In MM the survival time was 402 days for dendritic pattern and 1458 days in the absence of a dendritic pattern (p = 0.003). In addition, in lung AdCa there was a correlation between the dendritic pattern and lymph node metastases (Pearson chi-square = 5.5, p < 0.02). ILT3 tumor cell immunostaining was weak to absent overall and not correlated with survival. Non-tumor stromal staining was associated with survival in lung AdCa (HR 1.45 CI 1.01-2.06, p = 0.04), but not in MM. ILT3 immunostaining in alveolar macrophages was not associated with survival in lung AdCa. The dendritic pattern of immunostaining was associated with overall survival in lung AdCa (HR 1.24 CI 1.06-1.45, p = 0.007), and the association was significant in the subgroups of stage I&II tumors (n = 148), and stage I alone (n = 121). In MM the association between dendritic pattern and survival was also significant (HR 1.35 CI 1.08-1.69, p = 0.009).

**Conclusions:** These data show that the dendritic pattern of ILT3 expression by IHC is inversely correlated with survival in lung AdCa and MM. Since ILT3 is known to induce T suppressor cells, this result could be explained by the immune system becoming anergic to the tumor because of high ILT3 expression.

### 1585 Amphiregulin-2 Immunoreactivity Is Superior to *EGFR* FISH for Predicting Favorable Response to Tyrosine Kinase Inhibitors in *EGFR*-Wild Type Lung Adenocarcinoma

*J Beheshti, Y Xiao, K Yonesaka, D Jackman, V Joshi, C Lee, P Janne, N Lindeman.* Brigham and Women's Hospital, Boston, MA; Dana-Farber Cancer Institute, Boston, MA; Harvard Partners Center for Genetics and Genomics, Boston, MA; Harvard Medical School, Boston.

**Background:** Somatic mutations in the epidermal growth factor receptor (*EGFR*) are present in 20% of lung adenocarcinomas, where they confer a favorable response to treatment with anti-EGFR tyrosine kinase inhibitors (TKIs: erlotinib, gefitinib). However, in 15-25% of patients with favorable TKI response, no mutation in *EGFR* is detected. Some of these *EGFR*-wild type responsive tumors have increased immunohistochemical (IHC) expression of amphiregulin (AR), an alternative ligand for EGFR. It has also been suggested that increases in *EGFR* copy number may explain the clinical responses to TKIs in wild type tumors, but a direct comparison of *EGFR* FISH and AR IHC in these tumors has not been performed.

**Design:** A microarray with 48 lung adenocarcinomas from patients treated with TKIs was immunostained for AR and tested for *EGFR* mutation by PCR-sequencing and for *EGFR* copy number by FISH. FISH was scored by the method of Varella-Garcia, et al (Diag Pathol, 2006). An IHC score was calculated by multiplying the modal intensity (0-4+) by the % of tumor cells staining, to give a score from 0-400; >100 was considered positive. Test results were compared with clinical outcome, per RECIST criteria.

**Results:** 19 samples had wild type *EGFR* sequence, of which 13 had progression of disease on therapy and 6 had stable disease without progression; no responses were seen in *EGFR* wild type tumors. Of the six patients with stabilization of cancer on therapy, 5 (83%) had AR IHC positivity, while only 1 (17%) had *EGFR* FISH positivity. Of the 13 patients with disease progression, 7 had AR IHC positivity and 6 had *EGFR* FISH positivity. 6/7 (85%) cases with negative AR IHC had progressive disease.

**Conclusions:** In patients without *EGFR* mutations who are treated with TKI, positive IHC for AR was a sensitive (83%) test for disease stabilization, and negative AR IHC was a predictive (85%) marker of progression. If corroborated in larger studies, TKI therapy may benefit *EGFR* mutation negative patients with strong AR staining, but tumors with weak/no AR expression are likely to resist TKIs. *EGFR* copy number, as measured by FISH, is of no value in predicting likelihood of stable/progressive disease on TKI therapy in *EGFR* wild type tumors.

### 1586 MicroRNA-based Assay for Differential Diagnosis of Mesothelioma

*H Benjamin, D Lebanony, K Ashkenazi, S Tabak, I Leizerman, D Nonaka, S Morgenstern, Z Bentwich, S Rosenwald, A Chajut, D Cohen.* Rosetta Genomics, Rehovot, Israel; NYU School of Medicine, New York, NY; Rabin Medical Center Belinson Campus, Petach Tikva, Israel.

**Background:** Malignant mesothelioma is an aggressive pleural neoplasm, strongly linked to environmental exposure, particularly asbestos exposure. Mesothelioma in the lung pleura is difficult to differentiate from other tumors in the lung pleura, such as primary lung adenocarcinoma protruding into the pleural space and metastatic adenocarcinoma from other tissues in the same environment. Here we addressed the increasing need for accurate differential diagnosis of these tumors using expression profiles of microRNAs, a family of small, non-coding RNAs whose tissue-specificity has a proven applicability for the identification of cancer tissue type and histology.

**Design:** RNA was extracted from dozens of FFPE samples of mesothelioma, lung adenocarcinoma, and additional tumor types, using proprietary protocols that preserve the small RNA fraction. Initial profiling using microRNA microarrays identified potential microRNA biomarkers that can be used to differentiate mesothelioma from other tumors and tumor types. A qRT-PCR assay was developed that utilizes the microRNA markers for this differential diagnosis, and was validated using an independent blinded validation set.

**Results:** A small set of microRNA biomarkers was identified, that is strongly differentially expressed between mesothelioma, lung adenocarcinoma, and other confounding tumor types. A diagnostic qRT-PCR assay based on the expression level of the specific microRNAs in FFPE samples exceeded 90% accuracy in the differential diagnosis of mesothelioma on blinded validation samples.

**Conclusions:** MicroRNAs are highly efficient biomarkers for cancer diagnosis. A robust and simple assay based on the expression level of a few microRNA biomarkers can accurately differentiate mesothelioma from other possible tumors in the lung pleura.

### 1587 Comparative Analysis of Napsin-A and TTF-1 Expression in Non-Small Cell Lung Carcinoma, and Adenocarcinomas of the Breast, Pancreas and Colon

*J Bishop, R Sharma, P Illei.* Johns Hopkins Medical Institutions, Baltimore, MD.

**Background:** Owing to recent advances in the treatment of pumonary adenocarcinomas there is an increased need for accurate diagnosis of non-small cell lung cancer types. Immunohistochemistry for TTF-1 is widely used in the diagnosis of pulmonary adenocarcinomas since it marks approximately 75% of pulmonary adenocarcinomas and is negative in most other adenocarcinomas. Napsin-A is an aspartic proteinase involved in the maturation of surfactant protein B that is homologous with TA02, a polypeptide that is associated with primary lung adenocarcinomas. Napsin-A is expressed in the cytoplasm of epithelial cells of the lung and kidney and may be a good candidate marker for pulmonary adenocarcinomas.

**Design:** We have performed immunohistochemistry for TTF-1 (Cell Marque, USA) and Napsin-A (Novocastra, UK) on tissue microarrays (TMAs) of adenocarcinomas of the colon (5), pancreas (31), breast (17), as well as primary adenocarcinomas (95) and squamous cell carcinomas (47) of the lung. The TMAs also included 3 carcinoid, 1 atypical carcinoid tumors and 3 small cell carcinoma and 15 different benign tissues. The staining intensity was evaluated on a scale of 0-3+, and the extent as 1+-3+ (<25%, 25-50%, >50%).

**Results:** Pulmonary adenocarcinomas were TTF-1 positive in 67 of 95 cases (71%) and Napsin-A positive in 83 of 94 cases (88%). There were 2 TTF-1 positive and Napsin-A negative tumors increasing the number of cases that were positive with at least one of the markers to 85 of 94 (90%). All 47 squamous cell carcinomas and all adenocarcinomas of the colon, panceas and breast were negative for both markers. All benign tissues except for lung were negative for Napsin and TTF-1 (nuclear staining). In addition, weak cytplasmic TTF-1 staining was observed in samples from the GI tract, liver and kidney. In normal lung, Napsin-A positivity is seen type 2 pneumocytes and alveolar macrophages. The intensity and extent of Napsin-A and TTF-1 staining showed significant discordance in a subset of cases. TTF-1 was positive in 1 of 3 carcinoid, 1 of 1 atypical carcinoid and 1 of 3 small cell carcinomas, on the other hand, all neuroendocrine tumors were Napsin-A negative.

**Conclusions:** Immunohistochemistry for Napsin-A is a sensitive and specific marker for adenocarcinomas of lung origin. The combined use of TTF-1 and Napsin-A detects an increeased number adenocarcinomas than either marker alone, and based on a limited number of cases all pulmonary neuroendocrine tumors are Napsin-A negative.

### 1588 High Frequency of Gammaherpesvirus Infection in Idiopathic Pulmonary Fibrosis: Clinicopathological Correlations

*F Calabrese, F Lunardi, B Nordio, G Marulli, M Della Barbera, M Saetta, F Rea.* University of Padua Medical School, Padua, Italy.

**Background:** Idiopathic Pulmonary Fibrosis (IPF) is a progressive fibrotic interstitial lung disease of unknown etiology. Several studies reported chronic viral infection as a potential cause of lung injury in IPF particularly focusing on herpesvirus infection. The aim of the study was to investigate the presence of different types of viruses, many of them with a well known tropism for the respiratory system (Epstein-Barr virus-EBV-; cytomegalovirus-CMV-; human herpes virus 6-HHV6-; adenovirus; rhinovirus; influenza A and B viruses; parainfluenzae viruses; parvovirus B-19; metapneumovirus and respiratory syncytial virus).

**Design:** Frozen lung samples from 30 patients with end-stage IPF requiring lung transplantation (mean age 55.9; 20 males and 10 females) were analyzed by polymerase chain reaction (PCR) and reverse transcriptase-PCR for detecting respiratory viruses. Lung samples from 10 patients (5 non implanted donor lungs and 5 other forms of interstitial lung diseases) were used as control group. Molecular findings were then correlated with different clinico-functional and morphological data.

**Results:** A higher frequency of viral infection was detected in IPF patients than the control group (14/30, 46.6% versus 1/10, 10%; p=0.04). Genomes of gammaherpesviruses were the only ones detected (7 EBV; 6 HHV-6; 3 CMV; 2 patients had double infections).Viral IPF cases showed increased value of mean pulmonary arterial pressure (mPAP 35.1±6.9 versus 16.8±2.1; p=0.04) and partial pressure of $CO_2$ (43.8±2.1 versus 29.6±4.8; p=0.01) compared to the non viral IPF group. Muscular arteries were significantly thickened in viral compared to non viral IPF cases (58±11.1 versus 39.9±11.2; p=0.03).

**Conclusions:** Our data confirm the association between IPF and gammaherpesviruses. The higher values of mPAP and a more extensive vessel remodeling in viral IPF cases could be related to a well known endotheliotropism of gammaherpesviruses.

### 1589 Expression of Focal Adhesion Kinase (FAK), Phosphorylated Focal Adhesion Kinase (pFAK) and Paxillin in Malignant Pleural Mesothelioma (MPM)

*S Charoenthammaraksa, M Tretiakova, L Faoro, S Loganathan, R Salgia, T Krausz, AN Husain.* University of Chicago, Chicago.

**Background:** FAK and paxillin, focal adhesion proteins, function as positive regulators of cell adhesion and migration via integrin regulated signaling system. We have recently shown paxillin mutations and amplification in lung cancer. Overexpression of these proteins causes cell detachment and migration which is essential for tumor invasion and metastasis. This overexpression correlates with poor clinical outcome as has been reported in many metastatic carcinomas. The expression of FAK, pFAK and paxillin in MPM has not been previously investigated. The aim of our study is to analyze expression of these focal adhesion kinases in MPM compared to normal adjacent lung.

**Design:** With IRB approval, we used 2 tissue microarray (TMA) blocks containing a 1 mm in diameter tissue cores of MPM (50 epithelioid, 16 sarcomatoid, 1 mixed) and 40 normal adjacent lung parenchyma (pneumocytes and endothelial cells). The TMAs were sectioned and stained by IHC for FAK, pFAK and paxillin. Quantitative staining intensity was analyzed using automated cellular imaging system (ACIS) by calculating average intensity in 500 cells as an integrated optical density (IOD), which is directly proportional to antigen content in cytoplasmic compartment. Fisher's exact test and Pearson correlation were used for statistical analysis in SPSS software, v15.0.

**Results:**

| Histology | Number | FAK_IOD | pFAK_IOD | pFAK ratio | Paxillin_IOD |
|---|---|---|---|---|---|
| MPM, Epithelioid | 50 | 273.85 | 14.65 | 0.053 | 268.47 |
| MPM, Mixed | 1 | 231.4 | 8.07 | 0.0348 | 139.39 |
| MPM, Sarcomatoid | 16 | 218.78 | 12.61 | 0.057 | 331.17 |
| Normal Lung | 40 | 45.17 | 13.54 | 0.299 | 118.95 |

There was a significant increase in FAK and paxillin expression in MPM compared to normal adjacent lung ($p<0.01$). No significant difference in expression levels of FAK and paxillin between epithelioid and sarcomatoid MPM subtypes was found. High positive correlation between pFAK and paxillin at the 0.01 level (2-tailed) was observed.

**Conclusions:** Upregulation of FAK and paxillin was present in the majority of MPM of both epithelioid and sarcomatoid types suggesting that these molecules may be good candidates for targeted therapy. Furthermore, we found statistically significant

correlation between paxillin and pFAK expression levels. These findings may indicate importance of pFAK and paxillin overexpression in MPM progression, and require further studies in a larger pool of patients to correlate with survival.

### 1590 Pathology and PET-FDG Correlation Studies Determine False Positive and Negative Malignancies of the Lung

*C Chuang, D Raymond, JC Wandtke, H Xu.* University of Rcohester Medical Center, Rochester.

**Background:** Positron emission tomography (PET) with glucose analog F-18-flurodeoxyglucose (FDG-PET) is used to screen for tumors of the lung. It has been recognized that benign lung lesions can have an elevated FDG uptake with a standardized uptake value (SUV) >2.5 and low-grade cancers can have a SUV<2.5, but extensive analysis of neoplastic and non-neoplastic lesions of the lung correlated with FDG-PET studies are limited.

**Design:** 218 resected lung specimens from 1/2005 to 12/2007 were examined with their corresponding PET-CT scans. Of the 218, 34 were non-neoplastic [16 necrotizing granulomas (NG), 1 non-necrotizing granuloma (NNG), 14 pneumonias, 2 hemorrhages, 1 hyalinized nodule], 26 benign to low grade malignancies [1 hamartoma, 2 solitary fibrous tumors, 2 lymphomatoid granulomatosis, 7 carcinoids, 8 BACs, 7 well-differentiated (WD) adenocarcinomas (AC)] and 158 were moderately (MD) to poorly (PD) differentiated malignancies [77 ACs, 53 squamous carcinomas (SC), 6 adenosquamous carcinomas, 17 large cell carcinomas, 1 carcinosarcoma, 3 metastatic pleomorphic sarcomas]. SUV and size of the lesion determined by CT were obtained. Sensitivity and specificity were determined based on SUV>2.5, which is radiologically suspicious for malignancy. One-sided ANOVA correlated the histologic grade with the corresponding SUV.

**Results:** Sensitivity and specificity for suspected malignancies using SUV>2.5 cut-off was 0.87 (95% CI 0.81-0.91) and 0.58 (95% CI 0.41-0.74), respectively. Fifteen false positives (8 NGs, 1 NNG, 5 pneumonias, 1 hyalinized nodule) were non-neoplastic. There were 24 false negatives (3 carcinoids, 5 BACs, 2 WD ACs, 12 MD ACs, 2 MD SCs). A SUV>7.6 was 100% specific for malignancy. Utilizing criteria of SUV<2.5 and lesion size>12 mm as a predictor of benign pathology, the positive predictive value was 0.36 (95% CI 0.21-0.55) and the negative predictive value was 0.88 (95% CI 0.82-0.92). Mean SUVs of WD, MD, and PD carcinomas were 3.5 ($\sigma$ 3.2), 6.7 ($\sigma$ 4.2), 10.8 ($\sigma$ 5.9), respectively. One-sided ANOVA analysis of SUVs comparing the histologic grades of ACs and SCs showed a 3.2 SUV (95% CL 0.7-5.8) increase from WD to MD carcinoma and a 4.1 SUV (95% CL 2.2-5.9) increase from MD to PD carcinoma with a $p$ value <0.01.

**Conclusions:** SUV utilization in the lung as a screening tool, although effective, has numerous pitfalls. False positives were often observed in necrotizing granulomas and pneumonias. False negatives not only included low-grade neoplasms but also high-grade malignancies.

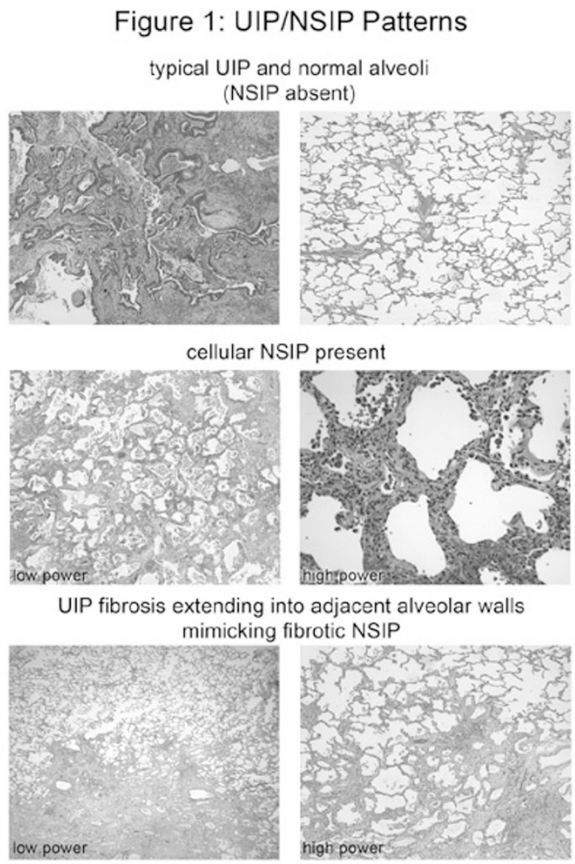
### 1591 Pieces of the Puzzle: Using Nonspecific Interstitial Pneumonia (NSIP) as a Clue to the Diagnosis of Connective Tissue Disease in Usual Interstitial Pneumonia (UIP)

*NA Cipriani, S Bhorade, E Garrity, W Vigneswaran, T Krausz, AN Husain.* University of Chicago, Chicago.

**Background:** UIP is the most common form of chronic interstitial lung disease, comprising up to 70% of cases. It can occur idiopathically (I-UIP) or in the setting of connective tissue disease (CTD-UIP). Patients with CTD have a better prognosis than those without, although both populations often undergo lung transplantation. Prior studies have demonstrated a preponderance of the NSIP pattern in CTD patients, or even coexisting NSIP and UIP. We aim to evaluate the utility of finding coexisting multi-lobar NSIP and UIP as a tool to aid in the differentiation between I-UIP and CTD-UIP.

**Design:** With IRB approval, explanted lung tissue from 8 patients (4 I-UIP, 4 CTD-UIP) was identified using an existing database: 2 I-UIP patients with bilateral explants, 1 with right and 1 with left-sided explants; 4 CTD-UIP patients with bilateral explants. Slides from all lobes were evaluated for the presence of NSIP in areas away from fibrosis/honeycombing.

**Results:** 1 of 4 I-UIP patients had cellular NSIP in all lobes and 1 had patchy fibrotic NSIP. 3 of 4 CTD-UIP patients had cellular NSIP in all lobes. In both groups, patients without multi-lobar NSIP demonstrated widened alveolar walls only in the areas adjacent to fibrosis, which was not seen in areas distant from fibrosis.




**Conclusions:** Although the presence of coexisting multi-lobar cellular NSIP is not exclusive to CTD-UIP patients, it does occur with a higher prevalence (25% in I-UIP versus 75% in CTD-UIP), and therefore may serve as a clue to the presence of CTD. Common to both groups are widened alveolar walls near areas of fibrosis, which may mimic fibrotic NSIP. Hence, alveoli near fibrotic/honeycombed areas cannot be used to evaluate for NSIP, and pathologists must be strict in examining pulmonary parenchyma at some distance from typical UIP fibrosis.

### 1592 Time after Time: The Temporal Progression of Usual Interstitial Pneumonia (UIP) from Biopsy to Explant or Autopsy

*NA Cipriani, M Strek, I Noth, J Charbeneau, JA Krishnan, T Krausz, AN Husain.* University of Chicago, Chicago.

**Background:** UIP is the most common form of interstitial lung disease, and has a 3-year median length of survival after diagnosis. The histologic appearance of end-stage UIP can be varied. Studies have previously shown evidence of acute lung injury, organizing pneumonia, type II pneumocyte hyperplasia and numerous, large fibroblastic foci. The presence of diffuse alveolar damage is inconsistently reported. Occasionally, UIP undergoes acceleration or acute exacerbation with no apparent inciting factor, with mortality as high as 50%. The natural history of UIP and the cause of this rapid deterioration remain elusive. We use a previously-reported quantitative methodology to evaluate the temporal progression of UIP from diagnosis to transplant or death.

**Design:** With IRB approval, lung tissue from 35 patients (23 biopsies, 8 explants, 4 autopsies) was evaluated: The number of fibroblastic foci (FF), lymphoid aggregates without (LA) and with germinal centers (GC) per total tissue area and the ratios of FF area, LA area, and GC area to total area were determined. The presence of superimposed diffuse alveolar damage (DAD) was recorded. Statistical analysis was performed using a Wilcoxon rank sum test.

