Results: The results of immunohistochemistry and EBER are shown in the table. Survivin and Bax were expressed strongly in all the cases, and the pattern of staining was both nuclear and cytoplasmic. No apparent correlation was seen between the expression of EBER and p53.

	CC3	p53	Bax	Survivin	Bcl-2	EBER
Absent	56%	6%	0%	0%	63%	64%
Weak	42%	92%	0%	0%	35%	--
Strong	2%	2%	100%	100%	2%	36%

Conclusions: Our study demonstrates abnormalities in the apoptotic pathways in HRS cells in pediatric cHL. Down-regulation of p53 may contribute to the over-expression of survivin, which then suppresses the final apoptosis effector CC3 and contributes to the resistance of HRS cells to apoptosis, despite the strong expression of the upstream Bax. Survivin may therefore be a potential target for the treatment of pediatric cHL.

1524 Significant Decrease Expression of pAkt in Mass Screening Neuroblastoma

H Sartelet, S Ohta, S Barette, M Brevet, A-L Rougemont, I Harvey, C Bernard, G Vassal. CHU Sainte Justine, Montreal, QC, Canada; Shiga University of Medical Science, Shiga, Japan; CHU Amiens, Amiens, France; Children Hospital, Quebec, QC, Canada; Children Hospital, Montreal, QC, Canada; Gustave Roussy Institute, Villejuif, France.

Background: Neuroblastoma (NB) is a pediatric solid tumour with a poor outcome except in children younger than one year. Based on catecholamines urinary excretion, several mass screening programs have been organised in infants. The AKT pathway contributes to tumor aggressiveness in many cancers. The objective was to analyse expression AKT activation in between mass screening NBs in comparison to standard NBs.

Design: A first tissue microarray with standard NB was performed with 101 primary tumors and 39 paired metastases (median age of 30 months (range, newborn to 151 months, 16 stage 1, 7 stage 2, 22 stage 3, 48 stage 4 and 8 stage 4S). A second tissue microarray with mass screening NB coming from Quebec and Japan contained 55 primitive tumors and 21 metastases (median age of 7 months (range 1 to 14 months), 16 stage 1, 15 stage 2, 9 stage 3, 6 stage 4 and 9 stage 4S). Immunohistochemical staining was performed using antibodies against, AKT, phosphoAKT and TRKB which is known as a poor prognosis factor of NB. Immunostaining intensity was evaluated by a semi-quantitative score based on the percentage of positive cells. The t-student test was applied for the comparison of protein expression between standard and mass screening NB.

Results: The expression of phosphoAKT was significantly higher in primitive tumors, in metastases, in stage 1 and in patient under one year of standard NB (mean intensity 1.92, 2.1, 1.54 and 1.97 in 96%, 92%, 94% and 100% of tumors cells, receptively) than in mass screening NB (mean intensity 1.18, 1.05, 0.96 and 1.22 in 75%, 68%, 62% and 78% of tumors cells, receptively). AKT was only significantly more present in primitive and metastases of standard NB than in mass screening NB, as for TRKB.

Conclusions: The activation of AKT pathway is significantly higher in standard than in mass screening NB independently of age, stage and primitive of metastatic status of the tumor. This confirmed that NB diagnosed through mass sceening differ biologically from standard NB.

1525 Morphoproteomic Confirmation of Activated mTOR, ERK and NF-kappaB Pathways in Ewing's Sarcoma Tumor Family with Cell Cycle and Genomic Correlates: A Preliminary Study

MJ Zenali, PL Zhang, RE Brown. University of Texas, Houston, TX; Geisinger Medical Center, Danville, PA.

Background: Ewing's sarcoma (ES), a highly aggressive pediatric tumor is associated with translocations that fuse the EWS gene to one of the ETS family of genes/transcription factors.The resultant fusion proteins effect transcriptional activation contributing to tumorigenesis.Studies on cell lines implicate the EWS/FLI-1 fusion in the activation of the extracellular signal-regulated kinase (ERK) 1/2 pathway and also, link it to mammalian target of rapamycin (mTOR) activation. Finally, nuclear factor (NF)-kappaB, has been shown to have constitutive activity and to be responsible for the tumorigenicity of ES cells in a xenograft model. Therefore, we investigated:a) the state of activation of components of the mTOR, ERK and NF-kappaB signal transduction pathways in ES cases; and b) cell cycle and genomic correlates.

Design: Archival materials from three (3) patients with ES were studied with prior IRB approval. Clinically,all had metastatic disease to the mediastinum or lung. Cytogenetic and molecular studies were performed in two of the patients. Immunohistochemical probes were utilized for the detection of the following: phosphorylated (p)-mTOR (Ser 2448); p-p70S6K (Thr 389); p-ERK 1/2 (Thr202/Tyr204); p-NF-kappaBp65 (Ser 536); and Skp-2. Chromogenic signal and cellular compartmentalization were assessed by bright-field microscopy (0-3+); the S phase-associated protein kinase (Skp-2) was quantified by an automated imaging system.

Results: Moderate to strong (2+ up to 3+) expressions of p-mTOR and p-p70S6K, of p-ERK 1/2 and of p-NF-kappaBp65 and with nuclear translocation of the latter three were noted. Nuclear Skp-2 expression levels were 14,12 and 16%, respectively. Cytogenetic analysis in one disclosed a translocation (t[7;22]), which has been associated with EWS/ETV1 fusion. EWS/FLI transcripts were documented in another.

Conclusions: Morphoproteomic analysis reveals the activation of the mTOR, ERK and NF-kappaB pathways in ES cases as evidenced by: phosphorylated mTOR, p70S6K, ERK 1/2 and NF-kappaBp65 using phosphospecific probes directed against sites of activation; nuclear translocation of p-p70S6K, p-ERK 1/2 and p-NF-kappaBp65; and correlative expression of Skp-2 protein consistent with cell cycling consequent to such signal transduction. These observations, although preliminary, appear to be the first on primary ES specimens and coincide with cytogenetic, molecular and preclinical data in ES.