**Results:** Compared to biopsy specimens, the explants had a higher FF count, higher FF area, similar LA count, higher LA area, and similar GC counts and areas. The difference in FF area approached statistical significance (p=0.08); none of the differences met statistical significance. The autopsy specimens were excluded from the quantitative analysis because fibroblastic foci were indistinct. None of the biopsy or explant specimens demonstrated DAD. All of the autopsy specimens demonstrated DAD, varying from mild organizing DAD with focal hyaline membranes to diffuse acute DAD with extensive hyaline membranes.

**Conclusions:** In this longitudinal study of the histology of UIP over time, late-stage UIP (explants) demonstrated increased numbers of larger fibroblastic foci and larger lymphoid aggregates compared to early-stage UIP (biopsies at diagnosis). Acute exacerbation was not a feature of biopsies or explants, but was uniformly present on autopsy specimens, suggesting superimposed acute lung injury as a terminal event. Furthermore, it is worthwhile to note that in the setting of DAD, fibroblastic foci are indistinct, making post-mortem diagnosis of UIP difficult.

### 1593 EGFR/KRAS Mutational Profiling of Lung Adenocarcinomas in a Clinical Practice

*S Dacic, SA Yousem, PN Ohori, M Nikiforova.* University of Pittsburgh, Pittsburgh.

**Background:** Screening for EGFR and KRAS mutations in patients with lung adenocarcinomas can be used to predict patient's response to EGFR TKIs, but there is a lack of guidelines for testing in a clinical practice. Significance of EGFR FISH testing is uncertain. Recent literature suggests possible correlation between morphology and EGFR/KRAS mutational profile of lung adenocarcinoma, which may be useful selection criteria for molecular testing. We analyzed morphologic and clinicopathologic characteristics of surgically treated primary lung adenocarcinomas in the absence of neoadjuvant or targeted therapies with respect to their EGFR and KRAS mutational profile and EGFR gene amplification.

**Design:** 337 consecutive newly diagnosed and surgically treated primary lung adenocarcinomas were assessed by PCR amplification and direct nucleotide sequencing for presence of mutations in EGFR exons 19 and 21 and KRAS codons 12/13. Each case was analyzed by FISH for EGFR using a standard method. Amplification was defined as EGFR gene to chromosome 7 ratio ≥ 2. High polysomy was defined as 4 or more EGFR gene copies in ≥40% of the cells. The results were correlated with tumor morphology and clinicopathologic characteristics including tumor stage, size, presence of scar, inflammatory response, angiolymphatic and pleural invasion.

**Results:** Mutational analysis demonstrated 33 (10%) EGFR+, 78 (23%) KRAS+ and 226 (67%) EGFR-/KRAS- lung adenocarcinomas. EGFR mutations were more frequently seen in women (77%), while equal sex distribution was observed in KRAS+ and EGFR-/KRAS- tumors. Mixed type of adenocarcinoma was the most common histologic type observed in all 3 groups. The primary pattern in EGFR + group was acinar (50%), while papillary (31%) was the most common secondary pattern. The primary and secondary patterns in KRAS+ group were acinar (27% and 32%). Similarly, acinar type was the most common in EGFR-/KRAS- group (42% primary; 29 % secondary). EGFR amplification was seen in only 5 EGFR + tumors which were all positive for exon 19 deletion. 1 KRAS + and 3 EGFR-/KRAS-tumors showed EGFR amplification. High polysomy was seen in 6 EGFR + tumors, 6 KRAS + and 17 EGFR-/KRAS- tumors. There was no difference between the tumor groups in respect to analyzed clinicopathologic characteristics.

**Conclusions:** Histologic type of lung adenocarcinoma and EGFR FISH status are poor predictors of tumor mutational profile. Determination of KRAS mutational status as the first step, followed by EGFR mutational analysis if necessary, should be considered in a clinical practice.

### 1594 miRNA Expression Profiling of Lung Adenocarcinomas: Correlation with Mutational Status

*S Dacic, S Chiosea, L Aspden, MN Nikiforova.* University of Pittsburgh, Pittsburgh, PA.

**Background:** MicroRNA (miRNA) expression is deregulated in many types of human cancers and expression of some miRNAs is associated with prognosis and survival. In this study we investigated the miRNA expression patterns in lung adenocarcinomas with different oncogenic mutations.

**Design:** 2 KRAS+, 2 EGFR + and 2 KRAS-/EGFR- stage matched primary lung adenocarcinomas and corresponding normal lung tissues were analyzed for expression levels of 328 human mature miRNAs. Total RNA was extracted from frozen tissue using Trizol reagent (Invitrogen). Quality of RNA was evaluated on 2100 Bioanalyzer (Agilent). Expression profiling was performed using Flexmir MicroRNA Human Panel (Exiqon) and analyzed on Luminex 200. Analysis of miRNA expression was performed using Luminex IS™ software v.2.3 (Luminex) relatively to normal lung tissue.

**Results:** Overall, all tumor groups revealed similar miRNA expression profiles, with most highly overexpressed miRNAs being miR-18b, miR-20a, and miR-328. Similarly, miR-32, miR-137 and miR-342 were most downregulated in all tumors. However, a number of miRNAs were differentially expressed between the groups: miR-25 and miR-30a-3p were significantly more overexpressed in EGFR + tumors (>12 folds) and miR-495 in KRAS + tumors (>10 folds). In KRAS-/EGFR- tumors, miR-155 and miR-154 were upregulated more than 12 folds whereas found at normal levels in KRAS and EGFR positive tumors. Expression of miR-155 and let-7 is of particular interest since it has been shown to correlate with poor prognosis in lung cancer. In our study, EGFR+ tumors demonstrated normal levels of miRNA-155 and down regulation of let-7g; KRAS-/EGFR- tumors showed severe upregulation of miR-155 and down regulation of let-7g and KRAS + positive didn't demonstrate deregulation of these miRNAs.

**Conclusions:** Our results indicate that number of miRNAs was differentially expressed in lung adenocarcinomas. Correlation between expression of several miRNAs and somatic mutations was found. miR-155 and let-7g (negative prognostic factors in lung cancer) were differentially expressed in tumors positive and negative for EGFR mutations, but remained at normal levels in KRAS positive tumors. Larger number of cases should be analyzed to validate potential role of miRNAs expression in prediction of lung adenocarcinomas mutational profile.

### 1595 Clear Cell Change in Lung Adenocarcinoma: A Cytologic Change Rather Than a Histologic Variant

*CG Deshpande, A Yoshizawa, N Motoi, W Gerald, M Kris, B Park, V Rusch, WD Travis.* Memorial Sloan-Kettering Cancer Center, New York, NY; Thoracic Surgery.

**Background:** Clear cell change has been recognized as a variant of lung adenocarcinoma (AD) in the 1999 and 2004 WHO classifications. However, clear cell changes have not been well characterized in the spectrum of adenocarcinoma histologic subtypes and the clinical significance is unknown.

**Design:** We reviewed 560 stage I lung adenocarcinoma to determine the frequency and clinical significance of clear cell change. Histologic subtyping was evaluated by comprehensive semiquantitation and classified by major subtype. Statistical correlations were made with SPSS v 16.

**Results:** Clear cell change was seen in 54 of 560 (9.6%) adenocarcinomas. There were 33F and 21M and mean age was 69 (46-90) years; no significant differences were found with sex and age in non-clear cell change adenocarcinoma patients. Clear cell change was present in 12/166 papillary (7.2%), 2/58 bronchioloalveolar (BAC) (3.4%) and 25/245 acinar (10.2%) adenocarcinoma. It was not seen in 10 colloid and 2 mucinous cystadenocarcinomas. Clear cell change was significantly greater in 15/79 solid adenocarcinomas (19%) compared to other subtypes 39/481 (8.1%) (p=0.002). Tumors with clear cell change were larger (3.0 cm) than those without it (2.4 cm, p=0.002). Clear cell changes affected 5 and 10% of the BAC component, 5-90% of the papillary, 5-90% of the acinar and 5-90% of the solid component of the adenocarcinoma. Five year survival for patients with some clear cell change in their adenocarcinoma was 93% which was not significantly different than for those without clear cell change: 85%, (p=0.51).

**Conclusions:** Clear cell change can occur in any of the four major subtypes of lung adenocarcinoma. It is present significantly more often in solid adenocarcinoma and larger tumors but it does not appear to have an impact on survival. We recommend that the next revision of the WHO classification drop the variant of clear cell adenocarcinoma and that clear cell changes be recognized when observed as a cytologic change rather than a separate category of lung adenocarcinoma.

### 1596 Grading of Lung Adenocarcinoma: Architectural Versus Nuclear Approach

*CG Deshpande, K Geisinger, I Petersen, A Moreira, M Zakowski, R Shen, J Suh, N Motoi, W Gerald, M Kris, V Rusch, WD Travis.* Memorial Sloan-Kettering Cancer Center, New York, NY; Wake Forest University, Winston-Salem, NC; Universitätsklinikum Jena, Germany; Stonybrook Stony Brook University Medical Center, Stonybrook, NY.

**Background:** Well established grading systems exist for carcinomas arising in the breast, prostate & kidney. However, there is no widely accepted detailed grading system for non-small cell lung carcinoma including adenocarcinoma.

**Design:** We reviewed 98 lung adenocarcinomas to evaluate architectural & nuclear (size, nucleoli, pleomorphism & mitoses) approaches to grading in tissue sections. Architectural grading was performed using semiquantitation of histologic subtypes and with classification by major subtype. Bronchioloalveolar (BAC) was compared to solid and a category of "glands & papillae" with a combination of acinar/papillary/micropapillary patterns. A subset were evaluated by 5 pathologists for reproducibility analysis with Fleiss kappa. Statistical correlations were made between grading features and survival, EGFR and KRAS mutation data & RNA gene expression profiling data (Affymetrix, HG-U133) using R software and SPSS v 16.

**Results:** Architectural grading showed moderate reproducibility (0.54, N=44) and significant prognostic correlation with solid vs BAC/acinar/papillary/micropapillary tumors demonstrating a 46% vs a 64% 5-year survival (p=0.014). Nuclear grading showed slight reproducibility for nuclear size (0.06, N=44), pleomorphism (0.09, N=45) and nucleoli (0.14, N=45). Mitotic count showed fair reproducibility (0.36, N=45). No survival correlations were found with nuclear features. All 15 EGFR mutations were found in acinar/papillary/micropapillary tumors 15/67 (22.4%) rather than BAC (0/8) or solid (0/22) tumors (p=0.014, Fisher exact test). Solid pattern was associated with gene expression profiling Cluster 3 (p=0.001) and BAC was associated with Cluster 2 (p=0.005). No correlation was found with KRAS mutation.

**Conclusions:** Architectural grading largely based on histologic subtyping shows moderate reproducibility and is useful for predicting prognosis. It also demonstrates significant correlations with EGFR status & gene expression profiling clusters. Nuclear grading shows only slight reproducibility & we could not find any survival or molecular correlations. Further work is needed to determine if grading (eg. combined architecture & nuclei) provides any advantage to histologic subtyping with predicting prognosis.

### 1597 Evaluation of microRNA Markers To Predict Outcome in Low Stage Lung Cancer

*EJ Duncavage, B Goodgame, R Govindin, JD Pfeifer.* Washington University, Saint Louis, MO.

**Background:** Low stage (I and II) non-small cell lung cancer (LS-NSCLC) currently presents a major treatment dilemma. While the overall 5 year survival rate is around 70%, approximately half of all patients who undergo initial surgical resection will die from their disease. However, as prognostic markers for LS-NSCLC are lacking, many patients receive unnecessary chemotherapy with its side effects. By measuring miRNA expression, which can control hundreds of downstream targets, we aim to discover its relationship to patient outcome.

**Design:** We selected a set of 5 miRNAs and one control miRNA from previous studies that linked outcome or invasiveness to expression in carcinomas of the lung and other organ systems. Of the 5 experimental miRNAs, increased expression of 3 (*hsa-miR-155*, *hsa-mir-210*, and *hsa-miR-21*) has been associated with a poor outcome/invasiveness. In contrast, decreased expression of 2 selected miRNAs (*hsa-let-7a* and *hsa-mir-221*) has been associated with invasiveness. Using a set 44 patients with surgically resected T1 or T2 LS-NSCLC and an average follow-up of 7 years, we measured expression of the selected miRNAs by Real Time RT-PCR. Briefly, comparable areas of viable cellular tumor were punched from paraffin blocks. RNA extracted and quantitated. Approximately 1-10ng of RNA from each sample was reverse transcribed using primers specific to each miRNA. The resulting cDNA was then assayed by Real Time PCR on an ABI 7500 using TaqMan FAM labeled primers. Expression was normalized to *rnu-6B*.

**Results:** Triplicate data on a subset of patients was averaged and correlated with outcome using Pearson correlation coefficients. One miRNA, *hsa-miR-210*, showed a direct relationship with recurrence that approached significance (p=0.056), consistent with previous reports. Expression of *hsa-miR-21* showed significant correlation with levels *of hsa-miR-155* (p=0.030) and *hsa-miR-210* (p=0.0018).

**Conclusions:** As the role of the more than 800 human miRNAs becomes better understood, it is clear that they play an important part in carcinogenesis. We have shown that increased expression of one miRNA, *hsa-miR-210*, is correlated with recurrence in LS-NSCLC as it is in carcinomas of other organs. In addition the 3 miRNAs associated with recurrence are correlated with one another. We aim to uncover further relationships as we add additional patients to the study.

### 1598 High Grade Neuroendocrine Carcinomas of the Lung Highly Express EZH2 but Carcinoids Do Not

*J Findeis-Hosey, J Huang, F Li, Q Yang, LA McMahon, H Xu.* University of Rochester Medical Center, Rochester, NY.

**Background:** Enhancer of zeste homolog 2 (EZH2) is the catalytic subunit of polycomb repressive complex 2 with histone methyltransferase activity. EZH2 has been shown to be important in transcriptional regulation and overexpressed in a number of malignancies, including prostate cancer, breast cancer, hepatocellular carcinoma, oral mucosal squamous cell carcinoma and melanoma. The aim of this study was to determine if there is differential EZH2 expression in a spectrum of neuroendocrine tumors of the lung (NETL), including typical carcinoid (TC), atypical carcinoid (AC), and large and small cell neuroendocrine carcinoma (LCNEC, SCLC).

**Design:** Forty surgically resected NETL including 16 TCs, 6 ACs, 9 LCNECs, and 9 SCLCs were immunohistochemically studied using a monoclonal antibody against EZH2. Nuclear staining was considered positive. The percentage of positively stained tumor cells was recorded and the staining intensity was graded as weak, moderate, or strong. A case was considered positive if >5% of tumor cells had nuclear staining. A $p$ value of <0.05, as determined by two-tailed Fisher exact test, was considered statistically significant.

**Results:** Immunohistochemical studies showed 8 of 9 (88.9%) LCNECs were moderately to strongly positive for EZH2, with 6 demonstrating staining in >80% of tumor cells and 3 showing staining in 10-30% of tumor cells. All 9 SCLCs demonstrated moderate to strong nuclear staining with 8 showing >90% of tumor cells staining and 1 exhibiting staining in 60% of tumor cells. There was not a statistically significant difference in EZH2 staining between LCNEC and SCLC. In contrast, all 16 TCs and 6 ACs were considered negative for EZH2, although some demonstrated rare scattered individual EZH2 positive cells totaling <1% of tumor cells. In non-neoplastic lung, EZH2 immunoreactivity with a variable degree of staining intensity was detected in scattered cells within the basal layer of bronchiolar epithelium and germinal center lymphocytes. Pneumocytes and other stromal cells were completely negative.

**Conclusions:** EZH2 is strongly and diffusely expressed in LCNEC and SCLC but not in carcinoids. These findings indicate that EZH2 may play an important role in the regulation of biological behavior of LCNEC and SCLC. In addition, immunohistochemical detection of EZH2 expression may serve as a useful diagnostic tool in the distinction between high grade neuroendocrine carcinoma and carcinoid, in particular when the diagnostic tissue is limited in a small biopsy with crush artifact.

### 1599 Field Effect in Lung Carcinogenesis Associated with Pulmonary Fibrosis

*T Fujii, A Miyamoto, K Kishi, K Yoshimura, K Ohashi.* Toranomon Hospital, Tokyo, Japan; Okinaka Memorial Institute for Medical Research, Tokyo, Japan.

**Background:** Idiopathic pulmonary fibrosis (IPF)/usual interstitial pneumonia (UIP) is associated with high prevalence of lung carcinogenesis especially in smokers, which is somewhat reminiscent of the hypercarcinogenic state in chronic hepatitis. To investigate the potential genetic or epigenetic changes in IPF/UIP, we performed microsatellite instability assay using mono- or tetranucleotide markers and methylation-specific polymerase chain reaction (PCR) on video-assisted thoracoscopic lung biopsies.

**Design:** Five video-assisted thoracoscopic lung biopsies of IPF/UIP were included in the present study. Genomic DNA was extracted from formalin fixed paraffin embedded tissue sections that were manually dissected for cancer, non-neoplastic lung tissue with or without UIP, and/or lymph node (if available). The presence or absence of microsatellite instability was assessed by fragment analysis on capillary electrophoresis using fluorescent labeled primers for mono- or tetra-nucleotide repeat markers, which included the following loci: NR21, BAT26, BAT25, NR24, UT5037, MYCL1, D9S303, D21S1436, D20S82 and D2S443. Lymph node or lung tissue with no or minimal fibrosis were regarded as normal for microsatellite analysis. The status of promoter CpG methylation was examined by methylation-specific PCR after bisulfite conversion using primers corresponding to the unmethylated and methylated alleles of the following genes: APC, ATM, CDKN2A, DAPK, ECAD, hMLH1, MGMT, RASSF1A, TGFBR2 and TCF21.

**Results:** No microsatellite instability was identified in four cases of IPF/UIP with/without associated lung cancer. DNA methylation was identified in four of five cases with IPF/UIP.

| Patient (age/sex) | Diagnosis | Source | CDKN2A | DAPK | ECAD | RASSF1A | TCF21 |
|---|---|---|---|---|---|---|---|
| 1 (71/M) | IP ca(+) | N (lung) | U | U | U | U | U |
| | | IP | U | M | U | U | U |
| | | T (adenoca) | U | U | U | U | U |
| 2 (77/M) | IP ca(+) | N (lung) | U | U | ND | U | ND |
| | | IP | U | U | U | U | U |
| | | T (squamous ca) | U | U | ND | U | ND |
| 3 (69/M)† | IP | IP (slight) | U | ND | ND | U | ND |
| | | IP (severe) | U | M | U | U | U |
| 4 (56/M)† | IP | N (LN) | U | U | ND | ND | ND |
| | | IP (slight) | U | M | M | U | U |
| | | IP (severe) | U | M | ND | U | U |
| 5 (69/M) | IP | N (lung) | U | U | ND | ND | U |
| | | IP (slight) | U | U | ND | U | U |
| | | IP (severe) | U | M | U | ND | ND |

IP: pulmonary fibrosis; N: normal; T: tumor; U: unmethylaed; M: methylated; ND: not determined.

**Conclusions:** The presence of epigenetic alteration (DNA CpG methylation) may be involved in lung carcinogenesis in pulmonary fibrosis in this preliminary study.

### 1600 Diagnostic Accuracy of Malignant Pleural Effusion Cytology in 2008

*F Galateau-Salle, N Le Stang, V Abonnet.* CHU Caen, Caen, France.

**Background:** The reported diagnostic accuracy of pleural effusion cytology has varied from 60 to 95% for the diagnosis of metastatic carcinoma [MC] to unsatisfactory (30%) for mesothelioma [MM]. The aim of the study was to evaluate, in 2008 the diagnostic accuracy of conventional pleural effusion cytology [PEC] in our institution, and to assess the usefulness of p16/*CDKN2A* deletion by FISH as a diagnostic marker for the distinction between benign and malignant mesothelial cells.

**Design:** A series of 3483 exfoliative PEC and 1659 biopsies were collected from the department of Pathology of CHU Caen during the period 1998-2008. They were systematically analyzed and compared with clinical and or histological follow up. Additionnally a series of 143 patients with a diagnosis of MM and of 347 patients with a diagnosis of MC made either by cytology and /or by a biopsy were selected according to the French system of codification ADICAP code. A dual color FISH for p16/*CDKN2A* and chromosome 9 centromere was performed either on frozen or paraffin embedded cell blocks from 20 reactive mesothelial hyperplasia [RMH] and 17 positive effusions for MM.

**Results:** From the 3483 PEC, a diagnosis of malignancy was performed in 12% (n=411), and of benign disease in 88% (n=3072). Among the 411 patients with a positive cytology for malignancy a diagnosis of MM was observed in 9% of cases, of MC in 84% and of lymphoma in 7%. From the series of 143 patients with a positive PEC for MM, the diagnosis was made by thoracoscopy/surgical biopsy in 50% (n=71), by cytology alone in 3% (n=5) and by both in 47% (n=67). From the series of 347 patients with a diagnosis of MC, the diagnosis was made respectively by thoracoscopy/surgical biopsy in 38% (n=133), by cytology alone in 17%(n=58), by both in 45% (n=155). The sensitivity of cytological diagnosis was respectively 36% with a predictive positive value [PPV] of 88% for MM and 52% with a PPV of 90 % for MC. When we compare the period of time from 1998 -2003 to the period from June 2003-2008, the sensitivity and PPV were respectively 19% with a PPV of 83% and 47% with a PPV of 90% for the diagnosis of MM ($p<0.05$) and of 45% with a PPV of 91% and 57% with a PPV of 90% for MC ($p=0.17$). Homozygous *CDKN2A* deletion was detected by FISH in 7 /17 MM cases and in none of RMH (0/20).

**Conclusions:** Our results show that the diagnostic accuracy of PEC is improving due to a better definition of criteria, and to the use of immunohistochemistry. The detection of homozygous deletion *CDKN2A* by FISH could be extremely useful for the diagnosis of MM over RMH.