1526 Pediatric Yolk Sac Tumors: A Comparison of the Sensitivity of Alpha-Fetoprotein to Glypican 3

DL Zynger, ND Dimov, JC McCallum, PM Chou, XJ Yang. Northwestern University Fienberg School of Medicine, Chicago, IL; Children's Memorial Hospital, Chicago, IL.

Background: Yolk sac tumor (YST) can display numerous histological patterns which can be confused with other germ cell components or other maligancies. As such, a marker with high sensitivity for YST is needed. Alpha-fetoprotein (AFP) is a commonly used marker for pediatric YST. However, reports of the sensitivity of AFP for YST vary, and sample sizes were small. Glypican 3 (GPC3), a new oncofetal protein, has been recently reported to be highly expressed in the YST component of adult testicular germ cell tumors. We sought to compare the immunohistochemical expression of AFP to GPC3.

Design: Sections from paraffin embedded blocks from 33 pediatric YST were obtained from the following organs: 12 testicular, 8 sacrococcygeal, 5 ovarian, 3 intracranial, 1 mediastinal, and 4 lymph node metastases. The sections were subjected to immunohistochemistry with a monoclonal antibody specific to AFP and GPC3. Immunoreactivity was semi-quantitatively assessed for percent of cells stained (0, <5%; 1+, 5-10%; 2+, 11-50%; 3+, >50%) and intensity (0-3).

Results: Immunohistochemical results are summarized in the table. The positivity of AFP was 66% (22/33) compared to 100% for GPC3 (33/33) in YST. In addition, the background staining of AFP was often high. In contrast, all YST expressed GPC3 with most cases showing diffuse expression. The mean staining intensity of GPC3 was also higher than that of AFP (2.8 vs. 1.2).

AFP and GPC3 immunoreactivity in YST

	0 (<5%)	1+ (5-10%)	2+ (10-50%)	3+ (>50%)	Total	Intensity (mean)
AFP	11 (33%)	8 (24%)	11 (33%)	3 (9%)	33	1.2
GPC3	0	1 (3%)	4 (12%)	28 (85%)	33	2.8

Conclusions: As YST has a variety of growth patterns, a marker with high sensitivity for this tumor is necessary. We found that GPC3 had a higher sensitivity and more diffuse expression than AFP, suggesting greater clinical utility of GPC3 than AFP in pediatric gonadal and extra-gonadal YST.

Pulmonary

1527 Value of Immunohistochemical Markers in Differentiating Benign from Malignant Mesothelial Lesions

A Acurio, Q Arif, P Gattuso, M McIntere, T Krausz, AN Husain. Univ. of Chicago, Chicago, IL; Rush Univ, Chicago, IL.

Background: Histologic distinction between benign reactive mesothelium and malignant mesothelioma (MM) often presents a diagnostic challenge. Characteristic features of malignancy are not always reliable in a small tissue biopsy. Multiple immunohistochemical (IHC) markers including desmin, epithelial membrane antigen (EMA) and p53 protein have been studied. Recently, it has been suggested that GLUT-1, a member of the glucose transporter family, can serve as an unequivocal IHC marker of malignant transformation. This study examines the diagnostic value of the aforementioned IHC markers in distinguishing benign from malignant mesothelial lesions.

Design: We examined the IHC profile of 85 mesothelial tissue samples including: normal (20), hyperplastic (20) and MM (45) using desmin, EMA, p53 protein and GLUT-1.

Results: The results of IHC staining are summarized in Table 1.

Table 1. IHC Profile of Mesothelial Tissue.

	Normal (%)		**Hyperplastic (%)**		**Malignant (%)**		
IHC marker	Negative	Weak positive	Negative	Weak positive	Negative	Weak positive	Strong positive
Desmin	13 (65)	7(35)	13 (65)	7 (35)	42 (98)	1 (2)	0 (0)
p53	11 (55)	9 (45)	10 (50)	10 (50)	16 (36)	26 (60)	2 (5)
EMA	19 (95)	1 (5)	19 (95)	1(5)	15 (34)	17 (39)	12 (27)
GLUT-1	20 (100)	0 (0)	20 (100)	0 (0)	9 (20)	24 (53)	12 (27)

No benign mesothelium showed strong positivity with any IHC marker studied. No significant difference in staining of benign versus malignant tissue was found for p53 (p=0.26).Desmin expression, although weak, was greater in benign lesions and was statistically significant in distinguishing them from MM (p=0.0013). The immunoreactivity of EMA and GLUT-1 was significantly different between benign and malignant lesions (p <0.0001, Fischer's exact probability test). The sensitivity and specificity in identifying malignant cells were similar for both EMA and GLUT-1.

Conclusions: In this study, p53 failed to distinguish benign from malignant mesothelial lesions. Desmin identified benign mesothelium and distinguished it from MM. EMA and GLUT-1 were positive in the majority of MMs and negative or only weakly positive in the benign mesothelial tissues studied. Since some malignant lesions were negative for EMA and GLUT-1, diagnosis should not be based exclusively on immunoreactivity. Instead, desmin, EMA and GLUT-1 may be part of an IHC panel and used as adjuncts to histomorphology in the diagnosis of MM.

1528 Granulomatous Pleuritis: A Series of 57 Cases

R Albadine, N Le Stang, E Bergot, B Malbruny, A Jehan, JP Le Rochais, F Galateau-Salle. University Hospital, Caen, France; Mesonat Register, Caen, France; ERI3 INSERM, Caen, France; Mesopath Group, Caen, France.

Background: Granulomatous pleuritis (GP) have been rarely reported on pleural biopsies with a wide range of etiologies.

Design: The aim of this study was to analyze the clinicopathologic features of the largest series to date of GP collected from the files of the pathology department, University Hospital, Caen, France, from 1992 to 2007. Cases were retrospectively reviewed [Mesopath group]. The diagnosis were established on the clinical and radiographic features, pleural fluid examination including cytology, biochemistry, acid fast bacilli

screening and mycobacterial cultures. The occupational histories were evaluated by a structured questionnaire. The diagnosis was confirmed by histological findings including special staining.

Results: Fifty-seven cases were identified (mean age 57±18 years) of those 15 were female (range 18-90 years) and 42 were male (range 23-91 years). Thirty cases were attributed to infectious etiology: tuberculosis (TB) 29/57 (53%), and aspergillosis 1/57 (2%). The other cases were: sarcoidosis 5/57 (9%), sarcoidosis with silicosis 1/57 (2%), silicosis 5/57 (9%), rheumatoid nodules 2/57 (4%), GP associated with cancer 8/57 (14%), and other miscellaneous cases 3/57 (5%). Three cases of non-necrotizing GP remained without etiology. Lymphocytic effusion was observed in 17/19 (89%) of the pleural TB cases, the biopsies showed granulomas with necrosis in 83% (24/29). On these paraffin embedded samples, mycobacteria were detected by Ziehl–Neelsen (ZN) staining in 9/29 (31%), confirmed by cultures in 6/9 cases (67%). In cases negative for ZN staining on paraffin sections, cultures were performed in 17/20 and were positive in 82% (14/17) cases.

Conclusions: Our series shows that tuberculosis is the most common cause of granulomas (51%) located to the pleura. Silicosis was observed in 11 % of cases and despite extensive investigations, 5% of pleural granulomas remains of undetermined etiology.

1529 Osteopontin, p-GSK-3β, β-Catenin, NF-kB, and HIF-1 Expression in 60 Diffuse Malignant Mesothelioma (DMM) Cases: Correlation with Asbestos Exposure and Patient Demographics

TC Allen, PT Cagle, L Garza, M Pacienca, V Rouleau, F Morlais, G Launoy, F Galateau-Salle. University of Texas Health Center-Tyler, Tyler, TX; The Methodist Hospital, Houston, TX; CHU, Caen, France.

Background: Although most DMM occur as the result of asbestos exposure in older males, DMM are not a monolithic disease. DMM may arise in the absence of asbestos exposure and occur in women and younger patients. Several biomarkers have been previously reported by us and others in DMM, including the following, and are of interest in regards to pathogenesis. p-GSK-3 has been implicated in β-catenin activation, and β-catenin has been identified in DMM. NF-kB has been shown to play a role in DMM carcinogenesis via TNF-α activation. HIF-1, a regulator of oxygen homeostasis, is reportedly expressed in DMM. Osteopontin is overexpressed in various cancers and may be a serum biomarker for DMM. Signaling pathways of these proteins have a number of interconnections and these molecular markers have the potential to serve DMM patients diagnostically and therapeutically. They have not been studied previously in DMM in which extensive demographic and exposure data were available to determine if they might correlate with any of these factors.

Design: 60 DMM were collected as part of the French Programme National De Surveillance Des Mesotheliomes, and formalin-fixed, paraffin-embedded sections were immunostained with antibodies to p-GSK-3β (1:25, Cell Signaling), NF-kB (1:100 Cell Signaling), β-catenin (1:100, BD Biosciences), HIF-1 (1:2000, Abcam), and osteopontin (1:100, Calbiochem). The results were correlated with demographic, exposure and survival data by Kaplan-Meier analysis.

Results: The 60 cases consisted of 22% women, 78% men; 73% epithelial, 22% biphasic and 5% sarcomatoid cell types; 80% with a significant asbestos exposure history and 20% without exposure. Tumor nuclei were positive with p-GSK-3β (44%), β-catenin (4%), NF-kB (53%), HIF-1 (24%), and osteopontin (71%). Immunopositivity did not correlate with age, sex, histologic type, asbestos exposure history, or survival for any molecular marker.

Conclusions: Expression of p-GSK-3β, NF-kB, β-catenin, HIF-1 and osteopontin occurs in DMM regardless of whether the patients have a history of asbestosis exposure or not and regardless of patient gender or age. This suggests that the pathogenesis of mesothelioma has some common pathways regardless of etiology or demographics.

1530 Osteopontin (OPN) and HIF-1 Expression in Diffuse Malignant Mesothelioma (DMM) with Long-Term (>3 Year) Survival (LS) Versus Short-Term Survival (SS)

TC Allen, HH Popper, H Kothmaier, I Halbwedl, P Morbini, B Murer, CE Comin, F Galateau-Salle, F Demirag, H Zeren, R Attanoos, A Gibbs, PT Cagle. University of Texas Health Center-Tyler, Tyler, TX; Institute of Pathology, Graz, Austria; Instituto di Anatomia Patologica, Pavia, Italy; Ospedale Umberto, Mestre-Venezia, Italy; University of Firenze, Firenze, Italy; CHU Cote de Nacre, Caen, France; Ataturk Chest Disease Hospital, Ankara, Turkey; Adana University, Adana, Turkey; Llandough Hospital, Cardiff, United Kingdom; The Methodist Hospital, Houston, TX.

Background: DMM is fatal within a year of diagnosis in the vast majority of cases, and survival >3 years is extremely rare. Proteins which are differentially expressed in DMM with LS as compared to DMM with SS may be a basis for understanding why a few patients are able to survive their disease and this may be a basis for targeted therapy in patients with typically aggressive DMM. OPN is a glycoprotein overexpressed in various cancers that mediates cell-matrix interactions and cell signaling. It is regulated by cell-signaling pathway proteins associated with asbestos-induced carcinogenesis and may be a serum marker for DMM. HIF-1 is a transcriptional activator important in oxygen homeostasis and may contribute to tumor progression, angiogenesis, and anti-apoptosis via tumor adaptation to hypoxia. OPN and HIF-1 expression have not been investigated in DMM with LS in comparison to more typical aggressive DMM.