### 1601 Asbestos Bodies in Lung Tissue Are Associated with Survival Duration for Epithelial, but Not Non-Epithelial, Mesothelioma in Patients Undergoing Extrapleural Pneumonectomy

*JJ Godleski, WG Richards, M Katler, J Mueller, DJ Sugarbaker.* Brigham and Women's Hospital, Boston, MA.

**Background:** Prior reports which have inconsistently linked asbestos exposure to outcome in malignant pleural mesothelioma (MPM) have often been under-powered to stratify for histological subtype or type of therapy. We analyzed patient survival in relation to asbestos body (AB) counts obtained in lung tissue from MPM patients treated with extrapleural pneumonectomy (EPP), stratifying by histology.

**Design:** Lung tissue for AB analysis was collected as part of the pathologic assessment of EPP specimens and reported in the clinical record. Clorox® digestion was carried out by standard methods and counts made using phase contrast light microscopy. The logrank statistic was used to compare Kaplan-Meier estimates of overall survival from diagnosis for patients grouped by AB count within epithelial and non-epithelial histological subgroups defined by WHO criteria.

**Results:** 559 patients were treated with EPP between 1988 and 2005. Of these 365 had tumors with epithelial histology and 194 had non-epithelial tumors. Among epithelial cases, (72% male, median age 57) 234 (64%) had AB counts prospectively determined compared to 142 (73%) non-epithelial cases (90% male, median age 60). Median AB counts were 265 per gram wet weight for epithelial compared to 1084 per gram for non-epithelials. Median control AB count for our laboratory is 20 per gram. Among patients with epithelial MPM, 105 patients with AB counts ≤200/gm had a 27-month median survival, compared to 17.1 months for 129 patients with >200/gm ($p=0.0003$). By contrast, median survival of patients with non-epithelial MPM did not differ between the 32 patients with ≤200/gm (9.5 months) and the 109 patients with >200/gm (12.7 months; $p=0.58$). A similar pattern of results was obtained by dichotomizing AB count at 100, 300, 500 or 1000/gm.

**Conclusions:** Patients with epithelial MPM have lower lung asbestos fiber AB burden than those with non-epithelial MPM. Smaller AB counts were associated with longer survival among patients with epithelial tumors, but AB burden was unrelated to survival among those with non-epithelial MPM. These results suggest that AB counts have prognostic significance and support the view that apart from the defining histological differences, epithelial and non-epithelial MPM represent disease entities with distinct biology, clinical behavior, and response to therapy.

### 1602 Airway Inflammation in Adult Patients with Symptoms of Asthma or COPD Does Not Distinguish Those with Fixed Airway Obstruction

*IO Gordon, JA Krishnan, J Charbeneau, A Mazurek, DK Hogarth, T Krausz, AN Husain.* The University of Chicago Medical Center, Chicago, IL.

**Background:** A substantial proportion of patients with asthma and chronic obstructive lung disease (COPD) have inadequate symptom control, and many have fixed airflow obstruction (FAO) despite treatment with inhaled bronchodilators and corticosteroids.

The objective of this study was to determine if patterns of airway inflammation observed in specimens obtained via bronchoscopy (bronchoalveolar lavage (BAL) and endobronchial biopsy (EBBx)) distinguish patients with and without FAO.
**Design:** Using an IRB-approved protocol, we prospectively collected data (2007-2008) at the University of Chicago Asthma and COPD Center on adult patients undergoing bronchoscopy for evaluation of inadequate respiratory symptom control despite optimal therapy. Standard spirometry studies were used to define patients with FAO (Group I; n=10), restriction (Group II; n=5), and normal lung function (Group III; n=6). Smoking history and percentage of eosinophils and neutrophils in BAL were recorded. Formalin fixed paraffin embedded EBBx specimens were stained with hematoxylin and eosin, and eosinophils and neutrophils were counted in the epithelium and submucosa, and given a corresponding grade. Significant differences were defined as a 2-sided p-value<0.05 using Kruskall Wallis and Fisher's Exact Tests on SAS System 9.1.3.
**Results:** The median % eosinophils (Group I vs. II vs. III: 2 vs. 8 vs. 1; p=0.6) and % neutrophils (25 vs. 30 vs. 12, p=0.4) in the BAL; epithelium (% with any eosinophils: 60 vs. 40 vs. 50, p=0.9; % with any neutrophils: 60 vs. 60 vs. 50, p=1.0), and submucosa (% with more than rare eosinophils: 60 vs. 60 vs. 83, p=0.7; % with more than rare neutrophils: 50 vs. 100 vs. 50, p= 0.2) did not significantly differ across groups. There were no differences in the number of pack-years smoked among the three groups (median: 0 vs. 0.5 vs. 0, p=0.6).
**Conclusions:** In patients with poorly controlled symptoms of asthma or COPD despite optimal therapy, patterns of airway inflammation do not distinguish those with or without FAO. These findings may help explain why the benefits of treatment intensification with anti-inflammatory agents based on an evaluation of airway inflammation have had inconsistent effects across studies. We conclude that a better understanding of pathologic alterations in airway structure (e.g., epithelium, submucosa, smooth muscle) is needed to evaluate the pathophysiology of FAO in patients with inadequate asthma or COPD symptom control.

### 1603 Thymoma Type According to World Health Organization Classification Does Not Provide Significant Prognostic Value by Stage: An International Study of Thymomas

*R Gupta, M Okumura, O Rena, C Casadio, DJ Kim, NA Jambhekar, AM Marchevsky.* Cedars Sinai Medical Center, LA, CA; Osaka University Graduate School of Medicine, Osaka, Japan; University of Eastern Piedmont, Maggiore della Carità General Hospital, Novara, Italy; Yonsei University College of Medicine, Seoul, Korea; Tata Memorial Hospital, Mumbai, India.
**Background:** The World Health Organization (WHO) has proposed to categorize thymomas into A, B1, B2, B3 and AB types. Recent studies with metaanalysis have suggested that only 3 classes, A-B1-AB, B2 and B3 appear to be associated with significant prognostic differences. However, there is limited information regarding the prognostic value of thymoma WHO type, by Masaoka stage.
**Design:** 811 cases of thymoma classified by Stage and WHO type were collected from 6 medical centers in India, Italy, Japan, Korea, Germany and U.S. They included 317, 267, 144 and 73 patients in stages I-IV respectively and 73, 233, 141, 265 and 99 by WHO types A-B3 respectively. A minimum of 5-year follow up information was obtained as participating centers did not have 10-year follow-up information for the majority of their patients. Survival proportions were analyzed with Comprehensive Metaanalysis software version 2 ((Biostat, Inc, Englewood, NJ) to determine the prognostic value of WHO type by Stage.
**Results:** Metaanalysis of survival proportions using multiple comparisons of WHO types by Stage showed that in none of the 4 Stages, WHO class provided significant prognostic information.

Evaluation with Meta analysis of the Prognostic Value of WHO Classification of Thymomas: P values obtained by multiple paired comparisons of thymomas by WHO type and Stage

| Stage | A vs. B | AB vs. B1 | B1vs. B2 | B2 vs. B3 |
|---|---|---|---|---|
| I | 0.84 | 0.56 | 0.59 | 0.12 |
| II | 0.28 | 0.46 | 0.64 | 0.4 |
| III | 0.51 | 0.95 | 0.7 | 0.86 |
| IV | Not possible* | 0.56 | 0.7 | 0.55 |

*None of the institutions encountered type A thymoma in stage IV.

**Conclusions:** Quantitative metaanalysis is difficult to perform when the data is split into 20 categories, including 4 stages and 5 WHO types and is limited by probable interobserver variability problems. A 5-year follow-up period is not optimal for tumors that are known to have an indolent clinical course. There is no current evidence to support the hypothesis that WHO classification provides significant prognostic 5-year survival information by Masaoka stage.

### 1604 The Prognostic Significance of Isolated Tumor Cells and Micrometastases in Patients with Non-Small Cell Carcinoma of the Lung: A Prospective Study of 233 Patients with Immunohistochemistry and Survival Analysis

*R Gupta, RJ Mckenna Jr, D Kusuanco, J Mirocha, AM Marchevsky.* Cedars Sinai Medical Center, LA, CA.
**Background:** Current AJCC staging guidelines stratify lymph node metastases into isolated tumor cells (ITC), micrometastases (MM) and metastases. It remains uncertain whether the nodal status of NSCLC patients should be upstaged in the presence of ITC or MM.
**Design:** 233 NSCLC patients with pN0 at mediastinoscopy underwent VATS lobectomy along with mediastinal lymph node dissection. The presence of ITC and MM was evaluated in 2197 lymph nodes using keratin AE1/AE3 antibody. Selected lymph nodes were also immunostained for calretinin to exclude the possibility of mesothelial inclusions. Nodal status were classified as pN0, pN0 (i+), pN1 (mi), pN1, pN2 (mi), or pN2. Overall survival data were analyzed with Kaplan-Meier statistics by nodal status. Survival for patients with pN0, pN0 (i+), pN1(mi), pN2(i+) and pN2(mi) and pN1 with pN2(i+) and pN2(mi) were compared. Power analysis was performed to determine the sample size required to exclude the possibility of a type II statistical error.
**Results:** Median follow-up was 56 months. Lymph nodes resected by mediastinal dissection after lobectomy showed nodal metastases in lymph nodes that were not sampled during initial mediastinoscopy in 48 pN1 and 28 pN2 patients. ITC and MM were detected in 63 lymph nodes from 51 patients (21.9%). This finding would have "upstaged" 4, 24 and 23 patients to pN0 (i+), pN1 (mi) and pN2 (mi) respectively. As expected, there were significant survival differences for patients with pN0, pN1 and pN2 disease. Survival analysis estimated a hazard ratio of 0.76. Change of nodal status resulting from the detection of ITC or MM was not associated with significant survival differences. Power analysis estimated that 304 patients with MM and ITC followed for 60 months would be required to obtain 80% power.
**Conclusions:** Lymph nodes obtained by lobectomy or mediastinal resection changed the nodal status to either pN1 or pN2 of 32.6 % of patients that were diagnosed as pN0 at concurrent mediastinoscopy. ITC and MM were detected in 21.9% of patients studied with IHC but were not significant prognostic features for NSCLC patients. The results underscore the need to perform power analysis in studies that evaluate the association between ITC-MM and survival.

### 1605 High Discordance Rate in Lung Transplant Rejection Grading: A Review of 414 Transbronchial Surveillance Biopsies

*KJ Henriksen, K Suzue, S Bhorade, E Garrity, AN Husain.* The University of Chicago, Chicago, IL.
**Background:** Transbronchial biopsies are routinely performed in lung transplant recipients to assess for rejection, and are graded according to a standardized schema established by The International Society for Heart and Lung Transplantation (ISHLT). Acute rejection is characterized by perivascular inflammation, and is graded according to severity from Grade A0 to A4. Airway rejection, or lymphocytic bronchiolitis, is manifested by inflammatory infiltration of the small airways, and is similarly graded from Grade B0 to B4. We assessed the concordance rates between independent pathologists' readings of transbronchial biopsies obtained from patients enrolled in a large multicenter randomized trial.
**Design:** Transbronchial biopsies were obtained at 6 weeks, 3, 6 and 12 months post-lung transplant from 181 patients enrolled in 7 U.S. transplant centers in the AIRSAC study. A total of 414 surveillance biopsies were obtained. The biopsies were read at each center by various pathologists utilizing the ISHLT 1996 grading criteria. The biopsies were then re-graded by one central pathologist blinded to the original reading.
**Results:** Of 310 biopsies considered sufficient for Grade A rejection, 60% (186) were graded similarly by two independent pathologists. Of those graded differently, 69% (87) differed by one grade and 31% (39) differed by two grades. 76 of 87 (87%) and 37 of 39 (95%) were downgraded by the central pathologist, respectively. Of the 141 biopsies sufficient for Grade B rejection, 70% (99) were graded similarly by the two independent pathologists. Of those graded differently, 76% (32) differed by one grade and 24% (10) differed by two grades. 24 of 32 (75%) and 10 of 10 (100%) were downgraded by the central pathologist, respectively.
**Conclusions:** In this study, there was a high discordance rate among pathologists' interpretations of lung transplant biopsies. These results suggest that consistency and experience may be enhanced by designating a single pathologist to interpret lung transplant biopsies in each center. The study also emphasizes the importance of recognizing bronchial associated lymphoid tissue (BALT), which is often hyperplastic. Failure to recognize BALT can lead to an over-diagnosis of acute rejection.

### 1606 IgG4 Plasmacytosis Is a Common Histological Association in Aspergillosis

*Y Iinuma, Y Kashima, S Ishizawa, R Hayashi, T Tanaka, Y Iwata, S Kageyama, M Hofer, J Fukuoka.* Toyama University Hospital, Toyama, Japan; Toyama University, Faculty of Medicine, Toyama, Japan; Brigham and Women's Hospital, Boston; Harvard Medical School, Boston.
**Background:** IgG4 related plasmacytic disease (IgG4-RPD) is a newly recognized disorder of uncertain etiology which shows various clinical and histological features. Inflammatory pseudotumor, autoimmune pancreatitis, and sclerosing cholangitis are known variants of IgG4-RPD. The common histological features seen in IgG4-RPD are abundant plasma cell infiltration along with dense hyalinizing fibrosis and frequent obliterative venulitis. Areas of chronic scarring seen in aspergillosis show similar histological patterns except the presence of cavitary lesions. To examine the histological overlap between aspergillosis and IgG4-RPD, we immunohistochemically analyzed the 13 cases of continuous cases with aspergillosis.
**Design:** 13 cases with aspergillosis were collected from case archive at Toyama University between 1999 and 2008 which includes eight male and six female with age ranged 48 to 90 years. Eight patients were diagnosed as chronic sinusitis. Three patients had cancer apart from the area with aspergillosis. Other two patients were diagnosed as pulmonary aspergillosis The specimens were immunostained with anti-IgG and IgG4 antibodies. We selected three fields for each specimen randomly and counted number of IgG4-positive plasma cells and IgG positive plasma cells. We calculated ratio of IgG4-positive plasma cells and IgG-positive plasma cells.
**Results:** All 13 cases of aspergillous infection showed marked plasmacytic infiltration along with chronic fibrosis, tissue eosinophilia, and destruction of the basic architecture, which overlap histological characteristics of IgG4-RPD. IgG4 general is the rarest component among the IgG subclasses and it only accounts for less than 6% of the total IgG fraction. In our cohort with aspergillous infection, all cases showed significant increase of IgG4 positive plasma cells in which IgG4/IgG was ranged from 39.8 to 78.8% (mean 58.3%).

**Conclusions:** The histological similarity between aspergillous and IgG4-RPD suggests a common pathogenetic process. Considering the vast histological variations what aspergillous infection show, our results should alert pathologists that chronic aspergillous infection should be ruled out when IgG4-RPD is considered in the differential diagnosis.

### 1607 WITHDRAWN

### 1608 The Development of Pulmonary Fibrosis in a Murine Model of Experimental Hypersensitivity Pneumonitis Is Not Associated with a Dominant Th2 Adaptive Response

*MM Kelly, B Atkinson, L Maxwell, C Gwozd, P Kubes.* University of Calgary, Calgary, AB, Canada.

**Background:** Hypersensitivity pneumonitis (HP) is an inflammatory interstitial lung disease caused by repeated inhalation of a wide variety of antigens, with ~ 5% developing chronic fibrosis and respiratory failure. Chronic HP is recognized as being a cause of a usual interstitial pneumonia pattern of fibrosis, with a peripheral distribution of collagen deposition. A murine model of experimental HP (EHP) involves repeated exposure to antigens from *Saccharopolyspora Rectivirgula* (SR Ag), the main causative agent of Farmer's Lung. Studies with this model suggest that the adaptive immune response is skewed towards a Th1 (high IFN-γ) environment. A generally held paradigm is that fibrosis is suppressed by a Th1 environment and promoted by a Th2 (high IL-4) environment. We hypothesized that the development of fibrosis would be accompanied by increasing concentrations of Th2 type cytokines. The aim of this study was to establish a robust model of pulmonary fibrosis in EHP and to examine the immune-mediated mechanisms involved in the development of chronic fibrosis.

**Design:** C57BL/6 mice were exposed to SR Ag for 3 D/wk. A precise dose of SR Ag was administered by the use of a microsprayer device and aerosolizer. Mice were harvested at the end of weeks 1-3, 5 and 6, when lungs were inflated and fixed in formalin, processed to paraffin and sections cut and stained with H&E and picrosirius red (PSR). Bronchoalveolar lavage (BAL) fluid was stored for subsequent measurement of cytokines using multiplexing xMAP technology.

**Results:** Sustained pulmonary inflammation developed by 2 weeks of SR Ag exposure, with bronchial associated lymphoid tissue (BALT) and airway goblet cell epithelial metaplasia. By the 3rd week, peripheral pulmonary fibrosis was present identified by PSR staining viewed under polarized light. These changes persisted at least until the 6$^{th}$ week, the longest timepoint examined. Concentrations of Th1 (IFN-γ, IP-10, IL-12) and Th2 (IL-4, IL-13, IL-5) mediators in BAL fluid were not significantly different between timepoints and neither showed dominance.

**Conclusions:** We have demonstrated a valid model of EHP with many of the characteristics of chronic HP in humans (BALT, airway goblet cell metaplasia, peripheral fibrosis). We found no evidence of Th1 or Th2 dominance at any timepoint examined, at least with regard to measurements in BAL fluid.

### 1609 A Panel of Immunohistochemistry To Differentiate Various WHO Types of Thymic Neoplasms

*T Khoury, G Wilding, R Chandrasekhar, S Alrawi, D Tan.* Roswell Park Cancer Institute, Buffalo; University of Florida, Jacksonville; MD-Anderson Cancer Center, Houston.

**Background:** Classifying thymic neoplasms according to the World Health Organization (WHO) scheme is somewhat difficult. Recognizing specific types, particularly B3 and C is crucial in patient management and outcome. The purpose of this study is to integrate histologic features adopted by WHO with immunohistochemistry to help properly classify thymic neoplasms, particularly types B3 and C.

**Design:** A series of 63 thymic neoplasms were reviewed and classified according to the WHO scheme. Three tissue microarray blocks were constructed. The following immunostains were studied, D2-40, Thromomodulin, CK5/6, Podoplanin, Mesothelin, CD57, CD20, CD5, TdT, CD1a, CD138, CD117, EMA, CD15, MOC-31 and BerEpi-4. Proper scoring system was used for each marker. Presence or absence of tumor eosinophilia was recorded. Statistical analyses for categorical variables were performed using Fisher's exact test.

**Results:** There were 8 type A, 16 type AB, 8 type B1, 5 type B2, 16 type B3, and 10 type C thymomas. Table1 summarizes the statistically significant markers that differentiate various thymoma types.

**Conclusions:** We suggest an algorithm combining morphology and IHC to properly classify thymic neoplasms with emphasis on types B3 and C.

Panels of statistically significant IHC in various WHO types combinations

| WHO type | Markers | Frequency #positive (%) |
|---|---|---|
| A vs. AB | CD138* | 7 (87.5) vs. 0 (0) |
| | EMA** | 6 (75) vs. (0) |
| B1 vs. B2 and B3 | CD57 | 0 (0) vs. 11 (52.4) |
| B1 and B2 vs. B3 | None | NA |
| A and AB vs. B1, B2 and B3 | CD20*** | 10 (41.7) vs. 2 (7) |
| | TdT^ | 14 (58.3) vs. 26 (89.7) |
| | CD1a^ | 11 (45.8) vs. 26 (89.7) |
| | CD138^^ | 20 (83.3) vs. 13 (44.8) |
| A and AB vs. B3 | CD1a^ | 11 (45.8) vs. 13 (81.3) |
| B3 vs. C | CK 5/6 | 15 (93.8) vs. 3 (30) |
| | Mesothelin | 0 (0) vs. 5 (50) |
| | CD57 | 8 (50) vs. 0 (0) |
| | CD5 | 0 (0) vs. 6 (60) |
| | TdT^ | 13 (81.3) vs. 1 (10) |
| | CD1a^ | 13 (81.3) vs. 2 (20) |
| | CD117 | 1 (6.3) vs. 7 (70) |
| | Eosinophilia | 1 (6.3) vs. 9 (90) |

*CD138 has to be diffuse and strong to be positive; ** EMA positive when luminal expression; ***CD20 positive expression in epithelium ; ^TdT and CD1a positive when more than 10% of lymphocytes; ^^CD138 positive when at least mild staining

### 1610 Anti-Oxidant Taurine Treatment Reduces Pulmonary Inflammation Induced by Allergen and Diesel Particulate Matter Challenge in a Mouse Model of Asthma

*J Kim, F Chiem, S Natarajan, L Vaickus, D Remick.* Boston University, Boston, MA.

**Background:** Many studies show a link between ambient particulate air pollution and exacerbation of pre-existing pulmonary diseases such as asthma. Air pollutants, including diesel particulate matter (DPM), exerts exogenous oxidative stress on biological systems exacerbating lung injury and inflammation in animals and humans. We investigated the pathophysiologic pathways and mediators responsible for exacerbation of asthma-like pulmonary inflammation and airway hyperresponsiveness (AHR) by DPM suspension in a mouse model of asthma and subsequent alleviation of asthma-like symptoms by the anti-oxidant, taurine.

**Design:** A house dust sample containing high concentrations of cockroach allergens and endotoxin was used for this study. BALB/c mice were exposed to 3 pulmonary challenges via hypopharyngeal administration of the house dust extract (HDE) on day 0, 14 and day 21. Groups of mice received DPM or PBS, 1 hr before each allergen challenge. On day 22, bronchoalveolar lavage (BAL) fluid was collected for cells and cytokines, with AHR measured prior to sacrifice. Whole lungs were collected for myeloperoxidase assay (MPO). For taurine treatment, groups of mice received taurine or the inactive analog alanine following DPM and HDE challenge as describe above.