Design: We constructed a tissue microarray (TMA) of 19 DMM with rare LS and 21 typical DMM cases with SS. Due to the rarity of LS-DMM, these cases were collected from 9 different institutions in Austria, Italy, France, Turkey, UK and USA. Sections of the TMA were immunostained for OPN (Calbiochem 1:100) and for monoclonal HIF-1 alpha antibody (Abcam 1:2000). Nuclear staining was graded as: 0= no staining; 1= <10% staining; 2= ≥10% -50% staining; 3= ≥50% staining.

Results: For OPN, immunopositivity in DMM with LS showed: 0=2 (11%), 1=0, 2=4 (21%), 3=13 (68%). In DMM with SS: 0=none, 1=2 (10%), 2=4(19%), 3=15(71%). For HIF-1, immunopositivity in DMM with LS showed: 0=1 (5%), 1=3 (16%), 2=4 (26%), 3=10 (53%). In DMM with SS: none=0, 1=3 (14%), 2=4(19%), 3=14 (67%).

Conclusions: OPN and HIF-1 are commonly over-expressed in DMM, including both DMM with LS and DMM with SS. However, absence of OPN and HIF-1 was observed only in LS-DMM, suggesting that lack of OPN and HIF-1 expression may have some role in some cases of prolonged survival.

1531 Stage I Adenocarcinomas of the Lung: A Single Institution Study of 287 Patients Evaluating the Relationship of Histopathologic Subtypes and Tumor Recurrence

AV Arrossi, S Reznik, S Murthy, E Blackstone, CF Farver. The Cleveland Clinic Foundation, Cleveland, OH.

Background: The World Health Organization (WHO) classification for adenocarcinomas of the lung made significant changes from the 1981 classification, including a strict definition of bronchioloalveolar carcinoma (BAC) and the addition of a mixed type to account for the frequent histologically heterogeneity of pulmonary adenocarcinomas. Previous studies suggest a better survival of BAC; however, the prognostic significance of the adenocarcinoma subtypes according to the new WHO classification has not been well studied.

Design: We reviewed the files from the departments of Anatomic Pathology and Cardiothoracic Surgery at the Cleveland Clinic of 287 patients who underwent surgical resection for stage I adenocarcinomas between 1991-2000. All slides on the surgical resection were reviewed and the adenocarcinomas were reclassified according to the 2004 WHO classification. The adenocarcinomas were categorized as pure or mixed; mixed tumors were further classified by the major component present and presence of other minor components. Other data collected included tumor size, presence of angiolymphatic and pleural invasion and the presence of atypical alveolar hyperplasia (AAH). We correlated the pathologic findings with tumor recurrence.

Results: There were 139 males and 148 females. The age range was 36 to 87 years old (mean 66.7 years old). Thirty one (10.8%) tumors were pure adenocarcinomas (fifteen acinar, five papillary, six BAC, two solid, two colloid and one signet ring cell adenocarcinomas) and 256 (89.2%) were mixed. The major component of the mixed adenocarcinomas was acinar in 150 tumors, BAC in 26 tumors, papillary in 21 tumors, solid in 46 tumors, micropapillary in 6 tumors, cribriform in 4 tumors, and colloid in 3 tumors. Median follow-up was 64.3 months. Patients with tumors with BAC as a major component had better recurrence free survival than those patients with other major components. AAH was present in 44 cases and did not have effect on tumor recurrence. Increased tumor size was associated with decreasing tumor recurrence at five years.

Conclusions: Patients with mixed adenocarcinomas with a BAC as a major component have better recurrence free survival than those patients with mixed adenocarcinomas with a non-BAC major component. Increased tumor size correlates with decreased tumor recurrence. The presence of AAH has no effect on five year tumor recurrence

1532 Clinical Significance of TTF-1 Protein Expression and TTF-1 Gene Amplification in Lung Adenocarcinoma

JA Barletta, BA Weir, S Perner, L Johnson, MA Rubin, BE Johnson, M Meyerson, LR Chirieac. Brigham and Women's Hospital, Boston, MA; Dana-Farber Cancer Institute, Boston, MA.

Background: Molecular alterations in non-small cell lung cancer (NSCLC) are investigated to define new prognostic markers and therapeutic targets and to elucidate the molecular mechanisms underlying NSCLC development and progression. TTF-1 expression by immunohistochemistry is commonly used to confirm a pulmonary origin of carcinomas. It has recently been found that the TTF-1 gene is amplified in 12% of lung adenocarcinomas (Weir, in press). The clinical significance of TTF-1 gene amplification and the relationship with TTF-1 protein expression have not been determined.

Design: We studied 125 consecutive patients with lung adenocarcinomas treated at Brigham and Women's Hospital between 1997 and 1999. TTF-1 protein expression by immunohistochemistry was assessed semiquantitatively by two pathologists and recorded as: no expression (no staining=0); low expression (weak staining=1); high expression (strong staining=2). The TTF-1 gene amplification status by fluorescence in situ hybridization (FISH) was recorded as amplified or non-amplified. We examined the relationship between TTF-1 expression and gene amplification status and compared the findings with overall survival.

Results: TTF-1 was amplified in 10 (14.1%) of 71 cases that had successful probe hybridization. TTF-1 expression was 0 in 17 (15.0%) cases, 1 in 37 (32.7%) cases, and 2 in 59 (52.2%) cases for the 113 cases with assessable TTF-1 staining. TTF-1 expression was strongly associated with TTF-1 amplification status ($p<0.001$). Patients with adenocarcinomas with high TTF-1 expression had a better outcome than those with low or no TTF-1 expression (median overall survival 89, 42 and 31 months, respectively, $p = 0.01$). Surprisingly, in subset analysis of the 59 patients with lung adenocarcinomas with high TTF-1 expression, TTF-1 gene amplification was a predictor of poor outcome (median overall survival 36 vs 100 months, $p=0.02$).

Conclusions: TTF-1 expression by immunohistochemistry is highly correlated with TTF-1 gene amplification status and is associated with a better overall survival. In patients with high levels of TTF-1 expression, TTF-1 gene amplification is a predictor of poor outcome. Our results indicate that both TTF-1 expression by immunohistochemistry and TTF-1 gene amplification by FISH may be significant prognostic factors for patients with lung adenocarcinomas.

1533 Clinicopathological Review of 50 Patients Receiving Hematopoietic Stem Cell Transplantation: Pulmonary Graft-Versus-Host Disease and Its Histological Mimics

MG Bayerl, WU Todd, RD Bruggeman, DS Zander. Penn State COM / MS Hershey Medical Center, Hershey', PA.

Background: The lung is a common site of injury following hematopoietic stem cell transplantation (HSCT). The most common etiologies include infections, drug reactions and graft-versus-host disease (GVHD). GVHD manifests as perivascular, alveolar septal and airway-centered lymphocytic infiltration with or without epithelial and endothelial cell apoptosis. However, these morphologic changes are not specific, so specific markers of GVHD would have substantial clinical value.

Design: Sixty-four lung biopsies from 50 HSCT patients were reviewed blinded to diagnosis and type of HSCT. Twenty-four biopsies from 18 patients demonstrated perivascular, septal or airway-centered lymphocytic infiltrates with or without lymphocyte exocytosis. Clinical data for these 18 patients was obtained. Of the specimens with lymphocytic infiltrates, 15 came from patients following allogeneic HSCT and were thus at high-risk for GVHD. Five specimens came from patients following autologous HSCT and 4 from patients before allogeneic HSCT; these served as controls. IHC for cleaved caspase 3 was performed on all 24 biopsies, as a marker of apoptosis, and evaluated for reactivity in bronchiolar epithelium, pneumocytes and endothelial cells, the cells targeted in GVHD. Kruskal-Wallis tests were used to compare caspase 3 staining in high-risk vs. control samples and GVHD vs. other pulmonary complications.

Results: The primary clinicopathological diagnoses obtained for each specimen are listed in Table 1.

Table 1. Clinicopathological Diagnoses

Etiology	Control Group	High-Risk Group
Infectious	5	9
GVHD	0	4
Drug Reaction	3	0
PTLD	0	1
Trauma	0	1
Foreign Body	1	0
Unknown	0	1

Infection was the most common primary diagnosis in both groups. GVHD was second in frequency in the high-risk group, whereas drug reaction was second in the control group. As expected, no histological features were specific for GVHD. Cleaved caspase 3 expression in more than one cell type was more common in the high-risk group (4/15, 27%) than in the control group (1/8, 13%), but the difference was not significant (p=0.32) and did not differentiate GVHD from other pulmonary complications of HSCT (p=0.56).

Conclusions: 1) Infections are the most common findings in lung biopsies from HSCT patients. 2) No morphologic features are specific for GVHD and IHC for cleaved caspase 3 does not differentiate GVHD from its histologic mimics. 3) GVHD remains a diagnosis requiring clinicopathological correlation.

1534 Expression of HIF-1 Alpha in Pulmonary Non-Small Cell Carcinomas and Neuroendocrine Carcinomas – A Tissue Microarray Analysis of 442 Cases

MB Beasley, CY Castro, L Garza, TC Allen, PT Cagle. Mt. Sinai Med Ctr, New York, NY; Holmes Reg Med Ctr, Melbourne, FL; The Methodist Hospital, Houston, TX; Univ of Texas Health Ctr, Tyler, TX.

Background: Hypoxia inducible factor-1 alpha (HIF-1) is a cellular transcription factor that mediates cellular response to hypoxia and appears to be associated with aggressive tumor behavior. HIF-1 induces angiogenesis via induction of vascular endothelial growth factor (VEGF). As such, inhibition of HIF-1 activity has been shown to decrease tumor growth in experimental studies. HIF-1 activity is also increased by epidermal growth factor receptor (EGFR), which further enhances cell survival. Expression of HIF-1 has been correlated with a worse prognosis in non-small cell lung carcinomas (NSCLC). The aim of this study is to evaluate HIF-1 expression in pulmonary neuroendocrine carcinomas in comparison to NSCLC.

Design: 187 adenocarcinoma (ADC)(141 conventional and 46 pure/predominatly bronchioloalveolar (ADC/BAC), 90 squamous cell carcinomas (SCC), 70 NSCLC not further classified (NSC-NOS), 40 typical carcinoids (TC), 5 atypical carcinoids (AC), 11 large cell neuroendocrine carcinomas (LCNEC) and 39 small cell carcinomas (SCLC) (33 pure and 6 combined SCLC) were evaluated for HIF-1 alpha expression. Sections from paraffin embedded tissue microarray blocks of ADC, SCC, NSC-NOS, TC/AC, LCNEC and SCLC were stained with monoclonal HIF-1 alpha antibody (Abcam 1:2000) using an automated stainer. Tissue sections were incubated for 60 minutes following antigen retrieval in a steamer using citrate buffer.

Results: HIF-1 expression was demonstrated in 53/131 (38%) conventional ADC, 6/46 (13%) ADC/BAC, 44/90 (57%) SCC, 40/70 (57%) NSC-NOS, 39/45 (87%) carcinoids (35/40 TC, 4/5 AC), 10/11 (91%) LCNEC and 31/39 (80%) SCLC. Diffuse cytoplasmic staining was observed in all positive cases.

Conclusions: HIF-1 is expressed in the majority of pulmonary neuroendocrine carcinomas regardless of grade, and is seen more frequently in neuroendocrine carcinomas than NSCLC. The finding of HIF-1 in the majority of TC would indicate that HIF-1 in not necessarily an independent marker for aggressive behavior in neuroendocrine carcinomas. In contrast, the low level of expression of HIF-1 in ADC/BAC compared to NSCLC as a whole is reflective of the less aggressive behavior typical of this group of tumors. The high level of expression in neuroendocrine carcinomas may indicate a role for HIF-1 targeted therapy in these tumors.