**Results:** Compared to vehicle, DPM significantly increased the airway infiltration of eosinophils (22590±5162 vs 74192±15693, $p \leq 0.0049$), lymphocytes (39966±6091 vs 72522±13814, $p \leq 0.042$), and neutrophils (307850±29270 vs 513314±80458, $p \leq 0.025$). MPO (94.5±12.4 vs 192.3±9.2, $p \leq 0.0001$) and AHR (0.50±0.05 vs 5.50±1.70, $p \leq 0.0011$) in DPM treated mice were also substantially elevated compared to the vehicle group. Pulmonary expression of chemokines and cytokines were significantly increased by DPM challenge. Histological examination of the lung demonstrated a substantial increase of mucus production in airway by DPM challenge. The anti-oxidant taurine significantly reduced AHR (6.05±0.1 vs 2.74±0.17, $p \leq 0.001$) as well as pulmonary infiltration of neutrophils (39500±5383 vs 19100±5360, $p \leq 0.05$) and eosinophils (20145±8598 vs 8860±3372, $p \leq 0.05$) in the allergen plus DPM model.

**Conclusions:** These results demonstrate that intratracheal administration of DPM exacerbates asthma-like pulmonary inflammation. Treatment with the anti-oxidant taurine significantly reduces the pulmonary inflammation in a mouse model of asthma, suggesting that oxidant stress may be an important component of the asthmatic response.

### 1611 Subtyping of Non-Small Cell Lung Cancer in the Era of Bevacizumab

*TU Lang, PA Navarro, B Weinstein, AE Fraire, M Garcia-Moliner.* Tufts Medical Center, Boston, MA; University of Massachusetts Medical School, Worcester, MA.

**Background:** The management of advanced non-small-cell lung cancer (NSCLC) has evolved considerably in recent years with the emergence of Bevacizumab as an adjunct therapy. Bevacizumab showed overall progression-free survival benefit in Phase I & II trials for the treatment of recurrent or refractory NSCLC. However, it is not free of side effects. A reported rare complication associated with necrotic cavitated squamous cell carcinoma is major pulmonary hemorrhage. Therefore in this era, the pathologists role to accurately subtype NSCLC is critical to the appropriate use of Bevacizumab in non-squamous NSCLC.

**Design:** Representative H&E stained slides of 37 patients accessioned between 2001-2007 with initial fine needle aspiration (FNA) and subsequent resection of NSCLC lung tumor were retrieved. The slides were independently reviewed by 3 pathologists. Each pathologist subtyped the tumors into 5 categories: squamous, adeno, adenosquamous, undetermined/NOS or others. Statistical analysis was used to determine the degree of concordance.

**Results:** Our study comprised of 16 male and 21 female patients with a mean age of 65 years (range 38-82). The κ statistic of concordance for 3 pathologists and 5 possible outcomes was 0.31 and 0.69 for FNA and resection specimens respectively. A κ value of this magnitude is defined as being fair agreement for FNA specimens and substantial agreement for resection specimens. In 71% of FNA and 97.3% of resection specimens, there was unanimous agreement or only one discordant designation. 29% of FNA and 2.7% of resection specimens had two or more discordant interpretations. The correlated subtyping of FNA to resected specimen for each pathologist was 70.3%, 78.4% and 86.5%.

**Conclusions:** Today, pathologists face the critical task of subtyping NSCLC using FNA materials. This allows the patient to be treated with the appropriate regime and to avoid pulmonary hemorrhage. Surprisingly, the concordance rate for subtyping NSCLC on FNA materials is fair at best. Reasons attributed to high rate of discordance are diagnostic language, mixed tumor differentiation, poorly differentiated tumor, unusual tumor morphology, tumor necrosis and inflammation. Our study highlights several issues for pathologists in the current era of bevacizumab use. Is NSCLC an acceptable diagnostic category for FNA? Should bevacizumab be used in a mixed tumor with adeno and squamous features? What is the role of immunohistochemistry to distinguish the tumor on FNA materials?

### 1612 Telomere Shortening, Telomere Protein TRF1 and TRF2 Activation, and DNA Damage Response Impairement during Bronchial Carcinogenesis

*S Lantuejoul, C Raynaud, S Gazzeri, D Moro-Sibilot, D Salameire, JC Soria, E Brambilla.* CHU A Michallon, Grenoble, France; CEA, Fontenay aux Roses, France; INSERM U823, IAB, University J. Fourier, Grenoble, France.

**Background:** Telomere shortening is an early event in bronchial carcinogenesis, preceding P53/Rb pathway inactivation and telomerase reactivation. It is also perceived as double strand break, leading to DNA damage responses (DDR), frequently inactivated in carcinogenesis to overcome G1 arrest or apoptosis. Concomitantly, telomere proteins

TRF1 and TRF2 are potentially overexpressed due to their involvement in telomere stabilization and DDR inactivation.
**Design:** To establish the chronology of these abnormalities in bronchial carcinogenesis, we have assessed telomere length by FISH and evaluated by immunohistochemistry TRF1 and TRF2 expression, besides DDR proteins phosphorylated (p)-CHK2, p-ATM and p-H2AX expression in 109 preneoplastic lesions, including 15 mild dysplasia, 19 moderate dysplasia, 31 severe dysplasia, and 44 in situ carcinoma, and in 35 normal or metaplastic epithelium, and 32 squamous invasive carcinoma.
**Results:** Telomeres critically shorten in bronchial metaplasia to increase from mild to moderate and severe dysplasia to invasive carcinoma (p= 0.03), along with TRF1 and TRF2 overexpression (p< 0.0001 and p=0.0007 respectively). In response, p-H2AX, p-CHK2 and p-ATM expressions are already observed at metaplasia stage, with an increased overexpression in preneoplastic lesions, but a reduced expression in invasive carcinoma (p< 0.0001 for p-CHK2 and p-ATM, and p=0.0002 for p-H2AX).
**Conclusions:** Telomere attrition is associated with DDR activation at the earliest stage of bronchial carcinogenesis, and precedes TRF1 and TRF2 overexpression. In contrast, DDR inactivation represents the latest event, occurring in invasive carcinoma.

### 1613 Protein Overexpression and Gene Amplification of Epidermal Growth Factor Receptor in Nonsmall Cell Lung Carcinomas: Comparison of Four Commercially Available Antibodies by Immunohistochemistry and Fluorescence In Situ Hybridization Study

*HJ Lee, XH Xu, GY Choe, DH Chung, JW Seo, CT Lee, SH Jheon, SW Sung, JH Chung.* Seoul National University College of Medicine, Seongnam-Si, Gyeonggi-do, Republic of Korea; Seoul National University Bundang Hospital, Seongnam-Si, Gyeonggi-do, Republic of Korea.
**Background:** Epidermal Growth Factor Receptor (EGFR) overexpression in nonsmall cell lung carcinomas (NSCLC) is variable ranged from 17% to 71.6% and prognostic value for survival in patients remains controversial. We tried to investigate 1) EGFR protein expression using four different antibodies 2) the correlation between protin overexpression and EGFR gene amplification and 3) the correlation between EGFR genetic status and clinicopathologic features in NSCLC.
**Design:** We examined EGFR protein expression using four different antibodies including Dako EGFR pharmDx kit (Clone 2-18C9), Zymed EGFR kit (Clone 31G7), Novocastra (Clone EGFR 113) and Dako (Clone H11) by immunohistochemical analysis. The protein overexpression was compared to gene amplification status by fluorescence in situ hybridization (FISH).
**Results:** EGFR protein overexpression was observed in 51% of tumors with Dako EGFR pharmDx kit (Clone 2-18C9), in 56% with Zymed EGFR kit (Clone 31G7), in 18% with Novocastra (Clone EGFR 113) and in 5% with Dako (Clone H11), respectively (*p <0.01*). Both Zymed and Dako pharmDx kit were more sensitive than the Dako test using clone H11 and Novocastra Clone EGFR 113. EGFR overexpression is most prominent in squamous cell carcinomas (SCC) than adenocarcinomas (ADC) (71% vs. 48%, *p =0.001* in Zymed; 61% vs. 45%, *p =0.011* in Dako pharmDx kit, respectively). EGFR FISH-positivity was observed in 45% of the NSCLC patients. Protein expression levels significantly correlated with the gene copy number per tumor cell (*p =0.000*). EGFR overexpression with Zymed clone 31G7 had significant influence on shortened progression-free survival in ADC (*p =0.037*).
**Conclusions:** Our data showed a higher percentage of positive cells detected by Zymed and Dako pharmDx tests, whatever scoring system. EGFR protein overexpression rate varies from 5% to 71% according to different antibody clones and histologic types. EGFR protein expression detected by Zymed and Dako pharmDx was significantly associated with a high EGFR gene copy number. EGFR gene amplification predicted a poor prognosis in NSCLC, especially ADC. Protein overexpression bears only prognostic informative value in ADC.

### 1614 Notch Signaling in Non Small Cell Lung Cancers (NSCLC) Is Associated with Squamous Differentiation and Favorable Clinical Outcome

*L Li, CE Sheehan, JS Ross.* Albany Medical College, Albany, NY.
**Background:** Notch protein signaling via a complex network of transmembrane receptors, ligands and effectors plays a major role in the development, differentiation and growth control of human respiratory epithelia. Clinical outcome studies of Notch expression in NSCLC have not been previously performed.
**Design:** Formalin-fixed, paraffin embedded sections from 134 NSCLC, including 47 squamous cell carcinomas (SCC), 50 adenocarcinomas (AC), and 30 bronchioloalveolar carcinomas (BAC) were immunostained by automated methods (Ventana, Tucson, AZ) with polyclonal antibodies to Notch1, Notch2, Notch3 and Notch4 (Santa Cruz Biotech., Santa Cruz, CA). Cytoplasmic (C) and nuclear (N) immunoreactivity of each protein was semiquantitatively assessed for all cases. Scoring was based on staining intensity (weak, moderate, intense) and percentage of positive cells (focal <= 10%, regional 11-50%, diffuse >50%). Results were correlated with clinicopathologic variables.
**Results:** C+N Notch1 overexpression was greatest in SCC (38%) vs AC (16%) and BAC [16%] (p<0.0001). NSCLC N Notch1 overexpression correlated with lengthened survival overall [p=0.02] and low grade [p=0.05] and low stage [p=0.03] in SCC. C+N Notch2 overexpression correlated with low stage [p=0.038] in SCC and small tumor size [p=0.005] within AC. NSCLC C Notch 2 overexpression correlated with low stage (p=0.05); SCC tumor type (p=0.05), low stage in SCC (p=0.038) and small tumor size in AC (p=0.02). NSCLC C Notch3 overexpression correlated with lengthened survival (p=0.002), survival in SCC (p=0.016) and BAC (p=0.036), and tumor size in AC (p=0.007). NSCLC C+N Notch4 immunoreactivity was greatest in SCC [33%] vs AC [8%] and BAC [16%] (p=0.008) and correlated with small tumor size (p=0.02) overall and in SCC (p=0.016) and AC (p=0.05); with male gender (p=0.05) in SCC and lengthened survival within the BAC (p=0.016). N Notch4 overexpression correlated with SCC tumor type (p<0.0001), small tumor size (p=0.05), male gender (p=0.04) overall and in the SCC. Notch1 coexpression was significant with Notch3 (p=0.011) and Notch4 (p<0.0001), as well as Notch3 with Notch4 (p=0.002). On multivariate analysis, stage was the only independent predictor of survival.
**Conclusions:** NSCLC Notch protein expression is variable and associated with squamous differentiation, early pathologic stage and lengthened survival. The potential oncogenic roles for Notch proteins in NSCLC as prognostic factors and targets of therapy warrant further study.

### 1615 Prognostic Significance of Excision Repair Cross-Complementation Group 1 (ERCC1) Expression in Early Stage Resectable Non Small Cell Lung Cancer (NSCLC)

*L Li, CE Sheehan, JS Ross.* Albany Medical College, Albany, NY.
**Background:** ERCC1 is an enzyme in the nucleotide excision repair pathway associated with the removal of damaged DNA after exposure to DNA damaging agents. Although the overexpression of ERCC1 mRNA and protein have been consistently associated with resistance to platinin-based chemotherapy and shortened survival in patients with recurrent or metastatic NSCLC, the significance or ERCC1 expression in early stage resectable NSCLC has not been widely studied.
**Design:** Formalin-fixed, paraffin embedded sections from 134 NSCLC, including 47 squamous cell carcinomas (SCC), 50 adenocarcinomas (AC), and 37 bronchioloalveolar carcinomas (BAC) were immunostained by automated methods (Ventana Medical Systems, Inc, Tucson, AZ) with mouse monoclonal ERCC1 (clone 8F1; Neomarkers). Nuclear immunoreactivity of each protein was semiquantitatively assessed in the tumor for all cases. Scoring was based on staining intensity (weak, moderate, intense) and percentage of positive cells (focal <= 10%, regional 11-50%, diffuse >50%). Results were correlated with clinicopathologic variables.
**Results:** ERCC1 immunoreactivity was predominantly nuclear. ERCC1 overexpression was noted in 48/134 (35%) cases and correlated overall with tumor type [45% SCC vs 10% AC vs 50% BAC, p=0.013], small tumor size [43% <= 3.0cm vs 23% > 3.0cm, p=0.02], lengthened survival within the expired group [13% expired <12mos vs 33% expired 1 - 5 yrs vs 57% expired > 5yrs, p=0.001], and showed a trend toward lymph node negative status [42% LN negative versus 23% LN positive, p=0.062]. Within the SCC subgroup, ERCC1 overexpression correlated with small tumor size [75% <= 3.0cm versus 22% > 3.0cm, p=0.001] and lack of disease recurrence [50% non-recurrent vs 0% recurrent, p=0.05]. On multivariate analysis, only advanced tumor stage and positive lymph node status independently predicted patient survival.
**Conclusions:** In contrast with the adverse outcome for platinin-treated late stage, recurrent and metastatic ERCC1 positive NSCLC, in this study of early stage respectable NSCLC, overexpression of ERCC1 was a favorable prognostic factor for the disease. Further prospective validation of these findings in the prospective clinical trial setting including neo-adjuvant treatment selection appears warranted.

### 1616 HER3 and c-Met Co-Expression in Non-Small and Small Cell Lung Cancers

*M Li, J Song, L Faoro, M Tretiakova, R Salgia, T Krausz, AN Husain.* University of Chicago, Chicago.
**Background:** Gifitinib/erlotinib, tyrosine kinase inhibitors (TKIs) which target the epdidermal growth factor receptor (EGFR) pathway, are effective in the treatment of non-small cell lung cancers (NSCLC) harboring somatic mutations in EGFR. However, despite an initial response, most EGFR mutant NSCLCs ultimately become resistant to these agents. The persistent phosphorylation of HER3 and c-Met amplification are important mechanisms of this acquired resistance, through the PI3K/Akt pathway. To overcome this, ongoing clinical trials are evaluating irreversible EGFR inhibitors alone or in combination with c-Met inhibitors. Here we conducted the first large-scale study of HER3 and c-Met expression in EGFR TKI-naive NSCLC and small cell lung cancers (SCLC).
**Design:** With IRB approval, tumor tissue microarrays of 25 squamous cell carcinomas (SCC), 42 adenocarcinomas (AC), 34 SCLC, and 35 large cell lung carcinomas (LCLC), with at least 2 cores from each case were immunohistochemically stained for HER3 and c-Met. Among them, 60 cases (23 SCC, 19 AC, 7 SCLC and 11 LCLC) had cores from both tumor center and advancing edge. In addition, a separate set of 16 metastatic tumors (4 SCC, 7 AC and 5 LCLC) with cores from both primary and lymph node metastasis were studied. The staining intensity was scored as: 0 (negative), 1+ (weak), or 2+ (strong).
**Results:** Both HER3 and c-Met showed a predominantly cytoplasmic staining and were expressed in the majority of cases, except for c-Met in SCC (Table 1). HER3 expression was strongest in SCC and SCLC, and was weakest in LCLC (p<0.01). C-Met was strongest in AC and weakest in SCC (p<0.01). The two markers were frequently co-expressed (SCC 48%, AC 95%, SCLC 76%, LCLC 86%). There was no significant difference in the expression level between tumor center and advancing edge, or between primary tumor and metastasis.

Table 1. Expression of HER3 and c-Met in NSCLC and SCLC.

| Tumor type | HER3 | | | c-Met | | |
|---|---|---|---|---|---|---|
| | Negative | Weak | Strong | Negative | Weak | Strong |
| SCC | 4% | 32% | 64% | 52% | 40% | 8% |
| AC | 0% | 50% | 50% | 5% | 24% | 71% |
| SCLC | 6% | 29% | 65% | 21% | 41% | 38% |
| LCLC | 6% | 74% | 20% | 11% | 40% | 49% |

**Conclusions:** This study demonstrates that HER3 and c-Met are co-expressed in the majority of EGFR TKI-naive NSCLC and SCLC. Our data supports the potential use of combined TKIs in naive NSCLC to avoid drug resistance. This study is the first to show that both HER3 and c-Met are co-expressed in the majority of SCLC (76%), suggesting that SCLC patients with both HER3 and c-Met abnormalities may benefit from combined regimens targeting these two genes.

### 1617 Triple Platform Testing (IHC, FISH and Mutation) To Predict Response to Targeted Therapy in NSCLC

*D Lucas, R Camidge, R Doebele, J Haney, M Sugita, L Bemis, W Franklin.* University of Colorado at Denver and Health Sciences Center, Aurora, CO.

**Background:** Multiple molecular abnormalities in the EGFR pathway including overexpression of EGFR protein, high gene copy number and mutations in *EGFR* and *Ki-ras* genes are reported to correlate with improved survival in response to EGFR blockade. To assess all of these abnormalities in a single predictive profile, our lab developed a triple-platform molecular testing protocol that includes IHC (EGFR, HER2/neu, and ERCC1), FISH (EGFR and HER2/neu), and mutational tests (*EGFR, TP53* and *Ki-ras*) that are combined into a single interpretive report.

**Design:** 39 patients with NSCLC have been tested to date. IHC was considered positive if the product of the staining intensity (0-3 scale) times the percent of cells stained was >150. High gene copy in multicolor FISH was indicated by >40% of tumor cells with ≥4 signals/cell. Mutational analysis for *Ki-ras (exon 1), EGFR (exons 19-21)*, and *TP53 (exons 5-8)* mutations were evaluated in DNA extracted from a 1 mm core of tumor tissue removed from a paraffin block which was amplified by PCR and directly sequenced.

**Results:** Of the 39 patients evaluated for EGFR and ERCC1 protein overexpression, 20 (51%) and 9 (23%) showed strong expression for EGFR and ERCC1, respectively. Patients were also tested for a high gene copy number and identified 25 (64%) that were both EGFR and HER2-neu FISH-positive. Mutational analysis revealed a total of 21 (54%) somatic mutations which included 7 *EGFR* (18%), 7 *TP53* (18%), and 7 *Ki-ras* (18%) single mutations. Two patients revealed a double-mutant profile consisting of both a *TP53* and *Ki-ras* mutations. Many of the markers had seemingly contradictory results and at the present time a weighted approach is being developed to interpret the interaction among the markers in regard to prognosis and prediction of treatment response. In this algorithm, mutation is an initial filter followed respectively by FISH and protein expression.

**Conclusions:** Triple-platform testing provides an integrated approach to biomarker assessment that lends itself to clinical interpretation and documentation for further correlations with outcome. Triple platform testing is expected to aid in patient-specific targeted agent treatment planning.

### 1618 ERCC1 Expression, Gender and Leukocytosis as Prognostic Indicators in Advanced Stage Non-Small Cell Lung Carcinoma with Cisplatin-Based Chemotherapy?

*G Luo, H Bui, N Patil, J Reynolds, N Karim, M Anderson, P Pathrose, A Mehta.* VA Medical Center, Cincinnati, OH; University of Cincinnati, Cincinnati, OH.

**Background:** Excision repair cross-complementation group 1 (ERCC1) plays a pivotal role in resistance of non-small cell lung cancer (NSCLC) to cisplatin-based chemotherapy. Evidence of hematopoetic growth factors (G-CSF) secreted by NSCLC is associated with the increased production of leukocytes. Some studies also show gender association with prognosis in NSCLC. Our aim was to evaluate ERCC1 expression, gender and leukocytosis as prognostic indicators in advanced stage NSCLC patients given cisplatin-based therapy.

**Design:** We retrospectively reviewed advanced stage NSCLC patients from the VAMC and University of Cincinnati from 1998-2007. Two pathologists independently reviewed the tissue sections for adequacy and selected 29 patients treated with cisplatin-based chemotherapy. IHC for ERCC1 (Mouse monoclonal ab- 8F1, Thermo Scientific, 1:200) was independently graded by 2 pathologists (0=absent; 1=weak, focal; 2=strong/diffuse). Baseline pre-treatment WBC counts were obtained from electronic medical records. Death dates were confirmed by contacting their physicians and hospice centers. ERCC1 reactivity and gender were correlated with overall survival (Cox proportional hazards model).

**Results:** Out of 29 patients (mean age=64; range: 42-84; 7 females (24%), 22 males (76%), 5 patients (17%) had leucocytosis (>11,000/μL). ERCC1 expression was positive in 15/29 patients (52%). 67% of patients with positive ERCC1 expression showed leukocytosis. 7 patients, who survived >36 months (mean= 54 mths) did not express ERCC1. 7 patients with significantly shorter survival (mean=11 mths) strongly expressed ERCC1. ERCC1 negativity and female gender were significantly associated with longer overall survival (p=0.018 and 0.048 respectively). Leukocytosis trended towards shorter survival, without reaching statistical significance (p=0.58).