1535 TTF-1 Positivity Is a Sensitive Predictor of *EGFR* Mutation and Treatment Response in Pulmonary Adenocarcinomas, by Pathologist Interpretation and by Image Analysis

J Beheshti, E Sabo, PA Janne, V Joshi, LJ Wang, NI Lindeman. Brigham and Women's Hospital, Boston, MA; Rhode Island Hospital, Brown Medical School, Providence, RI; Dana-Farber Cancer Institute, Boston, MA; Harvard Medical School, Boston, MA.

Background: 80% of lung adenocarcinomas with *EGFR* mutations respond to treatment with tyrosine kinase inhibitors (TKIs; gefitinib, erlotinib). However, *EGFR* mutation analysis is not widely available, and alternate techniques, such as *in situ* hybridization and immunohistochemistry (IHC) have shown inconsistent results. Image analysis (IA) provides an objective means of assessing IHC, and was compared with two surgical pathologists' (SP) interpretations of TTF-1 expression, a marker that was previously suggested in our laboratory to predict *EGFR* mutation and outcome.

Design: A tissue microarray with 240 cores from 48 lung adenocarcinomas with known *EGFR* mutation and/or response to TKIs was stained with antibodies to TTF-1. The slide was scanned (Aperio ScanScope, CA) and extent (0-100%) and intensity (0-255; lower is stronger staining) were measured by digital IA software (Image-Pro Plus 6.0, Media Cybernetics, MA). These results were compared with two pathologists' interpretations (intensity 0-4+).

Results: IA and SP gave similar results, with 83% concordance (100% in responders, 73% in nonresponders). In both, strong TTF-1 expression predicted response to TKIs and *EGFR* mutation. Strong staining (SP:≥ 2+, IA: ≤155) was seen in all 14 (100%) responsive tumors by both SP and IA, with specificities of 58% (SP) and 70% (IA) in the 23 nonresponsive tumors (p=0.0003 for SP and p=0.03 for IA). Similar sensitivities (SP:88%, IA:100%) and specificities (SP:63%, IA:61%) were obtained for predicting *EGFR* mutation (p=0.001 for SP and p=0.005 for IA). Results for extent of staining were similar to those for intensity, but only TTF-1 intensity was an independent predictor of repsonse in multivariate analysis.

Conclusions: Pathologists and image analysis software performed comparably when assessing TTF-1 IHC. Both showed that strong/extensive TTF-1 staining is a statistically significant, sensitive predictor of *EGFR* mutation and response to TKI treatment. Weak/absent TTF-1 had a very high negative predictive value (NPV) for treatment response (100%, IA or SP) and mutation (IA:100%, SP:88%). In centers where *EGFR* analysis is not available, a pathologist's interpretation of weak/absent TTF-1 (score <2+) may help exclude patients from *EGFR* testing and/or TKI therapy.

1536 Outcome and Staging of Multifocal Adenocarcinoma (ADC) of the Lung

M Bhanote, MF Vazquez. NYPH-Weill Cornell, NY.

Background: Lung cancer is the leading cause of cancer mortality worldwide. Surgical pathologists are integral in lung cancer staging and this influences therapeutic decisions. Both the AJCC and the proposed IASLC (International Association for the Study of Lung Cancer) staging consider histologically similar tumors as metastatic.

Design: We reviewed the 5 year outcome in 14 patients with multifocal ADC, excised at NYPH-WCMC from 1999 to 2002, of similar and dissimilar morphology to determine if outcome was consistent with metastatic disease or if these tumors should be classified as stage indeterminate and treated according to the recommendations for stage I lung cancer.

Results: Eight patients with multifocal ADC-BAC are alive without disease. Five patients had two tumors, 3 patients had 3 tumors; one patient with two ADC-BAC (2.0 and 1.6 cm) in the same lobe; one patient with two ADC-BAC (2.0 and 0.7 cm) in the same lobe; one patient with two ADC-BAC (4.5 and 2.5 cm) in ipsilateral lobes; one patient with one ADC-BAC (1.2 cm) and one ADC, acinar type (1.0 cm) in ipsilateral lobe; one patient with ADC-BAC (1.5 cm) and an ADC, acinar type with signet ring morphology (0.5 cm); one patient with three ADC-BAC (1.6, 1.1 and 0.9 cm) had two tumors in the same lobe and one tumor in the ipsilateral lobe; one patient with three ADC-BAC (1.1, 0.7 and 0.5 cm) in ipsilateral lobes; one patient with three tumors, two ADC-BAC (2.6, 1.6 cm) in contralateral lobes and a third ADC, mixed type with acinar and papillary components (1.7 cm). Six patients with multifocal ADC are deceased on five year follow-up. Five had lobectomies: one patient with a 1.6 cm ADC, mixed type with BAC features (ADC-BAC) and two additional papillary ADC (2.0 and 0.7 cm) in the same lobe; one patient with three poorly differentiated ADC-BAC (2.6, 1.1, and 0.7 cm) in the same lobe; one patient with two ADC, mixed type with papillary features (1.5, and 1.5 cm) in the same lobe, and one positive lymph node; one patient had two ADC-BAC (1.5 and 0.9 cm) and a third poorly differentiated ADC, acinar subtype (0.9 cm) in the same lobe; and one patient with two ADC-BAC (1.7 and 0.4 cm) in ipsilateral lobes died of other causes. One of the six deceased patients had only wedge resection of two ADC-BAC (0.8 and 0.2 cm) in ipsilateral lobes and had a pleural effusion within two years.

Conclusions: In our series of multifocal ADC of the lung, 57.1% of patients are alive and well. This is contrary to the expected survival based on the current AJCC and proposed IASLC staging. This data supports multifocal ADC may not represent intrapulmonary metastasis.