**Conclusions:** 1. Increased ERCC1 expression may predict shorter survival following cisplatin-based therapy. 2. Female gender seems to be significantly associated with longer survival in cisplatin-treated advanced stage NSCLC. 3. Leukocytosis shows mild statistical trend of association with shorter survival. 4. Consideration of these factors can be helpful during selection of suitable chemotherapy for management of these patients. A larger sample in this particular treatment category, possibly with a multi-institution cohort study using ERCC1, gender and leukocytosis is warranted.

### 1619 Pulmonary Smooth Muscle Neoplasms: A Clinicopathologic Study

*H Mani, K Shilo, M Miettinen, JR Galvin, TJ Franks.* Armed Forces Institute of Pathology, Washington, DC; University of Maryland School of Medicine, Baltimore, MD.

**Background:** Pulmonary smooth muscle neoplasms are rare. We present a study of 51 low-grade pulmonary leiomyomatous tumors, the largest series to date.

**Design:** All pulmonary spindle cell tumors accessioned to our consultation service from 1972 to 2004 were retrieved. Low-grade smooth muscle tumors including leiomyomas, lipoleiomyomas and low-grade leiomyosarcomas were selected. Clinical histories, radiology reports, gross descriptions and histology slides were reviewed.

**Results:** Fifty-one cases included 7 men and 44 women ranging in age from 32-72 years, mean 48 years. Tumors were found incidentally in 74% (26/35) of cases. Fifty-nine percent (29/49) of patients had multiple tumors. All 7 men and 31% (13/42) of women had single tumors. Twenty-seven women had a history of uterine fibroids; 2 of these were identified following discovery of lung lesions, while in 25, the uterine fibroids had been diagnosed 2-18 years prior to lung lesions. Uterine fibroids were more commonly associated with multiple than single tumors (69% vs 23%). All patients with a history of mitotically active uterine leiomyomas (2) and/or intravenous leiomyomatosis (4) had multiple tumors. Tumors ranged in size from 0.4-10 cm, mean 2.2 cm, and were well-circumscribed. Five cases had small cystic areas visible on imaging or gross examination, while an additional 18 cases showed dilated to microcystic entrapped glands on microscopy. There were 43 leiomyomas, 7 lipoleiomyomas and 1 low-grade leiomyosarcoma. Forty-nine percent (25/51) of tumors showed prominent collagenization. Twenty cases were classified as benign metastasizing leiomyomas based on the presence of multiple tumors and a history of uterine fibroids. Among 41 patients with available demographic information, one patient died at 17 months and 8 died 14-24 years after diagnosis. Sixty-six percent (6/9) of those who died were women; all 6 had uterine fibroids and 5 had multiple lung tumors.

**Conclusions:** Low-grade pulmonary smooth muscle tumors are usually discovered incidentally and occur predominantly in women. Their association with uterine fibroids (recent or remote) and prolonged survival, even in the setting of multiple tumors, suggests that benign metastasizing leiomyomas are a distinct clinicopathologic entity. The solitary lesions in men likely represent primary neoplasms.

### 1620 Evidence-Based Pathology: Interobserver Variability Can Result in Significantly Different Estimates for Prognostic Outcome in Clinicopathologic "Entities" Such as UIP or NSIP

*AM Marchevsky, R Gupta.* Cedars Sinai Medical Center, LA, CA.

**Background:** Clinicopathologic "entities" are based on findings associated with significantly different prognoses. The effect of interobserver variability (IOV) on the prognostic estimates associated with overlapping entities like UIP and NSIP is unknown.

**Design:** UIP and NSIP studies providing prognostic estimates were systematically reviewed. A simulation was performed keeping the number of surviving patients in each cohort as a constant. The proportion of UIP cases that could have been classified as NSIP by putative "other pathologists" was arbitrarily changed at 10-30% IOV intervals, by study. The variable "simulated survival proportion" was analyzed with kappa and chi-square statistics to determine at what level of IOV and kappa value the different "diagnoses" by various "pathologists" would have significantly affected the prognostic estimates reported by each study.

**Results:** Seven level III studies reporting 518 UIP and 189 NSIP patients were analyzed. Two of these studies reported IOV resulting in kappa=0.59-0.42. Overall survival of UIP and NSIP patients ranged between studies from 11% to 58% and 39% to 100% respectively. Simulation showed that IOV at the 20% level and kappa=0.68 would have significantly changed the survival estimates of all studies at the $p<0.02$ level.

**Conclusions:** Prognostic estimates are dependent on IOV, effect size and sample size. IOV at agreement levels that would be classified as "moderate to substantial" by kappa statistics can significantly influence prognostic estimates. Definitions of overlapping entities such as UIP and NSIP need to be made more stringent to decrease IOV to lower levels in order to provide more consistent prognostic estimates.

### 1621 The Prognostic Significance of Isolated Tumor Cells and Micrometastases in Patients with Non-Small Cell Carcinoma of the Lung: Systematic Review of Current Best Evidence with Meta-Analysis

*AM Marchevsky, R Gupta.* Cedars Sinai Medical Center, LA, CA.

**Background:** The prognostic value of isolated tumor cells (ITC) and micrometastases (MM) remains controversial for non-small cell carcinoma of the lung (NSCLC) patients.

**Design:** The English literature for NSCLC was systematically reviewed for best evidence regarding the prognostic value of ITC and MM detected by IHC and/or molecular methods. The data were analyzed with metaanalysis (Comprehensive Meta-analysis Biostat, Englewood NJ).

**Results:** Sixteen retrospective cases series (level III studies) reported the prognosis of 906 NSCLC patients evaluated with IHC using antibodies to keratins, CEA, Ber-EP4 or p53. Three other studies evaluated 140 NSCLC patients with molecular methods. Metaanalysis of the survival information available from patients evaluated with IHC and/or molecular methods showed no significant survival differences between pN0, pN0 (i+) and pN1(mi) cases and between patients with pN0 or pN1 and pN2(mi) ($p>0.05$). Concordance between IHC and molecular findings was 76.9% in the single study where both methods were used. Evaluation of the data with funnel plots and Eggers regression intercept showed significant heterogeneity and asymmetry within the data resulting from publication and other biases.

**Conclusions:** Available best evidence shows that ITC and MM detected with IHC and molecular methods do not provide significant prognostic information for NSCLC patients. The available data shows significant heterogeneity. Additional evidence is needed before these tests are recommended for the routine pathologic staging of NSCLC patients.

### 1622 Comparison of Thyroid Transcription Factor-1 (TTF-1) Expression by Two Monoclonal Antibodies in Lung and Non-Lung Primary Tumors

*A Matoso, W Greaves, R Tavares, L Noble, MB Resnick, RA DeLellis, LJ Wang.* Rhode Island Hospital/Brown University, Providence, RI.

**Background:** TTF-1 is a nuclear protein that plays a role in the morphogenesis of the thyroid and lungs. Expression of TTF-1 has been used as a marker of lung and thyroid clinically. There are currently two commercially available clones of TTF-1 antibodies, 8G7G1/1 and SPT24. Comperat *et al.*(Mod Pathol 2005; 18:1371-6) reported TTF-1 positivity in 10% of metastatic and 5% of primary colonic carcinomas with the SPT24 antibody in contrast to no positive cases with 8G7G1. To further assess the comparative

specificities ans sensitivities of these antibodies, we examined TTF-1 expression in primary tumors of the lung, prostrate, pancreas, stomach and salivary glands.
**Design:** Tissue microarrays were created from archival paraffin embedded tissue samples from 374 patients with primary lung tumors, 104 prostate adenocarcinomas, 110 gastric adenocarcinomas, 110 pancreatic adenocarcinoma and 58 salivary gland tumors. None of the patients with non-lung tumors had a history of lung cancer. The microarrays were immunostained with monoclonal antibodies to TTF-1, clones 8G7G1/1(Dako) and SPT24 (Novocastra), using Dako Autostainer with Daki Envision Plus kit.
**Results:** Only cases with nuclear staining were considered to be positive.

Immunohistochemistry Results

| | SPT24 | 8G7G1 | P value |
|---|---|---|---|
| LUNG | | | |
| *Adenocarcinoma* (185) | 134(72.4%) | 121(65.4%) | 0.080 |
| *Large cell* (47) | 22(46.8%) | 17(36.2%) | 0.201 |
| *Carcinoid* (23) | 14(60.8%) | 4(17.4%) | 0.003 |
| *Squamous cell* (97) | 14(16.8%) | 1(1.0%) | 0.003 |
| *Unclassified* (22) | 10(45.5%) | 7(31.8%) | 0.260 |
| PROSTATE (104) | 2(1.9%) | 2(1.9%) | NA |
| STOMACH (110) | 1(0.9%) | 1(0.9%) | NA |
| SALIVARY GLAND (56) | 1(1.8%) | 1(1.8%) | NA |
| PANCREATIC ADENOCARCINOMA (110) | 0(0%) | 0(0%) | NA |

**Conclusions:** TTF-1 SPT24 clone is more sensitive than 8G7G1 for detection of primary lung tumors of all histologic subtypes. Importantly, the SPT24 clone detects a significant number of squamous cell carcinomas in contrast to 8G7G1. A small proportion of non-pulmonary tumors, i.e. 1-2% of prostate, gastric primary adenocarcinomas and salivary gland adenocarcinomas are positive with both clones. SPT24 is more sensitive than 8G7G1 for the detection of lung tumors, but both antibodies have similar specificities.

### 1623 Histological Characteristics in the Prediction of Acute Exacerbation of Idiopathic Pulmonary Fibrosis (IPF) Following Surgery for Primary Lung Cancer

*O Matsubara, T Ohba, H Ninomiya, Y Ishikawa, EJ Mark.* National Defense Medical College, Tokorozawa, Japan; Cancer Institute, Tokyo, Japan; Massachusetts General Hospital and Harvard Medical School, Boston, MA.
**Background:** Idiopathic pulmonary fibrosis (IPF) is associated with lung cancer with a reported frequency of between 13 and 31%. Although the postoperative mortality rate in patients with lung cancer has decreased with advances in clinical management, patients with IPF remain at a high risk of complications. The incidence of acute exacerbation of IPF after lobectomy has been as high as 20%. We sought to determine whether or not there are any histological characteristics that suggest acute exacerbation of IPF in surgically resected lung specimens.
**Design:** A retrospective study of 735 patients undergoing resection of lung cancer revealed that 27 patients had IPF based on clinical and radiological records. These cases were analysed by quantitative histopathology.
**Results:** Five of the 27 patients with IPF showed histologic evidence of acute exacerbation of IPF. Four of the five died 20 to 40 days after the lung resection, and one patient survived after steroid pulse therapy. The histologic findings in patients with IPF with and without acute exacerbation were temporal and spatial variation, patchy involvement, subpleural accentuation of disease, honeycomb change, fibroblastic foci and interstitial inflammation. There were three prominent findings when compared to the remainder of the cases: 1) more marked interstitial inflammation with neutrophil and eosinophil, 2) more frequent lymphoid aggregates, and 3) more extensive accumulation of macrophages. There were no significant differences in the frequency of fibroblastic foci, the degree of bronchiolar metaplasia, type 2 pneumocyte hyperplasia, squamous metaplasia, or honeycomb fibrosis between the two groups.
**Conclusions:** More prominent and numerous active fibroblastic foci have been shown to be a marker of a more ominous course including acute exacerbation in patients with IPF. In this study, however, the presence of interstitial pneumonitis is more predictive of the accelerated phase of usual interstitial pneumonia (UIP) than the presence of active fibroblastic foci.

### 1624 Genetic and Immunohistochemical Profiling of Lung Cancer in Young Adults

*N Motoi, H Nagano, K Nomura, Y Ishikawa, M Arai.* Japanese Foundation for Cancer Research, Kotoku, Tokyo, Japan; Cancer Instituite Hospital, Kotoku, Tokyo, Japan.
**Background:** The clinical features and phenotype of lung cancers can be strongly influenced by the underlying oncogenic molecular alterations. The goal of this study is to clarify the molecular alterations in lung cancer (LC) of young adults as a possible model of lung carcinogenesis, and to evaluate clinical relevance of molecular subtypes.
**Design:** "Young adults' lung cancer" (YLC) was defined as a LC occurring at the age below the average - 2 SD of all primary LC during 1987-2007 at our institution (i.e. 46 year old). Clinical data were collected from the medical record. YLC under the age of 40 were further analyzed for mutational status and immunohistochemical (IHC) profiles. EGFR and KRAS mutations were screened by high resolution melting method and confirmed by sequencing. IHC was performed for TP53 and ALK.
**Results:** Of all 2827 LCs, 173 YLCs were identified (6.1%), with 91 males and 82 females (39 and 33, respectively in the group of under 40 year old). Almost all the patients were no or light smokers. Adenocarcinoma (AD) was the most common histologic subgroup (n=55), followed by squamous cell carcinoma (SQ, n=6), small cell carcinoma (4), carcinoid (4) and large cell carcinoma (3). Among AD, BAC (7) and salivary gland type-AD (7) were the most common subtypes. Genetic analysis revealed 7 AD with EGFR mutations with preference of histologic BAC subtype. One AD (BAC) developed in the patient with Li-Fraumeni syndrome with TP53 germline mutation. All the studied cases showed wild type KRAS. IHC showed strong expression of ALK in 8 AD (14.5% of all AD), with preference of acinar and papillary subtypes. Overexpression of p53 was observed in 23 cases; 19 AD (34.5% of AD) and 4 SQ (66.7% of SQ). Five of EGFR-mutated AD showed overexpression of p53. Two AD showed both ALK and p53 expression. In summary, YLC showed higher incidence of EGFR and ALK alteration as compared to the older population. Of these changes, some EGFR or ALK alteration occurred with simultaneous p53 overexpression. KRAS mutation in YLC was exceptional.
**Conclusions:** Our data suggest that there are distinct genetically characterized subgroups in YLC. In addition, each of these subgroups seems to show different histopathologic features and immunohistochemical profiles. Although further validation is needed, this study suggests that molecular and clinicopathologic features may be combined to provide clinically and therapeutically relevant classification of lung cancer, especially in young population.

### 1625 Interobserver Variability in the Cytologic Diagnosis and Sub-Classification of Primary Lung Tumors

*AB O'Bannon, H Alatassi, MG Sanders, S Sahoo.* University of Louisville Hospital, Louisville, KY.
**Background:** CT-guided fine-needle aspiration biopsy (FNAB) is widely accepted as an accurate and safe procedure for characterizing lung nodules prior to treatment. The challenge for the pathologist is not only to distinguish malignant from benign lesions, but also to sub-classify the type of malignant tumors in order to facilitate management.
**Design:** Three pathologists (a cytopathology fellow, a general pathologist without fellowship training, and a board certified cytopathologist) blindly reviewed cytology smears from 62 consecutive cases of primary lung tumor in an attempt to distinguish small cell carcinoma (SCC) from non-small cell carcinomas (NSCC) and to further subclassify the NSCCs. To assess interobserver variation, the diagnoses of the 3 reviewers were compared to one another and to the final diagnosis which included evaluation of cell block, small core biopsy and immunostains.
**Results:** In all 62 cases, at least one of the pathologists made the correct diagnosis. Table 1 lists the interpretations made by each pathologist. Overall, the correct classification of SCC vs NSCC was high (90%). The fellow and practicing cytopathologist correctly categorized SCC in all cases whereas the general pathologist classified 3 SCCs as NSCC. All the NSCCs were correctly identified by the general pathologist and cytopathologist, however, the fellow misclassified 3 NSCCs as SCC. In the subclassifcation of NSCC, there was complete agreement by all three pathologists in 23/47 cases (49%) and agreement by two of three pathologists in 36/47 cases (77%). These include cases of squamous cell carcinoma with some degree of keratinization and cases of adenocarcinoma showing any glandular differentiation. The cases with discordant diagnoses were poorly differentiated NSCC (adenosquamous, adenocarcinoma and non-keratinizing squamous cell carcinoma).

Table 1. Summary of interpretations.

| Tumor Types | # of cases | Fellow | General Pathologist | Cytopathologist | % of cases with unanimous agreement |
|---|---|---|---|---|---|
| **SCC** | 13 | 13 | 10 | 13 | 77 |
| **NSCC** | 47 | 44 | 47 | 47 | 94 |
| Squamous cell carcinoma | 22 | 20 | 19 | 19 | 68 |
| Adenocarcinoma | 16 | 11 | 11 | 13 | 50 |
| NSCC, nos | 9 | 4 | 3 | 3 | 33 |
| **Mixed SCC & NSCC** | 1 | 1 | 1 | 1 | 100 |
| **Carcinoid tumor** | 1 | 1 | 0 | 1 | 0 |
| Total | 62 | 50 | 49 | 55 | 90 |

**Conclusions:** In majority of cases, FNAB alone can correctly classify lung tumors as SCC or NSCC. Subclassification of poorly differentiated NSCCs can easily be achieved by a consensus approach or by using ancillary studies if needed.

### 1626 Large Cell Carcinoma: A Revised Concept

*J Pardo, JJ Sola, A Martinez-Penuela, A Panizo, G Aisa, JM Martinez-Penuela, MD Lozano.* Clinica Universitaria, University of Navarra, Pamplona, Navarra, Spain; Hospital de Navarra, Pamplona, Navarra, Spain.
**Background:** Large cell carcinoma (LCC) accounts for 15.7% of lung cancer. Althought LCC includes some specific entities such as neuroendocrine LCC, basaloid LCC, lymphoepithelioma-like LCC, clear cell LCC, and LCC with rhabdoid features, most cases correspond to classic LCC, which is a diagnosis of exclusion, since it probable represents the end point of differentiation of various lung tumors This study aims to evaluate a panel of immunohistochemical markers in an attempt to reduce the number of LLCs by the identification of tumors belonging to other categories.
**Design:** We analyzed two tissue microarray platforms consisting of 121 LLC. 15 pulmonary squamous cell carcinomas and 22 adenocarcinomas were used as controls. A panel consisting of 31 monoclonal antibodies was evaluated.
**Results:** The tumors were classified as follows: 96 (79.2%) were classic LCC, 10 (8.1 %) were neuroendocrine LCC, 7 (5.5 %) were lymphoepithelioma-like LCC, 3 (2.7%) were basaloid LCC, 3 (2.7%) were clear cell LCC, and 2 (2.4%) was a LCC with rhabdoid phenotype. Characteristic classic LCC immunophenotype was loss of staining with CK5/6 and CK14, loss of MOC 31 expression, and the immunoreactivity to EGFR, PDGFRα and c-kit. 37 of 96 classic LCC (38.5%) were re-classified as adenocarcinomas, because they coexpressed TTF-1, CK7, and CK19, and were negative for p63. 42 (43.7%) of 82 classic LCC were reclassified as poorly differentiated squamous cell carcinoma, based on their immunoreactivity with 34betaE12, p63, thrombomodulin, and CD44v6. All neuroendocrine LCC were positive for AE3AE1, MOC-31, NSE, and PGP 9.5, and, at least, two additional neuroendocrine markers. Basaloid carcinomas were positive for AE3AE1, EGFR, CK5/CK6, CK19, Ber-EP4, and p63. Lymphoepithelioma-like carcinomas were positive for AE3AE1, MOC31, NSE, and PGP9.5.
**Conclusions:** The use of seven immunohistochemical markers, consisting of TTF-1, CK7, CK19, p63, 34betaE12, thrombomodulin and CD44v6, markedly reduces the number of classic LCC by readily identifying cases of poorly differentiated squamous cell carcinomas and adenocarcinomas. Routine use of such an immunohistochemical

approach in LCC will conduce to change the distribution of each type of lung cancer and will facilitate a more specific prognosis and treatment of each lung tumor.

### 1627 Type V Collagen Treatment Increases Caspase-9 Expression in Murine Lung Cancer

*ER Parra, CM Vargas, LC Bielecki, JMFP Ribeiro, FA Balsalobre, WR Teodoro, W de Souza, VL Capelozzi.* Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil.

**Background:** Type V collagen (COL V) is a component of the extracellular matrix (ECM) located in pulmonary interstitium and capillary basement membranes. Recently, this collagen has shown efficient as tumoral and endothelial apoptotic-promoter emerging promise as inductor of death response via caspase 9. In this study, we sought to examine the interface of COL V and endothelial apoptosis in experimental lung cancer.

**Design:** Four groups of mice Balb/c males were studied: a) control (n=5), b) animals that received two doses of 3g/kg intraperitoneal of Uretane (n=10), c) animals that received two doses of 3g/kg intraperitoneal of Uretane and treatment with COL V intranasal administration of 20 mug for 2 months after 2 months of uretane administration (n=10), and d) animals that received only intranasal COL V (n=5). The mice were sacrificed after 6 months. Lung histological sections underwent hematoxylin-eosin, immunofluoresce for COL V and immunohistochemistry for Caspase 9 methods for morphometric analysis.

**Results:** Collagen V (7.19 ± 2.81) and caspase 9 (5.99 ± 2.55) densities in tumoral area were lower when compared with normal surrounding parenchyma (12.31 ± 0.79) and control (9.67 ± 3.20) groups. The intranasal COL V administration in animals with lung cancer increased the caspase 9 expression rate by tumoral endothelial cells (16.06 ± 4.36) when compared with other groups ($p<0.01$).

**Conclusions:** Collagen V intranasal treatment induces increased endothelial immune expression of caspase 9 in tumoral areas of experimental lung cancer, thus leading to decreased angiogenesis by high endothelial death rate. Further studies will be required in randomized and prospective trials to validate the therapeutic efficacy of type V collagen. ***Financial supported:*** FAPESP.

### 1628 Therapeutic Biomarkers in Thymic Tumors – A Clinicopathologic Study from Single Institution

*DT Patil, N Dimov, AV Yeldandi, AL deHoyos, N Hou, A Rademaker, MG Blum, JD Patel.* Northwestern University, Chicago, IL.

**Background:** Stage and completeness of resection are the best prognostic indicators of thymic tumors. Advanced thymomas are often treated like non small cell lung cancers with cisplatin-based regimens. Excision-repair cross-complementation group 1 (ERCC1), a marker of resistance to cisplatin and its analogues, has not been evaluated in thymic tumors. In this study, we evaluate expression of a panel of markers including ERCC1 for their utility as potential therapeutic targets and correlate their expression with clinicopathological parameters.

**Design:** Surgical thymic resections (normal-21, hyperplasia-10 and thymomas-31) from 1998-2007 were evaluated for WHO type, size, myasthenia gravis (MG), Masaoka stage and follow-up status. Immunohistochemical expression of HER2/neu, c-kit, EGFR, ERCC1, Octreotide (OCT) and VEGF was evaluated using appropriate controls, scored semiquantitatively & statistically analyzed.

**Results:** The mean age of patients (15 males, 16 females) was 52 yrs. Subjects with MG (n=8, 45 yrs) were younger than those without MG (n=55 yrs, $p=0.06$). The tumor distribution per WHO type & Masaoka stage was: A(1), AB(6), B1(7), B2(6), B3(7) and C(4) and Stage I (12), II (7), III (9) and IV (3). Compared to normal/hyperplasia (8%), EGFR was the only biomarker significantly overexpressed in thymomas (92%, $p=0.001$). In contrast, nuclear ERCC1 expression was higher in normal/hyperplasia (85%/90%) compared to thymomas (A,AB,B -50% and C-100%, $p=0.002$). In addition, more ERCC1 expression was noted with progressive WHO type ($p=0.04$) and Masaoka stage ($p=0.03$). HER2/neu expression was noted in 3 cases (B1, 2 -type C) while only one case was c-kit positive (type C). VEGF was expressed normally in Hassall's corpuscles, all Type C and 80% of other thymomas. OCT expression was higher in thymomas (61%) compared to normal/hyperplasia (39%).

**Conclusions:** Our findings suggest that anti-EGFR, VEGF and Octreotide agents may be potential therapeutic targets as they are overexpressed in thymomas. Higher expression of ERCC1 in advanced thymomas indicates that cisplatin-based regimens may not be a good treatment option for these patients. EGFR is a good discriminatory marker between normal and abnormal thymic tissue, especially, in small biopsy samples.

### 1629 Increased RASSF1 C Expression Correlates with Tumor Recurrence and Shorter Patient Survival in High-Grade Pulmonary Neuroendocrine Carcinomas

*G Pelosi, C Fumagalli, A Sonzogni, P Maisonneuve, B Del Curto, M Manzotti, D Galetta, G Viale.* European Institute of Oncology and University of Milan School of Medicine, Milan, Italy; European Institute of Oncology, Milan, Italy.

**Background:** The Ras-association domain family 1 (RASSF1) gene regulates complex functions, such as cell proliferation, motility and apoptosis, under two promoters (1&2) and different mRNA isoforms. Little is known, however, about the role of this gene in pulmonary neuroendocrine tumors.

**Design:** Promoter hypermethylation, real-time PCR assay for different RASSF1 isoforms, RASSF1 A protein immunodetection, and FISH analysis for 3p21.3 loss were assessed in 58 snap-frozen pulmonary neuroendocrine tumors, including 20 typical (TC) and 11 atypical carcinoids (AC), 11 large-cell neuroendocrine (LCNEC) and 16 small-cell (SCLC) carcinomas, as well as in 20 non-small cell lung carcinomas (NSCLC) which were used as controls. Non-neoplastic lung tissue was also paired in all assays.

**Results:** Promoter 1 included two main CpG islands that were hypermethylated in neuroendocrine tumors but not in non-neoplastic tissue samples or NSCLC ($p<0.001$), with fine regulation being according to tumor grade (TC/AC vs LCNEC/SCLC, $p<0.001$). Island 1 but not island 2 hypermethylation correlated with down-regulation of RASSF1 A protein expression in AC ($p=0.0337$), and reduced RASSF1 A/E mRNA content was also observed in SCLC as compared with LCNEC ($p=0.041$). At variance, promoter 2 was never hypermethylated and up-regulation of its RASSF1 C transcript emerged as an adverse prognosticator in the LCNEC/SCLC group for both overall (HR: 2.271; $p=0.005$) and disease-free survival (HR: 1.763; $p=0.0169$), independent of tumor stage. Loss of RASSF1 locus at 3p21.3 did not differ between neuroendocrine tumors and NSCLC, but positive correlation was found between the FISH signal and the RASSF1 A/E isoform ($p=0.023$) and protein ($p=0.043$) expression in atypical carcinoids.

**Conclusions:** In conclusion, the RASSF1 gene is likely to play a relevant role in the development of pulmonary neuroendocrine tumors, with dual functions of the recessive oncogene for RASSF1 A/E in the growth of atypical carcinoids and SCLC, and of the dominant oncogene for RASSF1 C in the survival impairment of high-grade tumor patients.

### 1630 MicroRNA Biomarkers for Differential Diagnosis of Lung Tumors

*M Perelman, S Rosenwald, Y Spector, L Cohen, E Meiri, Z Bentwich, R Aharonov, I Barshack.* Sheba Medical Center, Tel-Hashomer, Israel; Rosetta Genomics, Rehovot, Israel.

**Background:** Lung cancer is a heterogeneous disease with many histological subtypes, further complicated by the frequent occurrence of lung metastases from various tumor origins. Classification of lung tumors often poses a diagnostic challenge, but correct classification is important for selecting the proper line of treatment. Specific markers for different subtypes are an important aid for pathologists. Various molecular markers have been suggested, but the potential role of microRNA has not yet been thoroughly explored. MicroRNAs are a family of short non-coding RNAs that play a central role in regulation of gene expression, and are strongly associated with tissue and cancer development. Here we studied the potential utility of microRNAs as biomarkers for classification of neuroendocrine, small-cell, non-small-cell, and metastatic lung malignancies.

**Design:** We used proprietary protocols for extracting high-quality RNA from formalin-fixed, paraffin-embedded (FFPE) and fresh/frozen samples. RNA, which includes the microRNA fraction, was profiled using microRNA microarray and qRT-PCR. We profiled dozens of samples from different histological subtypes of lung cancer including small cell carcinoma, different neuroendocrine lung cancers, non small cell lung cancer (NSCLC), and metastases to the lung. Expression levels of hundreds of microRNAs were measured and compared between the sample groups. Emerging differences in expression were validated by specific assays using a microRNA qRT-PCR platform.

**Results:** We found that a surprisingly small number of microRNAs can be used as biomarkers for differential diagnosis of various types of lung tumors. 1) A panel of microRNAs can distinguish between primary lung tumors and metastases to the lung. 2) A combination of few microRNAs is sufficient to distinguish between carcinoid and other neuroendocrine tumors. 3) An additional set of microRNAs distinguishes between neuroendocrine tumors and small cell lung cancer.

**Conclusions:** Our results highlight the specificity of microRNA expression in tumor subtypes. We found that combinations of small numbers of microRNAs can successfully aid in the differential diagnosis of lung tumors, and provide a basis for the development of simple and reliable assays in clinical oncology.

### 1631 C4d Immunohistochemistry in Surveillance Pulmonary Allograft Biopsies: A Helpful or Wasteful Practice?

*MK Potash, JA Weydert.* University of Iowa, Iowa City, IA.

**Background:** Immunohistochemical detection of C4d, a product of complement activation, has been touted by some as a useful marker of antibody mediated rejection (AMR) in pulmonary allograft recipients. However, interpretative criteria for assessing a 'positive' C4d stain have yet to be standardized, and there is no consensus recommendation from the ISHLT in this regard. We investigated the utility of routine C4d immunohistochemistry in the evaluation of surveillance biopsies in pulmonary allografts at our medical center.

**Design:** Immunohistochemistry for C4d (Bromedica) was performed as a routine test on pulmonary allograft surveillance biopsies (10 patients, 32 total biopsies, all within first year of transplant). IHC was done using the Dako EnVision Plus system, at 1:30 primary dilution, pressure-cooker antigen retrieval, and 40 minute primary incubation time. Staining was assessed by histoanatomic location (small vessel endothelium versus alveolar septal) and semi-quantitative extent of staining. Results were correlated with histologic findings, clinical information, and panel reactive antibodies (PRA) results.

**Results:** Alveolar septal staining was present in 9 of 10 patients (26 total biopsies). Alveolar septal staining was focal or limited in 24 of the biopsies and diffuse in 2 biopsies. Small vessel staining was seen to some degree in all biopsies. Two patients developed a positive PRA with specific antibodies identified on confirmatory testing, however no patients developed clinical evidence of, or were treated for, humoral mediated rejection. Extent of C4d staining did not correlate with the presence or absence of acute cellular rejection.

**Conclusions:** In our hands, C4d immunostaining shows variable reactivity within the alveolar septae and small vessel endothelium in most biopsies tested. Given these findings and a lack of prospectively validated interpretative criteria, it is difficult to justify routine C4d staining in surveillance biopsies. C4d may be better utilized as an adjunctive test in cases where clinical suspicion of AMR is high. Large multicenter studies with central review should be undertaken in order to determine diagnostic cut-offs for 'positive' C4d reactivity in this setting.

### 1632 Evaluation of 18 miRNAs in Non-Small Cell Lung Carcinoma According to Its Clinical Stage

*A Quera, A Navarro, A Huerta, R Marrades, N Vinolas, M Monzo, J Ramirez.* Hospital Clinic, Barcelona, Spain; Facultat Medicina UB, Barcelona, Spain.

**Background:** microRNA (miRNA) are small molecules of RNA that act as negative regulators in proteic synthesis. They are encoded in intronic or intergenic regions. Its function is performed in the cytoplasm where they bind specific messenger RNAs and downregulate its translation. They do participate in the regulation of multiple biologic processes, with cancer among them.

**Design:** Aim: To determine the expression of miRNA in normal lung tissue as well as in neoplasic tissue. To analyze the relationship among them in different stages of tumoral disease according to the TNM. Methods: We analyzed 62 bronchial biopsies (31 tumoral and 31 normal) obtained from fibrobronchoscopy corresponding to 31 patients with non-small cell lung carcinoma (NSCLC). We determined the expression of 18 miRNA by real time RT-PCR and performed the analysis of data with SPSS.

**Results:** The hierarchical clustering of miRNA expression showed statistically significant differences between the following groups: · Tumoral tissue vs. normal tissue: There is an underexpression of *let-7c* and overexpression of *miR 17-5p*, *miR 19a* and *miR-221*, that are involved in carcinogenesis and cell proliferation. · NSCLC stage I vs. all other stages: We found underexpression of miRNA of the *let-7* family (let-7a, let-7c amd let-7e) (p=0.015) and overexpression of *miR 17-5p*, *miR 19a* and *miR-221.* · NSCLC stage IV vs. all others: We found an overexpression of *miR 21*, *miR 491* and *miR 502* (miRNAs involved in the diminution of apoptosis and cellular polarization).

**Conclusions:** The results indicate that miRNA expression profiles are diagnostic and prognostic markers of NSCLC. The miRNA studies give us information about both the biology of these tumors and the staging. These miRNAs may be good targets for new therapeutic strategies. **With the support of**: FIS060087, SEPAR06, CIBER DE ENFERMEDADES RESPIRATORIAS 06/06/0028

### 1633 Analysis of *EGFR* and *KRAS* Mutations in High-Grade Neuroendocrine Carcinomas of the Lung

*N Rekhtman, M He, C Lau, K Nafa, GJ Riely, MF Zakowski, M Ladanyi.* Memorial Sloan-Kettering Cancer Center, New York, NY.

**Background:** *EGFR* and *KRAS* mutations are found in ~20% and ~25% of lung adenocarcinomas (AdCA), respectively. The prevalence of *EGFR* and *KRAS* mutations in small cell lung cancer (SCLC) and large cell neuroendocrine carcinoma (LCNEC) is not well established. Based on limited published data, these mutations are thought to be confined to AdCA. However, recent case reports have described three unusual *EGFR*-mutant SCLCs, arising in association with AdCA. These studies highlight the need for a more systematic analysis of *EGFR* and *KRAS* mutations in high-grade neuroendocrine carcinomas.

**Design:** Archival tissue blocks from SCLC (n=20) and LCNEC (n=10) were macrodissected and DNA was extracted. We screened for known point mutations in *EGFR* and *KRAS* by Sequenom (MALDI-TOF mass spectrometry genotyping platform). *EGFR* exon 19 mutations were tested by length analysis of fluorescently labeled PCR products. In addition, point mutations in other key signaling genes (*NRAS*, *HRAS*, *BRAF*, *HER2*, *PIK3CA*, *MEK1*, *AKT1*) were analyzed by Sequenom. The findings were correlated with clinicopathological parameters using a two-tailed *t* test or a Fisher's exact test.

**Results:** No *EGFR* mutations were identified. However, *KRAS* mutations were found in 1 of 20 SCLC (5%) and 4 of 10 LCNEC (40%). The only SCLC with *KRAS* mutation had a separate synchronous AdCA. *KRAS* mutations involved codons 12 (n=4) and 13 (n=1). Interestingly, the type of *KRAS* mutations paralleled the distribution of these mutations in smoking-related AdCA with G>T transversions (n=4) found more frequently than G>A transitions (n=1). In addition, within the group as a whole (n=30), *KRAS* mutations were associated with heavier smoking history: average pack-years 70 vs 43 for *KRAS*-mutated vs *KRAS*-non-mutated tumors (*P*=0.027). *KRAS* mutations were not associated with age, gender, tumor size, or metastasis. No mutations were found in any other tested genes.

**Conclusions:** This study uncovered a high rate of *KRAS* mutations in LCNEC (40%), whereas none of pure SCLC harbored either *KRAS* or *EGFR* mutations. One *KRAS*-mutated SCLC was associated with a synchronous AdCA, similar to previously reported SCLCs with *EGFR* mutations. Our findings suggest that SCLC and LCNEC are molecularly distinct, and that the *EGFR* or *KRAS* mutations in SCLC may be limited to tumors arising in association with AdCA. The significance of frequent *KRAS* mutations in LCNEC and their association with smoking warrants further investigation.

### 1634 Prognostic Significance and Reproducibility of Two Histological Classifications for Thymic Epithelial Tumors

*AC Roden, ES Yi, SC Bryant, JL Donovan, JE Lewis, RS Marks, SD Cassivi, YI Garces, MC Aubry.* Mayo Clinic, Rochester, MN.

**Background:** Thymomas can be complicated by invasion, recurrence, metastases and death. Several histological classifications have been proposed, however, their prognostic significance, ability to guide further treatment and reproducibility have been controversial.

**Design:** Medical records from 100 pts with thymoma (1942–1981) were reviewed. Two pts were classified having carcinoma and removed from analyses (N=98). Two pathologists blinded to outcome independently classified all cases. If more than one subtype, a predominant and a "worst" type were determined. Statistical analyses were performed.

**Results:** 50 men & 48 women had a median age of 53yrs. The median FU was 9.5yrs. In 13 pts recurrence and/or metastasis occurred. 11 died of disease.

Histological Classifications

| **WHO** | **# of pts** |
|---|---|
| A | 2 |
| AB | 8 |
| B1 | 35 |
| B2 | 33 |
| B3 | 20 |
| **Bernatz** | |
| Spindle | 6 |
| Lymphocyte predominant | 33 |
| Mixed epithelial & lymphocytic | 41 |
| Epithelial predominant | 18 |

Patients were staged according to Masaoka: I in 24, II in 48, III in 20, IVa in 5, IVb in1.

Results of Univariate Analyses

| | Recurrence free survival (RFS)* | Overall survival (OS)* | Death to disease survival (DDS)* |
|---|---|---|---|
| Myasthenia gravis | 0.78 | 0.54 | 0.85 |
| Sup. vena cava (SVC) syndrome | <0.001 | 0.018 | <0.001 |
| WHO,overall | 0.006 | 0.002 | 0.425 |
| WHO,worst | 0.012 | <0.001 | 0.32 |
| Bernatz,overall | 0.002 | 0.001 | 0.66 |
| Bernatz,worst | 0.009 | <0.001 | 0.35 |
| Invasion | 0.027 | 0.29 | 0.09 |
| Masaoka Stage | <0.001 | <0.001 | <0.001 |
| Complete Resection | 0.003 | <0.001 | <0.001 |
| Radiation neo-adjuvant | 0.32 | <0.001 | <0.001 |

*p-values

In multiple variable survival analysis, after adjusting for resection and neo-adjuvant therapy, only Bernatz classification (p=0.02) was an independent prognostic feature. Interobserver agreements were: WHO, κ 0.475; Bernatz κ 0.425, Invasion κ 0.293.

**Conclusions:** SVC syndrome, complete resection and neo-adjuvant radiation are strongest survival predictors. WHO and Bernatz classifications predicted RFS and OS but not DDS. Invasion into capsule predicts RFS, however not OS and DDS. After adjusting for resection and radiation, Bernatz classification was an independent factor. The interobserver agreement was only moderate in both classifications and poor for determining invasion into capsule.

### 1635 Phenotype of Lung Adenocarcinoma with *EML-ALK* Fusion in the Western Population

*SJ Rodig, S Dacic, BY Yeap, JA Barletta, K Law, N Lindeman, AJ Iafrate, LR Chirieac.* Brigham & Women's Hospital, Boston, MA; Massachusetts General Hospital, Boston, MA; University of Pittsburgh, Pittsburgh, PA.

**Background:** Approximately 3% of lung adenocarcinomas within the Asian population have an interstitial inversion in chromosome 2p resulting in the *EML4-ALK* fusion gene (ALK+) which is sensitive to ALK inhibitors. The prevalence, epidemiological and morphological characteristics of this abnormality in the Western population is unknown and establishing the appropriate confirmatory diagnostic tests is critical.

**Design:** We screened tumor specimens from 2 groups of patients with lung adenocarcinoma by immunohistochemical staining (IHC) for ALK protein expression and fluorescent in-situ hybridization analysis (FISH) for the *EML4-ALK* gene fusion. The first group consisted of 280 unselected cases from 2 institutions (BWH, Boston=155; University of Pittsburgh=125). A second group were lung adenocarcinomas from a selected cohort of patients accrued in a trial conducted at a third institution (MGH, Boston=83).

**Results:** Within the first group of cases, we identified only 1 tumor (1/280 cases; 0.4%) with an *EML4-ALK* fusion. Within the second group, we identified 11 tumors (11/83 cases; 13%) with *EML4-ALK* fusion. Relative to all patients with NSCLC in the United States, we find that patients with ALK+ tumors are more likely to be female (75% vs. 36%; p= .007), younger (median age 52 vs. 66 years; p= .019), and non-smokers (58% vs. 15%; p< .001). In addition, all ALK+ tumors were EGFR wild type (12/12 cases). Although a broad spectrum of histologic patterns were represented among the ALK+ tumors, we found a predominance of signet-ring cell/mucinous histology (4/12 cases).

**Conclusions:** ALK+ NSCLC is very rare in the Western population. Our results define the features of the molecular subtype of lung adenocarcinomas with *EML4-ALK* fusion. Suspected cases are best evaluated for the *EML4-ALK* fusion gene by both IHC and FISH to ensure an accurate diagnosis in triaging for the appropriate targeted therapy.

### 1636 Different TRAP220 Expression in Distinct Histologic Subtypes and Smoking Status of Lung Adenocarcinomas

*MS Roh, JH Yun, CH Son, DK Yang, PJ Choi, KE Lee.* Dong-A University, Busan, Korea.

**Background:** Adenocarcinoma (ADC) is becoming the most common histologic type of lung cancer in both sex. Although most cases are seen in smokers, it develops more frequently than other histologic types in individuals who have never smoked. Recent evidence now suggests that estrogen also contributes to the pathogenesis of lung cancer. TRAP220 can serve as a binding target for transactivators involved in cell growth and development. Since TRAP 220 is also an essential coactivator that interacts directly with estrogen receptor α, we examined the expression of TRAP220 protein to investigate its role in lung ADC, with particular attention paid to its different histologic subtypes and smoking history.

**Design:** We performed immunohistochemical detection of TRAP220 protein in eighty-three tissue samples from primary lung ADC patients using a tissue microarray and Western blotting was done to confirm the immunohistochemical observations.

**Results:** TRAP220 immunoreactivity was observed in 18 (21.7%) of the 83 ADC cases. Analysis of the TRAP220 expression in 6 ADC tissues and 2 normal lung tissues by Western blotting confirmed the immunohistochemical results. TRAP220 expression

was higher in ADC tissues than in adjacent normal bronchial epithelial cells. TRAP220 expression was more frequently detected in acinar and papillary subtypes than in solid subtype (p =0.029). The tumors with a positive TRAP220 expression more frequently showed lymph node metastasis (p=0.037). The incidence of TRAP220 expression was significantly lower in the heavy smokers with more than 20 pack-years (p=0.047). TRAP200 expression pattern had a negative association with the number of pack-years smoked (positive expression (14.4±11.3 pack-years) vs. negative expression (22.2±15.0 pack-years)) (p=0.043).

**Conclusions:** This study suggests that TRAP220 might have a distinct role in the pathogenesis of acinar and papillary histologic subtypes and in non-smokers. Because ADC is a very heterogeneous subgroup of lung cancers, our data may be useful to understand the different biologic basis of the development of subtypes of lung ADC.

### 1637 ERK Immunostaining Predicts *EGFR* and/or *KRAS* Mutation in Lung Adenocarcinomas, and Can Be Used To Prescreen Tumors for Subsequent Molecular Testing

*MB Ruzinova, V Joshi, D Jackman, P Janne, NI Lindeman.* Brigham and Women's Hospital, Boston, MA; Beigham and Women's Hospital, Boston, MA; Dana Farber Cancer Institute, Boston, MA; Harvard Medical School, Boston, MA.