1537 Tumor Anaplastic Lymphoma Kinase (ALK) Expression among Patients with Non-Small Cell Carcinoma of the Lung

JM Boland, S Erdogan, G Vasmatzis, P Yang, LS Tillmans, MC Aubry, ES Yi. Mayo Clinic, Rochester, MN.

Background: Non-small cell lung carcinoma (NSCLC) comprises 75-80% of all lung cancers with an overall 5-year-survival of 15%. Improvement in clinical outcome could be achieved by identifying novel therapeutic targets via understanding of molecular pathogenesis. A recent study reported a small inversion of chromosome 2p in NSCLC that leads to a fusion gene composed of the echinoderm microtubule-associated protein-like 4 (*EML4*) gene and the anaplastic lymphoma kinase (*ALK*) gene. This fusion transcript is a promising therapeutic target and was present in 6.7% of NSCLC patients in this study.

However, ALK protein expression in NSCLC has not been reported. We investigated the presence of ALK protein expression in NSCLC by immunohistochemistry (IHC) and correlated immunoreactivity with *ALK* gene expression.

Design: Gene expression profiling was performed on frozen tissue of 35 adenocarcinomas using Affymetrix chip technology. IHC was performed with an antibody directed against ALK protein (clone ALK1, Dakocytomation, Carpenteria, CA) on these 35 adenocarcinomas (ADC) using formalin-fixed, paraffin embedded lung tissue. We also examined ALK1 protein expression in an independent cohort consisting of 50 ADC and 50 squamous cell carcinomas (SQCC).

Results: High expression of *ALK* was identified in 6% (2/35) of ADC. These two cases also showed cytoplasmic ALK1 positivity by IHC, while the remaining 33 cases without high *ALK* gene expression were negative for ALK1 immunostaining. The underlying non-neoplastic lung tissue did not show any immunoreactivity for ALK1. In the independent cohort, cytoplasmic ALK1 positivity by IHC was seen in 1% of cases (1/50 SQCC and 0/50 ADC).

Conclusions: We observed ALK1 expression by IHC in 3 (2 ADC and 1 SQCC) of 135 NSCLC cases (85 ADC and 50 SQCC). Although the ALK1 immunoreactivity rate in our NSCLC cases was lower than the rate of the fusion transcript *EML4/ALK* reported in the previous study, ALK1 protein expression by IHC in ADC showed a 100% correlation with the high level of *ALK* as detected by gene expression profiling. Further analysis is needed to determine the correlation between overexpression of ALK protein and presence of the fusion transcript. However, IHC staining for ALK appears to be a promising tool to assess for the presence of the fusion transcript.

1538 Response to Neoadjuvant Therapy and Survival with Pulmonary Non-Small Cell Carcinomas

WK Brix, WD Ormsby, PW Smith, N Theodosakis, DR Jones, MR Wick, MA Conaway, EB Stelow. University of Virginia, Charlottesville, VA.

Background: Preoperative chemotherapy and radiation therapy have been shown to improve outcomes with subsequent surgery with a number of malignancies including esophageal and rectal carcinomas. With these, the histologic responses to these therapies have been well-characterized and the degree of down-staging and tumor response have both been shown to correlate with outcomes. Although neoadjuvant radiation therapy and chemotherapy have been shown to improve survival with high-stage non-small cell lung carcinomas, little has been reported regarding the histologic changes of the treated tumors and their relationships with outcomes. Here, we evaluated the histologic changes seen in pretreated pulmonary non-small cell carcinomas and compared them to outcomes.

Design: Thirty-six non-small cell carcinomas of the lung were identified that had undergone chemotherapy and / or radiation therapy prior to surgical resection. Tumors were evaluated for histologic type and grade. Degree of response (0-100%) was recorded and characterized. Pre-operative radiographic and biopsy-determined stage was compared to post-operative pathologic stage as assessed using viable tumor. All results were compared with outcomes.

Results: Most cases were classified as either squamous carcinoma or adenocarcinoma (77%), although 9% were considered large cell carcinomas. The histologic response was generally non-specific and characterized by necrosis, fibroelastosis, foreign body reaction and mixed inflammation. Nineteen percent of cases showed complete histologic response, 36% showed 90-99% response and 45% showed <90% response. Sixty-four percent of tumors were at least stage IIIA prior to neoadjuvant therapy. Following resection 19% were stage 0, 50% were stage IA or IB, 19% were stage IIA or IIB, 8% were stage IIIA or IIIB and 4% were stage IV. Patients with 100% tumor response had fewer deaths and recurrences than those patients with less response. With degree of downstaging assessed as a continuous variable, greater dowstaging was associated with both better survival time and longer time before recurrence (estimated hazard ratios of 1.41 and 1.46, respectively, for each step of downstaging, e.g., IIIA to IIB), although the numbers were not yet significant.

Conclusions: Pulmonary non-small cell carcinomas have a variable degree of histologic response to preoperative therapies. The degree of response and TNM downstaging both appear predictive of outcomes.

1539 IL32 Is Overexpressed in Lung Tissue of Patients with COPD

F Calabrese, S Baraldo, E Bazzan, F Lunardi, P Sfriso, CA Dinarello, R Zuin, M Saetta. University of Padua, Padua, Italy; University of Colorado, Colorado.

Background: Proinflammatory mediators such as IL1beta, IFN gamma and TNFalfa may have a crucial role in the persistence of the inflammatory response in COPD. IL32 is a recently described cytokine that may play an important role in the amplification of inflammatory reactions in autoimmune disorders. Up to now, the lung expression of IL-32 has never been investigated in COPD.

Design: To elucidate the role of IL32 in this disease, we applied immunohistochemistry to surgical specimens obtained from 42 subjects undergoing thoracotomy for solitary nodule or lung volume reduction surgery, grouped as follows: 22 smokers with COPD (GOLD stage I to IV), 11 control smokers and 9 nonsmokers.