**Background:** Somatic mutations in the gene for the epidermal growth factor receptor (*EGFR*) are found in ~20% of lung adenocarcinomas and are associated with clinical response to the tyrosine kinase inhibitors (TKIs) gefitinib (Iressa) and erlotinib (Tarceva). Another ~30% of lung adenocarcinomas harbor activating mutations in *KRAS*, the GTPase involved in signaling downstream of EGFR, but in contrast to *EGFR* mutations, *KRAS* mutations confer resistance to TKIs. Molecular analysis of both genes is needed to guide TKI therapy, but molecular analysis is costly and not widely available, and a simpler technique is desirable. Since both *EGFR* and *KRAS* mutations lead to increased levels of a common downstream effector, extracellular signal regulated kinase (*ERK*), IHC for ERK may be useful to select tumors to refer for molecular testing.

**Design:** ERK expression was studied by immunohistochemistry on a microarray containing 5 cores from each of 29 lung adenocarcinomas, and correlated with the mutational status of *EGFR* and *KRAS* determined by direct sequencing of corresponding tissue from dissected unstained 5-micron paraffin-sections.

**Results:** ERK immunoreactivity was seen in 15/20 (75% sensitivity) tumors with either *EGFR* or *KRAS* mutation, and in only 3/9 tumors without mutation in either gene (67% specificity). Positive predictive value in this selected population was 83.3% (15/18) . All three of the patients with ERK positivity but neither *EGFR* nor *KRA*S mutation failed to respond to TKI treatment, suggesting that perhaps an alteration in another signaling molecule downstream of KRAS (e.g., *BRAF*) may be the oncogenic stimulus in these tumors.

**Conclusions:** ERK immunostaining may be a useful predictive tool to identify tumors that have mutations in either *EGFR* or *KRAS*, and should go on to molecular testing of these genes. Larger studies are needed to confirm this result.

### 1638 Morphologic Findings in PET-Positive, Tumor-Negative Lymph Nodes in Patients with Lung Cancer

*DR Semmel, B Daly, CJ O'Hara.* Boston Medical Center and Boston University School of Medicine, Boston, MA.

**Background:** Positron Emission Tomography **(**PET) imaging has become a major preoperative tool used to assess lung cancer tumor burden in lung and extra-pulmonary sites, including lymph nodes. Not infrequently, PET scans exhibit positive FDG uptake in lymph nodes, indicating presumed nodal metastasis, which subsequently reveal no detectable tumor on routine histological evaluation. As little is known about the histology of these nodes, the goal of this study was to look at the morphologic characteristics of PET-Positive, Tumor-Negative (PPTN) lymph nodes and compare them to PET-Negative, Tumor-Negative (PNTN) nodes from similar sites.

**Design:** In total, 68 blocks of PPTN lymph nodes from 15 patients and 16 blocks of PNTN lymph nodes from 7 patients with known primary non-small cell lung cancer were evaluated for the following histologic parameters: Histiocytosis (SH), Anthracosis (AN) and Follicular Hyperplasia (FH). In addition immunohistochemical stains for cytokeratins (AE1/3) were included to detect possible missed metastasis and stains for Ki-67 to assess cellular proliferation.

**Results:** The most significant findings were morphologic and are summarized in the following table:

Sinus Histiocytosis (SH) and Anthracosis (AN)

| | Graded Score: | 0 | 1 | 2 | 3 |
|---|---|---|---|---|---|
| PPTN (68) | SH | 1 | 16 | 24 | 27 |
| PNTN (16) | SH | 0 | 7 | 8 | 1 |
| PPTN (68) | AN | 1 | 28 | 30 | 9 |
| PNTN (16) | AN | 2 | 5 | 6 | 3 |

SH: 0=none, 1=sinus expansion, 2=histiocytosis w/ aggregates, 3=sheetlike aggregates/granulomas.
AN: 0=none, 1=mild, 2=moderate, 3=severe

Follicular Hyperplasia

| Graded Score: | 0 | 1 | 2 |
|---|---|---|---|
| PPTN (68) | 23 | 35 | 10 |
| PNTN (16) | 6 | 8 | 2 |

0=none, 1=rare atrophic follicles, 2=prominent follicle formation

The most notable morphologic findings in the PPTN nodes were increased sinus histiocytosis and anthracosis. Well-formed granulomas were noted in 3 patients. Missed micrometastases (< 1mm) were observed in only two patients. Ki-67 staining (range from <5 to 15%) was found mostly in secondary follicles when present and was negligible in the histiocytic component.

**Conclusions:** The major histological correlate for the increased FDG uptake in PPTN nodes is sinus histiocytosis and anthracosis. The increased uptake appears not to be due to an increased proliferative activity of these cells. Nodal follicular hyperplasia, micrometastasis and granulomas are less of a factor.

### 1639 EGFR Mutation Is a Better Predictor of Response to TKIs in NSCLC Than FISH, CISH, or IHC

*LM Sholl, V Joshi, D Jackman, Y Xiao, C Lee, P Janne, N Lindeman.* Brigham and Women's Hospital, Boston, MA; Harvard University, Boston, MA; Dana Farber Cancer Institute, Boston, MA.

**Background:** ~10% of patients with advanced NSCLC respond to epidermal growth factor receptor (EGFR)-targeted tyrosine kinase inhibitor (TKI) therapy. >75% of the tumors that respond have activating mutations in the *EGFR* gene. However, mutation analysis is not widely available, and proposed alternative assays, such as FISH or CISH for *EGFR* copy number (CN), and immunohistochemistry (IHC) for protein expression have shown inconsistent associations with TKI therapy response.

**Design:** *EGFR* mutation, CN, and protein expression were studied in 43 NSCLC samples from patients treated with TKIs with known treatment response (per RECIST criteria). Stable nonprogression was included with partial response as a favorable outcome. DNA was extracted from unstained 5-um paraffin sections containing >50% tumor cells; EGFR mutations were detected by PCR-capillary sequencing. FISH, CISH and IHC assays were performed on a tissue microarray containing 5 cores from each sample. Modal *EGFR* copy numbers seen by CISH were categorized as either disomic (2 signals), low polysomy (<5), high polysomy (HP)(5-7), or amplified (8+ or homogenous staining regions). FISH was scored by the method of Varella-Garcia, et al. HP/amplified cases were considered CN positive. Modal membranous IHC intensity (0-4) was multiplied by % of cells staining to give an index from 0-400; scores ≥100 were considered positive.

**Results:** 24/43 (56%) patients had a favorable outcome after TKI treatment. Favorable outcomes were observed in 16/20 (80%) patients with mutations vs. 8/23 (35%) WT patients (p = 0.005); in 9/16 (54%) patients with high CN vs. 13/24 (56%) CN negative cases (p=1); and in 7/9 (78%) of IHC positive cases vs. 12/25 (48%) IHC negative cases (p=0.24). Moreover, neither IHC nor CN were associated with mutation: 9/17 (53%) IHC positive cases were mutated vs. 10/21 (48%) IHC negative cases, and 10/16 (63%) CN positive cases were mutated vs. 8/24 (33%) CN negative cases (p=0.10). There was a trend toward higher copy number in IHC positive cases (p=0.04).

**Conclusions:** *EGFR* sequence, copy number, and/or protein expression can all be altered in NSCLC. However, only mutation predicts response to TKI therapy. Increased *EGFR* copy number, but not mutation, correlates with protein overexpression.

### 1640 Fibrosing Lymphohistiocytic Lesions with Increased IgG4-Positive Plasma Cells as a Pulmonary Manifestation of Autoimmune Pancreatitis

*B Shrestha, TV Colby, MC Aubry, TC Smyrk, AL Feldman, LD Cornell, ST Chari, JH Ryu, ES Yi.* Mayo Clinic, Rochester; Mayo Clinic, Scottsdale.

**Background:** Autoimmune pancreatitis (AIP) is the prototype of a systemic, steroid-responsive IgG4-related disease featuring elevated serum IgG4 and characteristic fibroinflammatory changes accompanied by increased IgG4-positive plasma cells in the tissue. IgG4-rich fibroinflammatory infiltrates have been reported in many extrapancreatic sites including the lung, but the histologic features of pulmonary IgG4 disease are not well established.

**Design:** Four lung biopsies from documented AIP patients and additional 10 cases showing similar histopathology were identified in consult and surgical pathology files at Mayo Clinic. For comparison, Erdheim-Chester disease (ECD) (n=4), pulmonary Sjögren's diseases (SD) (n=11), variety of chronic interstitial pneumonia (CIP) (n=25) and nodal/extranodal Rosai Dorfman disease (n=8) were studied. All cases were stained for IgG4 and all but 3 for IgG (due to insufficient material). Average numbers of IgG- and IgG4-positive cells in 3 HPFs showing the highest density were counted. IgG4 score (0:<5, 1:5-10, 2:11-30, 3:>30), IgG4/IgG % and histopathologic findings were recorded.

**Results:** Three lung biopsies from AIP patients showed IgG4 score of 3 with the IgG4/IgG ratio ranging from 32 to 58%. The remaining AIP patient had IgG4 score of 2 and the IgG4/IgG ratio of 10%. All 4 cases had fibroinflammatory changes in a lymphangitic distribution and prominent histiocytic infiltrates: one case had a marked emperipolesis. Peribronchial lymphoid infiltrates, obliterative vasculitis and storiform fibrosis were also noted. Nine of 10 additional cases showing similar histopathology demonstrated IgG4 score 2 or 3 and the remaining 1 case had score 0. IgG4/IgG ratio in cases with IgG4 score 2 or 3 ranged from 16 to 71%. None of ECD and pulmonary SD had IgG4 score 2 or 3, while 4 of 25 CIP and 6 of 8 RDD cases showed IgG4 score of 2 or 3.

**Conclusions:** Our AIP cases demonstrated pulmonary lymphohistiocytic infiltrates in lymphangitic distribution, storiform fibrosis, obliterative vasculitis and occasional emperipolesis. Increased IgG4-positive cells were seen in the majority of cases showing similar histopathology to the pancreatic lesions of patients with AIP, suggesting these histologic findings as pulmonary manifestations of IgG4 disease. ECD or pulmonary SD probably is not IgG4-related. The significance of increased IgG4-positive cells in some CIP and nodal/extranodal RD cases merits a further study.

### 1641 Strong Correlation between Solid and Micropapillary Subtypes of Lung Adenocarcinoma (AD) in Primary and Metastatic Sites

*G Sica, W Travis, A Moreira.* Memorial Sloan Kettering Cancer Center, New York, NY.

**Background:** AD mixed subtype, the most common histologic type of lung cancer, is a heterogeneous group composed of a mixture of different patterns and pattern combinations. The prognostic significance of each individual type of AD within the mixed subtype is not clearly understood. In this study, the predominant pattern of AD in the primary tumor is compared with the pattern seen in the metastatic site to determine whether the presence of a specific pattern in a mixed subtype tumor correlates with higher stage of disease.

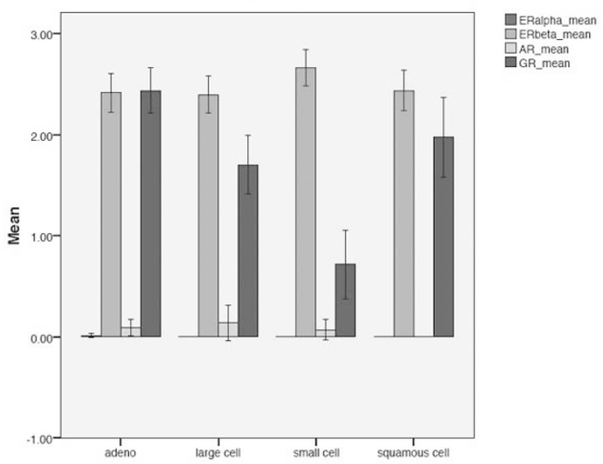
**Design:** This is a retrospective study of 59 patients with mixed subtype ADs that presented with regional lymph node metastasis. Histologic subtyping was evaluated by

comprehensive semiquantitation and classified by major subtype. The major subtypes were compared in the primary and the metastatic site.
**Results:** The patient's age ranged from 35 to 83 years (mean age of 65), 43 were women and 16 men. Acinar was the most common subtype, present in 100% of cases, followed by micropapillary (81%), papillary (78%), solid (60%) and bronchioloalveolar (18%). Acinar was the predominant pattern in 53% of the tumors, followed by papillary (20%), solid (17%), and micropapillary (10%). In contrast, the predominant metastatic pattern was solid (31%), acinar (27%), micropapillary (27%), and papillary (15%). The overall correlation coefficient between predominant pattern in the primary site and metastasis was of 0.489 ($p < 0.0001$). In the cases that micropapillary (n=6) or solid (n=10) types were the predominant patterns in the primary tumor, the corresponding lymph node metastasis had the same pattern as the primary tumor. Even when the micropapillary or solid types comprised a minor, 5-10%, of a primary tumor, these types were more likely to be seen in the lymph node metastases.
**Conclusions:** AD classification using the most prominent subtyping reveals that when a mixed subtype AD contains either solid or micropapillary pattern, these patterns are overrepresented in the metastatic sites, a finding that correlates with the known high grade nature of these subtypes. A predominant acinar or papillary pattern in mixed subtype does not appear to correlate with the pattern in the metastatic site. Therefore, the high grade solid and micropapillary subtypes of AD are more likely to metastasize to lymph nodes even if they are not the most prominent subtype in the primary tumor.

### 1642 Steroid Hormone Receptor Expression and Survival in Lung Cancer

*SM Sitterding, M Tretiakova, L Faoro, R Salgia, S Conzen, AN Husain, T Krausz.* University of Chicago, Chicago, IL.

**Background:** Steroid hormone receptor expression is used in the treatment of solid tumors and often correlates with patient outcome. For example, estrogen receptor (ER) reactivity of some lung cancers predicts poor survival. Glucocorticoids (GCs) are produced during physiologic stress and have medical utility for their immunosuppressive and anti-toxic effects as well as for the treatment of ALL. Studies in solid tumor cell lines have demonstrated that GCs inhibit growth and in some non-small cell lung cancers (NSCLC) GCR expression correlates with good outcome. However, in vitro studies illustrate that GCs antagonize chemotherapy-induced apoptosis; thus, the role of GCR expression and GC therapy remains unclear. Our study examines the expression of steroid hormone receptors in primary lung carcinomas and correlates these data with patient survival.
**Design:** Tissue microarrays (TMAs) of archived primary lung tumors from the University of Chicago, created after IRB approval, include 59 adenocarcinomas (AdCa), 48 squamous cell (SqCC), 47 small cell (SmCC), and 42 large cell carcinomas (LCC). Each TMA was stained with ERalpha, ERbeta, androgen receptor, and GCR. Scoring of each immunohistochemical stain was based on the Reiner 4-tiered system incorporating combined scores of intensity and percent of nuclei staining (score 0-3).
**Results:** GCR levels were highest in AdCas while SmCCs showed significantly lower scores. In patients with NSCLC, no significant difference in survival was seen based on low vs high GCR expression. However, in patients with SmCC, high expression of GCR correlated with better survival (8/9, 89% alive at a mean of 1 year) compared to those with low expression of GCR (11/32, 34% alive at a mean of 1 year).
**Conclusions:** GCR is differentially expressed on various primary lung carcinomas. In patients with SmCC, low expression correlates with worse prognosis, suggesting that GCR expression may be a useful predictor of survival in patients with SmCC. Further studies are needed analyzing survival data following GC treatment and chemotherapy to determine the combined effect on patient outcome.




### 1643 Primary Oncocytic Adenocarcinoma of the Lung: Clinicopathological and Immunohistochemical Study of 16 Cases

*LM Solis, MG Raso, C Behrens, I Wistuba, CA Moran.* The University of Texas MD Anderson Cancer Center, Houston, TX.

**Background:** Oncocytic carcinomas originating primarily in the lung parenchyma are rare and lack full characterization as primary pulmonary neoplasms.
**Design:** To investigate the frequency of lung adenocarcinomas having oncocytic features we reviewed 566 primary lung adenocarcinomas surgically resected with curative intent in our institution between 1997 to 2005, and performed a detailed histopathological and immunohistochemical analysis and correlation with patients clinical information.
**Results:** We classified 16 (3%) cases as oncocytic adenocarcinoma. The patients were 11 women and 5 men with age ranging from 47 and 81 years (median 75 years). Fourteen patients (88%) were tobacco smokers. Thirteen patients underwent lobectomy and 3 wedge resection as initial treatment. Thirteen patients (81%) were in pathological stage I; while the remaining 3 patients were in stages II, III, and IV, respectively. Macroscopically, the tumors ranged in size from 1.2 to 7 cm in greatest diameter. Histologically, the tumors showed four distinct growth patterns: bronchioloalveolar, acinar, solid, and papillary. The tumors were composed of cells with abundant eosinophilic cytoplasm, round to oval nuclei, and prominent nucleoli; while in 7 cases areas of necrosis were present. Mitotic figures were present in the range of 1 to 8 per 10hpf. Immunohistochemical studies were performed in 14 cases: all tumors showed positive staining for keratin 7 and TTF-1, and were negative for thyroglobulin. Keratin 20 immunostaining was observed focally in two cases. Four tumors that were analyzed for *EGFR* and 3 for *KRAS* mutations demonstrated to be wild-type. Clinical follow up for recurrence free and overall survival was performed for at least 2 years in all cases: 14 (88%) patients were alive, and 12 (75%) did not have recurrence.
**Conclusions:** The cases herein presented highlight an unusual feature in primary lung adenocarcinoma that may pose a problem in the evaluation of these tumors. The oncocytic characteristics present in our cases represent a distinct histological feature important to recognize in primary lung adenocarcinomas. The use of immunohistochemical studies may prove beneficial in the final evaluation of these tumors (Supported by DoD grant VITAL W81XWH-04-1-0142).

### 1644 Effect of Infection and Acute Cellular Rejection on T Cell Populations in Post-Lung Transplant Biopsy Specimens

*K Suzue, SM Bhorade, M Tretiakova, YX Fu, AN Husain.* Mount Sinai Hospital, Chicago, IL; University of Chicago, Chicago, IL.

**Background:** Episodes of infection and acute rejection are risk factors for subsequent chronic lung transplant rejection. A better understanding of the inflammatory milieu within lung allografts is crucial for improving immunosuppressive regimens and survival in lung transplant (LTx) recipients. Recent studies have suggested a role for CD4+ T cells expressing FoxP3 in inhibiting the CD8+ anti-cellular T cell response and inducing immunotolerance. This study assessed the populations of T cells present in LTx biopsy specimens.
**Design:** We evaluated 21 LTx transbronchial biopsy specimens, which were divided as: Group 1 (n=5) positive for acute cellular rejection (A1BX, A1BX, A2B0, A2B0, A2B2); Group 2 (n=8) positive for viral infection (CMV, influenza, RSV) and Group 3 (n=8) negative for infection and acute cellular rejection. We confirmed these diagnoses on H&E sections and by clinical history. We then immunostained for CD3, CD4, CD8, CD20 and FoxP3 and examined the interstitial and perivascular infiltrates. (Scored: '0' for no positive cells; '1' for 1 cell/40X; '2' for 2-8 cells/40X, '3' for >8 positive cells at 40X and '4' for diffusely positive cells at 4X.).
**Results:** All 21 cases contained a higher proportion of CD8+ T cells in the interstitium, relative to CD4+ T cells (avg CD4:CD8 of 1:6). In group 3 (no rejection or infection), further analysis of T cells was not done due to the minimal inflammatory cell infiltrate. In group 1 (acute cellular rejection group), 1-10% (mean 8%) of interstitial T cells were FoxP3+, and the perivascular FoxP3+ T cell infiltrate was 10%-20% FoxP3+ T cells. In contrast, in group 2 (viral infection group), rare to absent (0-4%; mean 1%) ($p=0.002$) interstitial FoxP3+ T cells were seen despite a 3+ to 4+ level of CD3+ T cell infiltration.
**Conclusions:** This preliminary study illustrates the difference in T cell populations in LTx allografts in patients with acute cellular rejection vs. infection. Specifically, viral infections led to lower numbers of FoxP3+ T cells and may be a mechanism by which viral infections contribute to chronic lung allograft rejection. Evaluation of regulatory T cells as well as other immunologic mediators and markers will contribute to our understanding of lung transplant immunopathology. Indeed, manipulation of specific T cell subsets may be an effective strategy for inducing lung transplant tolerance.

### 1645 NTRK2 Expression Predicts Improved Disease Specific Survival in Squamous Cell Carcinoma of Lung

*J Terry, A DeLuca, S Leung, G Peacock, YZ Wang, WM Elliot, D Huntsman.* BC Cancer Agency, Vancouver, BC, Canada; UBC-James Hogg iCAPTURE Centre, Vancouver, BC, Canada.

**Background:** The neurotrophic tyrosine kinase receptors NTRK1 (TRKA) and NTRK2 (TRKB) are oncoproteins belonging to a family of nerve growth factor receptors that normally regulate nervous system development. NTRK1 and 2 abnormalities have been reported in neoplasms of lung, suggesting a pathogenic role; however, the significance of NTRK1 and 2 expression in lung cancer is unclear.
**Design:** Normal control tissues and 713 individual lung cancer cases with long-term clinical outcome data in tissue microarray format are immunohistochemically stained for NTRK1 and NTRK2 using commercially available antibodies, automated staining and standard protocols. Positive staining is defined as any amount of membranous staining within tumor tissue in at least one tumor core. Statistical analyses are performed on SPSS v16.0.
**Results:** Both NTRK1 and NTRK2 show strong correlation to squamous carcinoma of lung (NTRK1: 211/282 positive, 95% specific and 75% sensitive, Kendall tau-b = 0.730 $p << 0.05$; NTRK2: 139/282 positive, 96% specific and 49% sensitive, Kendall tau-b = 0.529 $p << 0.05$). Positive staining is less frequent in adenocarcinoma (NTRK1: 12/248, NTRK2: 11/248), bronchioloalveolar carcinoma (1: 0/8, 2: 0/8), carcinoid tumor (1: 2/93, 2: 1/93), large cell carcinoma (1: 5/60, 2: 4/60), large cell neuroendocrine tumor (1: 0/6, 2: 2/6), and small cell carcinoma (1: 0/13, 2: 0/13). There is significant correlation between positive NTRK2 staining and improved disease specific survival in squamous carcinoma (Log rank test: $chi^2 = 14.4$, $p = 1.46 \times 10^{-4}$) while NTRK1 staining has no prognostic significance. Positive NTRK2 staining is not frequent enough in other

lung cancer subtypes to achieve statistically reliable correlations with patient outcome. NTRK1 staining is present in normal squamous epithelium from skin and tongue (3/3) but not normal lung (0/1) whereas NTRK2 staining is present in brain (1/1) but not in lung (0/1) or squamous epithelium (0/3) and appears to be cancer specific.
**Conclusions:** Positive NTRK2 staining predicts significantly improved disease specific survival in patients with squamous carcinoma of lung. Both NTRK1 and 2 are highly specific markers of squamous lung carcinoma; however, NTRK2 expression appears restricted to neoplastic squamous lung epithelium and may identify a subset of lung cancer with unique pathogenesis amenable to therapies targeting the NTRK2 signaling pathway.

### 1646 EGFR and K-ras Mutation Status and Correlations Histological Subtypes in Pulmonary Carcinomas. A Dutch Experience

*FB Thunnissen, DW de Jong, DA Heideman, EF Smit, S Burgers, PJ Snijders, J Berkhof, CJ Meijer, PM Nederlof.* VU University Medical Center, Amsterdam, Netherlands; The Netherlands Cancer Institute, Amsterdam, Netherlands.
**Background:** Emperical treatment with EGFR antagonists is highly effective in a subset of patients with advanced lung cancer. Subsequently, in these patients EGFR mutations were correlated with histopathological subtyping. Initially an association with bronchioloalveolar subtype and later with papillary subtype was suggested. The aim of this study was to determine the relation between histopathological subtypes of lung cancer and EGFR and K-ras mutation status.
**Design:** From 350 patients mutation analysis for K-ras / EGFR was performed as requested by pulmonologists, i.e. either because of cancer in non-smoking patient or for treatment stratification for recurrence after first line treatment. Paraffin embedded archive material was used for EGFR and K-ras mutation analysis. Histological classification and subtyping were performed according to the WHO classification (2004) with an additional distinction between micropapillary and papillary carcinoma. Odds ratios [OR] and Chi square p values were calculated .
**Results:** The archival material of 20 cases was qualitatively insufficient for mutation analysis. In addition, 19 cytology specimens were excluded as well. Mixed histological subtypes occurred to a variable extent. The glandular, bronchioloaveolar and micropapillary pattern were positively correlated (P<0.01). These patterns were inversely correlated with solid type adenocarcinoma and large cel carcinoma (p.<0.01). A dominant papillary pattern was present in 8 cases (2.6%). A dominant micropapillary pattern was seen 40 times.EGFR and K-ras mutations were mutually exclusive and detected in 82 and 57 adenocarcinoma cases, respectively. The presence of glandular subtype was positively related to EGFR mutations (OR 2, p<0.01), whereas a solid pattern was negatively related to EGFR (OR 0.35, p<0.05). A micropapillary pattern was positively related to K-ras mutations (OR 1.9, p =0.02). No significant associations were found for: a bronchioloaveolar subtype and EGFR (OR 1.5, p=0.1); a dominant papillary pattern and EGFR (OR 2.89, p=0.12); and intracellular or glandular mucin (n=12) and K-ras (OR 2.3, p=0.17).
**Conclusions:** In pulmonary adenocarcinoma a combination of glandular, bronchioloaveolar and micropapillary subtype was frequently observed. The glandular subtype was related to EGFR and the micropapillary subtype to K-ras mutations.

### 1647 Radiation-Induced Malignant Mesothelioma Following Treatment for Hodgkin Lymphoma

*MA Vasef, K Sheibani, D Lopez, S Sheibani.* University of New Mexico, Albuquerque, NM; Western Medical Center, Santa Ana, CA; University of Southern California, Los Angeles, CA.
**Background:** Recent epidemiologic studies have demonstrated an increased risk of malignant mesothelioma (MM) in patients who have received radiation therapy for testicular tumors and hematolymphoid malignancies. However, there have been no studies to determine whether there are any morphologic or immunohistochemical features that distinguish radiation-induced MM from asbestos-related MM. The purpose of our study was to compare morphologic and immunohistochemical features of radiation-induced MM with those of asbestos-related MM.
**Design:** We studied materials from ten patients (six men and four women with an age ranging from 35 to 74) who developed MM after radiation treatment for Hodgkin lymphoma (HL) at the site of radiation therapy. The diagnosis of MM had been immunohistochemically confirmed in all ten cases. The average duration between the treatment of HL and the development of MM was 29 years. Five patients had either a history of exposure to asbestos (occupational or non-occupational) or pleural fibrosis. These five patients were excluded from this study. The morphologic and immunohistochemical features in remaining five cases were compared with 100 cases of immunohistochemically confirmed MM.
**Results:** We found no morphologic or immunohistochemical features that could distinguish radiation-induced MM from those of asbestos-related MM.
**Conclusions:** Our study confirms an etiologic link between therapeutic radiation and the development of MM. However, we found no distinct morphologic or immunohistochemical features that can distinguish radiation-induced MM from asbestos-induced MM. Given the known DNA alterations induced by radiation, molecular genetic studies to screen for genetic alterations may prove helpful to determine whether there are any distinct genetic abnormalities that can separate radiation-induced MM from asbestos-related MM.

### 1648 TTF1 Expression in Non-Small Cell Lung Carcinoma: Association with *TTF1* Gene Amplification and Improved Survival

*PL Wagner, S Perner, A Soltermann, CL LaFargue, V Tischler, BA Weir, W Weder, M Meyerson, TJ Giordano, H Moch, MA Rubin.* Weill Medical College of Cornell University, New York, NY; University Hospital of Zurich, Zurich, Switzerland; University of Zurich, Zurich, Switzerland; University of Michigan, Ann Arbor, MI; Dana Farber Cancer Institute, Boston, MA.
**Background:** Acquired chromosomal aberrations play an important role in tumor development and progression. Such genetic alterations occur in a significant proportion of non-small cell lung carcinomas (NSCLCs), and include amplification of 14q13.3, which contains the *TTF1* gene. The aim of our study was to determine whether *TTF1* amplification is associated with increased TTF1 protein expression in NSCLCs, and whether TTF1 is associated with clinicopathologic features including patient survival.
**Design:** We used a FISH assay and quantitative immunohistochemical staining to interrogate a population-based cohort of 538 NSCLCs from Swiss patients for *TTF1* amplification and protein expression. Amplification status and protein expression levels were compared with patient and tumor characteristics, including overall survival using Kaplan-Meier and Cox multivariate regression analysis.
**Results:** *TTF1* amplification was found in ~13% of adenocarcinomas (ACs) and in ~9% of squamous cell carcinomas (SCCs). Amplification was associated with increased TTF1 protein expression. High-level TTF1 expression was significantly associated with smaller tumor size, female gender, and longer overall survival only among ACs (median survival 82 vs. 28 months; p=0.002). On multivariate analysis, high TTF1 expression was an independent predictor of favorable prognosis in patients with AC (hazard ratio 0.56 [95%CI 0.38-0.83]; p=0.008).
**Conclusions:** We are among the first to demonstrate that *TTF1* amplification is a mechanism of high-level TTF1 expression in a subset of NSCLCs. When expressed at high levels, this routinely used diagnostic marker is also an independent biomarker of favorable prognosis in AC.

### 1649 Clonality Analysis of Multifocal Lung Cancers by Loss of Heterozygosity, P53 Gene Sequencing and X Chromosome Inactivation Studies

*X Wang, M Wang, FW Abdul-Karim, GT MacLennan, JN Eble, TD Jones, F Olobatuyi, R Eisenberg, OW Cummings, A Lopez-Beltran, R Montironi, S Zheng, D Bratman, DD Davidson, L Cheng.* Indiana University, Indianapolis; Case Western Reserve University, Cleveland; Cordoba University, Cordoba, Spain; Polytechnic University of Marche Region, Ancona, Italy.
**Background:** The incidence of multifocality of lung cancer ranges from 0.2% to 2%. The knowledge of clonality of multiple anatomically separate, but histologically similar lung tumors is limited. Detailed characterization of genetic changes may provide insights into the mechanisms of multifocality of this malignancy.
**Design:** We analyzed 70 lung tumors from 30 patients (23 females, 7 males) (26 patients with non-small cell carcinomas and 4 patients with carcinoid/atypical carcinoid tumors) who underwent surgical resection for lung epithelial tumors. All patients had multiple tumors (two to five) involving one or both lungs. Genomic DNA was prepared from paraffin-embedded tissue sections using laser capture microdissection. Loss of heterozygosity (LOH) study was performed using a panel of microsatellite markers at 3p14-21 (D3S1766), 4q33-34 (D4S408), 9p21 (IFNA, D9S171), 11q13 (D11S970) and 17p13.1 (TP53). Exons 5, 7 and 8 of p53 gene were screened for mutations by direct DNA sequencing. X-chromosome inactivation was methylation and PCR based analysis.
**Results:** All thirty cases showed loss of heterozygosity in at least one of the six polymorphic microsatellite markers (ranging from one to four markers). Completely concordant LOH patterns between synchronous or metachronous cancers in individual patients were seen in 26 of 30 informative cases (87%). The identical p53 point mutations were present in 8 of 10 cases, who showed p53 mutation by sequencing. Nineteen of 23 (83%) informative cases showed identical X chromosome inactivation pattern. With the combination of LOH studies, p53 gene mutation screening and X chromosome inactivation analyses, 23 of 30 (77%) cases demonstrated identical genetic changes, consistent with monoclonal origin among separate tumors.
**Conclusions:** Our data indicate that the great majority of multifocal lung cancers have a common clonal origin, and that multifocality in lung cancer represents local and regional intrapulmonary metastasis.

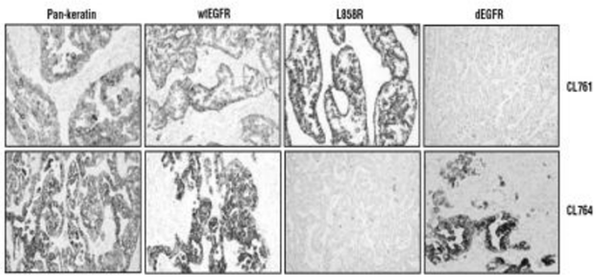
### 1650 Epidermal Growth Factor Receptor (EGFR) Gene Amplification in Non-Small Cell Lung Cancer: A Comparison of Fluorescence In Situ Hybridization and Chromogenic In Situ Hybridization Study

*SB Yoo, HJ Lee, JH Chung, DH Chung, JW Seo.* Seoul National University College of Medicine, Seoul, Republic of Korea; Seoul National University Bundang Hospital, Seongnam, Republic of Korea.
**Background:** Fluorescence in situ hybridization (FISH) is the most representative and standardized test for assessing gene amplification. However, FISH requires a fluorescence microscope, interpretation of FISH slides is time-consuming and rapid fading of the fluorescent signal is problematic. Recently, of chromogenic in situ hybridization(CISH) has emerged as a potential alternative to FISH. The aim of this study was to test the reliability of CISH technique for the detection of epidermal growth factor receptor (EGFR) gene amplification in non-small cell lung carcinoma (NSCLC), to compare CISH results with FISH and to assess interobserver reproducibility among three pathologists.
**Design:** A total of 170 formalin –fixed and paraffin embedded NSCLC tissue samples were retrieved from the surgical pathology archives at Seoul National University Bundang Hospital. FISH and CISH examinations were performed to test EGFR gene amplification status. FISH and CISH results were independently evaluated by the three pathologists.

**Results:** There was high concordance in the assessment of EGFR gene copy number between CISH and FISH tests and between observers ($p<0.000$). Also, there was substantial consistency between immunohistochemical results and CISH results, showing correlation of protein overexpression and gene amplification.
**Conclusions:** There was nearly perfect agreement between the CISH and corresponding FISH results, and interpretation of CISH results were highly reproducible among the pathologists. In conclusion, EGFR gene amplification status can be reliably assessed by CISH.

### 1651 Mutation-Specific Antibodies for the Detection of EGFR Mutations in Non-Small-Cell Lung Cancer

*J Yu, E Benedettini, DQ Li, T Gu, H Herebert, B Smith, R Polakiewicz, XM Zhou, M Loda, M Comb.* Cell Signaling Technology, Inc, Danvers, MA; Dana-Farber Cancer Institute, Boston, MA; Second Xiangya Hosptial, Changsha, Hunan, China.
**Background:** Activating mutations within the tyrosine kinase domain of the epidermal growth factor receptor (EGFR) are found in approximately 10-20% of non-small cell lung cancer (NSCLC) patients and are associated with response to the EGFR inhibitors Gefitinib and Erlotinib. The most common NSCLC-associated EGFR mutations are the 15 nucleotide in-frame deletion in exon 19 (E746-A750del) and the point mutation in exon 21 (L858R), together accounting for 90% of EGFR mutations. The ability to detect mutated gene products in cancer cells can identify patients most likely benefit from such therapies.
**Design:** We generated rabbit monoclonal antibodies (RmAb) against EGFR with E746-A750 deletions and L858R point mutation. We tested the antibodies by western blot, Immunofluorescence (IF) and immunohistochemistry (IHC) and used the antibodies staining 40 molecularly pre-typed tumors by IHC. Then, we used IHC by a panel of four antibodies (two EGFR mutation-specific antibodies, a control EGFR antibody and a pan-keratin antibody) to screen 340 cases of tumor samples with unknown genotype.
**Results:** The mutation-specific antibodies detect the corresponding mutant form of EGFR but not wild type EGFR by Western blotting, immunofluorescence (IF), and immunohistochemistry (IHC). IHC screening of a large panel of paraffin-embedded tumor samples of Non-Small-Cell Lung Cancer (NSCLC) patients shows that antibody reactivity is highly correlated with the presence of EGFR mutations.




DNA Sequencing and IHC Result of EGFR Mutation

| | DNA Sequencing | | | |
|---|---|---|---|---|
| IHC | L858R (+) | dEGFR (+) | Wild Type | Failed |
| L858R (+) | 24 | 0 | 2 | 2 |
| dEGFR (+) | 0 | 23 | 0 | 1 |
| L858R (-) | 2 | 0 | 193 | 25 |
| dEGFR (-) | 0 | 3 | 196 | 23 |

**Conclusions:** This simple assay for detection of EGFR mutations in diagnostic human tissues provides a rapid, sensitive, specific and cost-effective methodology to identify lung cancer patients responsive to EGFR-based therapies.

# Quality Assurance

### 1652 Do Amended Reports Get to Where They Should?

*B Arcarese, P Cohen, L Hao, M Pinto, V Parkash.* Yale University School of Medicine, New Haven, CT.
**Background:** Amended pathology reports (AmR) are issued to correct erroneous information. To avoid improper treatment based on the original incorrect report, the AmR needs to reach, and come to the attention of, the treating physician (phyT). However, the physician of record (phyR) is often not the phyT. Frequently, especially in cases with malignant diagnoses, the phyR is a radiologist or a surgeon who performed the biopsy or surgery, and the phyT is an oncologist or radiation therapist. Pathology offices typically send AmR to the phyR, and do not have measures in place to ensure that a copy of the corrected report gets to the phyT.
**Design:** All AmR with a change in the "final diagnosis" field of the report, and were issued >21 days after the original signout, were tracked. A chart review to determine receipt of AmR was performed in the phyR's office. The phyT was identified, and a similar chart review was done at the phyT's office. Both sets of physicians were asked to answer a short questionnaire.
**Results:** Of a total of 194 AmR over an 18 month period, 60 reports were amended due to changes in the final diagnosis field. Of these, 21 were amended 21 days or more after sign-out with a range of 3 weeks to 4 months. Of these, 16 pertained to malignant diagnoses - including 7 breast, 4 hematopathology, 2 gynecologic, and 1 each pulmonary, soft tissue and genitourinary cases. The phyT was the phyR (either as a primary or secondary clinician) in 9 cases. The remaining 7 had only the phyR listed in the pathology files. 5 of these had an AmR in the chart of the phyR. ONLY 1 had an AmR in the phyT chart. The phyR questionnaire revealed that, in general, phyR submitted AmR to the phyT if the AmR was received prior to the patient's referral to the phyT, but did not necessarily send it along if the AmR was received after the patients referral. Fortunately, only 1 case had a major amendment (a negative to positive lymph node (N0 to N1(mic)), and although this case did not have an AmR in the chart, the corrected stage was entered in the chart based on a discussion at Tumor Board. The phyT questionnaire revealed that failing to receive the AmR was not rare, but in general, major changes were communicated via Tumor Board conferences or other mechanisms.
**Conclusions:** An understanding of error-prone steps in a system is fundamental to achieving a planned and organized system for error reduction. We have identified an error prone step in the AmR transmission system that needs to be addressed.

### 1653 A Comparison of Sampling Techniques in the Pathologic Staging of Radical Prostatectomy Specimens: Part II

*KB Bell, M Kida, K Cooper.* University of Vermont College of Medicine/Fletcher Allen Health Care, Burlington, VT.
**Background:** The lack of standardized processing evaluating radical prostatectomy (RP) is well recognized, with only 12% of surveyed laboratories submitting the entire specimen (ES) for microscopic examination. (Am J Clin Pathol 1994;102:572-579). Employing 222 consecutive separate RP specimens (110 entire submission (ES) vs 112 representative sampling (RS)) we have previously demonstrated (Mod Pathol 2007; 20(supp2):340A) that complete submission of the entire prostate gland identified extraprostatic extension of tumor twice as often when compared to RS [40% ES vs 21% RS]; resulting in escalation of tumor stage from pT2 to pT3 (AJCC). The present study examines a cohort of ES RP, comparing ES vs RS within the same specimens.
**Design:** A consecutive series of ES RP specimens from 71 patients were reviewed. Slides/blocks were chosen per RS (representative blocks from apex, superior, mid, inferior, base, seminal vesicles) protocol and compared with the ES of the same RP specimen. This was conducted blindly without knowledge of the previously reported findings. The criteria for comparison included the following: extraprostatic spread, positive surgical margin, prostatic intraepithelial neoplasia (PIN), lymphovascular and perineural invasion, tumor volume and total Gleason score.
**Results:** The ages ranged from 43 – 78 years (mean 62 yrs). Weights ranged from 16 - 108 grams (mean 42g.).

ES vs RS

| Sampling Method | Extraprostatic Extension | Perineural Invasion | Margin (+) | PIN | LVI | Mean Tumor Volume (%) | Total Gleason score |
|---|---|---|---|---|---|---|---|
| ES (n=71) | 27 (38%) [p=0.003] | 54 (76%) | 25 (35%) | 62 (87%) | 5 (7%) | 14.50 | 6.81 |
| RS (n=71) | 18 (25%) [p=0.003] | 28 (39%) | 17 (24%) | 48 (68%) | 0 (0%) | 7.00 | 7.01 |

**Conclusions:** Both our previous study (Mod Path 2007; 20(supp2):340A) and the present study clearly demonstrate that total submission of the entire specimen of radical prostatectomy improves detection of extraprostatic spread (ES 38% vs RS 25%), [p=0.003], thereby escalating AJCC tumor staging pT2 to pT3, and positive surgical resection margins (ES 35% vs RS 24%). The detection of perineural and lymphovascular invasion was also higher in the ES than the RS RP specimens. Therefore, it would seem prudent that until such time that protocols for sampling RP have been standardized and management strategies for pT2 vs pT3 ironed out, it is best that the entire specimen of RP be subjected to histological examination.

### 1654 Amended Report Worksheets in Surgical Pathology

*C Bernadt, CS Jensen, SS Raab, JA Weydert.* University of Iowa, Iowa City, IA; University of Colorado Denver, Denver, CO.
**Background:** Amended reports represent a valuable resource for identifying and tracking deficiencies in the practice of surgical pathology. An amended report worksheet is a form that can be completed by the pathologist and allows the pathologist to document the discoverer of the error/deficiency, the mechanism of discovery, the error/deficiency type, and the type of revision made to the original report. We performed an audit of one year's worth of amended report worksheets in order to determine: 1) if pathologists were compliant with this tracking tool and; 2) if useful data could be obtained for the purposes of quality improvement in surgical pathology.
**Design:** Retrospective analysis of the amended report worksheets in general surgical pathology for the calendar year of 2007 at the University of Iowa Hospitals and Clinics. In addition, there was review of clinical records of all cases in which the amended report changed either the primary or secondary diagnostic characteristics to assess the clinical impact of such changes.
**Results:** A total of 170 amended reports were issued in general surgical pathology in 2007. Of these, amended report worksheets were completed for 151 cases (89% pathologist compliance). Clinician initiated review of a case was the most often marked mechanism of error discovery (35.5%). Defective reports, which includes erroneous and missing non-diagnostic information and errors in dictation and/or typing was the most common deficiency type (52%), which corresponded with editorial changes that do not change primary or secondary diagnostic information as the most common type of revision (78%). Revisions that changed either primary or secondary diagnostic information accounted for only 7.2% and 13.2% of amended reports, respectively. The most common types of deficiency in amended reports with revisions to either primary or secondary diagnostic information were interpretive errors (40%), followed by defective reports (31.4%) and inadequate specimen handling (20%). Review of clinical records of all cases that had revisions to either primary or secondary diagnostic information did not reveal any negative impact on clinical outcome.
**Conclusions:** Amended report worksheets appear to provide an easy and convenient mechanism of compiling categorical quality data related to amended reporting in surgical pathology. This type of data can be of use in targeting recurrent or over-represented deficiencies in surgical pathology reporting, and can serve as a bench mark for quality improvement programs.