Results: The percentage of IL32+ macrophages was different in the 3 groups of subjects (p=0.003), being significantly increased in smokers with COPD (median;range:93;3-100%) compared to both control smokers (67;0-99%, p=0.03) and nonsmokers (20; 0-93%, p=0.002). A similar pattern of expression was also observed in alveolar walls, with an increased expression of IL32 in smokers with COPD (1.5;0-6.5 cells/mm) compared to control smokers (0;0-3 cells/mm, p=0.03) and nonsmokers (0; 0-2.4 cells/mm, p=0.04). Moreover, IL32 expression in alveolar macrophages was correlated to that in alveolar walls (p<0.0001; r=0.81). Considering all subjects as one group, IL32+ macrophages (%) were inversely related to values of FEV1 % pred (p=0.02; r=-0.4) and FEV1/FVC % (p=0.002; r=-0.5).

Conclusions: In conclusion, our study provides, for the first time, information about IL32 expression in lung tissue of smokers with COPD, suggesting that this cytokine may play a role in the persistence of the inflammatory response. This observation supports the view that an autoimmune component may be present in COPD.

1540 Bcl-2 Expression in Pulmonary Neuroendocrine Neoplasms: A Tissue Microarray Study

MD Chiselite, NM Deal, IJ Check, SM Share, TC Pereira, JS Coon, TA Hensing, RK Orr, KW Lannert, MJ Liptay, JA Howington, CD Sturgis. Evanston Northwestern Healthcare, Evanston; Rush University, Chicago; Allegheny General Hospital, Pittsburgh.

Background: Neuroendocrine pulmonary neoplasms (NPNs) are a distinct morphologic group of tumors, ranging across a biological spectrum from typical carcinoids (TCs) to small cell lung carcinomas (SCLCs). TC 5 yr survival rates average 95%, while SCLC 5 yr survival rates are reported at 5%. Alterations in mechanisms of cellular senescence are fundamental in tumorigenesis. Bcl-2 (an apoptosis supressor) has not been extensively studied in all NPNs. Cautious optimism exists pertaining to an antisense oligonucleotide against the oncogene Bcl-2 for the treatment of SCLCs. We report findings of Bcl-2 expression across the NPN spectrum based on tissue microarray (TMA) immunohistochemistry (IHC).

Design: 34 TCs, 4 atypical carcinoids (ACs), 15 large cell neuroendocrine carcinomas (LCNCs) and 51 SCLCs (ENH and AGH 1999-2006) were used to create a TMA. Patients were 29%M, 71%F, mean age 65 yrs. Three (0.6 mm) cores of tumor were included for each case. Bcl-2 expression was evaluated by IHC (clone 100/D5, Biocare Medical, Walnut Creek, CA). Staining pattern was scored as 0%, <25% and >25% of tumor cells positive. Staining intensity was graded on a semiquantitative scale of 0-3. (All IHC studies and original tumor diagnoses were confirmed by two pathologists, MDC and CDS).

Results: Bcl-2 immunoreactivity patterns are shown in the table below.

Bcl-2 In Neuroendocrine Lung Neoplasms

Category	0%cells Bcl2+	<25%cells Bcl2+	>25%cells Bcl2+	% Cases Mod-Strong Intensity
TC (n=34)	35	12	53	29
AC (n=4)	0	0	100	100
LCNC (n=15)	0	0	100	93
SCLC (n=51)	0	2	98	92

Conclusions: Bcl-2 expression was noticeably different across the spectrum of NPNs. TC Bcl-2 intensity was less than AC intensity (Fisher's exact test, P = 0.0136). When combining the higher grade NPNs (ACs, LCNCs and SCLCs) as a group, Bcl-2 immunoreactivity was significantly higher than that of TCs (Fisher's exact test, P<0.0001). The same statistical significance was achieved in comparing LCNC to TC and SCLC to TC, respectively. When >25% of cells were Bcl-2+, regardless of intensity, higher grade NPNs were significantly more likely to be positive (Fisher's exact test, P<0.0001). Our findings indicate that Bcl-2 disregulation is greater in higher grade NPNs. Further studies of Bcl-2 targeted therapies may prove useful for patients with these tumors.

1541 An Integrated Analysis of Gene Expression, Copy Number Alterations, and Kinase Pathway Mutations in Lung Adenocarcinoma Reveals Frequent Loss of the MAPK Phosphatase Gene *DUSP4* in *EGFR* Mutant Tumors

D Chitale, Y Gong, S Broderick, C Brennan, BS Taylor, R Somwar, L Wang, N Motoi, J Szoke, C Sander, EV Seshan, A Hawley, PP Pandolfi, V Rusch, W Pao, W Gerald, M Ladanyi. Memorial Sloan-Kettering Cancer Center, New York, NY.

Background: Genomic analyses of lung adenocarcinoma have yielded striking advances that are already impacting on clinical management. Further advances in understanding of the biological heterogeneity of this disease will require integration of multiple types of genomic data. To this end, we have assembled a large integrated genomics dataset of lung adenocarcinomas.

Design: We studied 227 primary lung adenocarcinomas. Profiling of genomic gains and losses was done by array comparative genomic hybridization (aCGH) on Agilent 44K arrays. Expression profiling was based on Affymetrix U133A arrays. The sample set was annotated for mutations in *EGFR*, *KRAS*, *BRAF*, *PIK3CA*, *LKB1, PTEN,* and *P53*, using direct sequencing, mutation-specific PCR assays, and mass-spectrometry-based genotyping (Sequenom).

Results: Mutations in *EGFR*, *KRAS*, *ERBB2*, and *BRAF*, present collectively in 105/227 cases (46%), were completely mutually exclusive. By unsupervised analysis of the aCGH data, the tumors clustered into two or three patterns of co-occurring gains and losses. One aCGH cluster was strongly associated with *EGFR* mutation and was characterized by 7p gains (in the *EGFR* region) and 8p losses, further confirmed by 2-color FISH. Remarkably, by expression profiling the most consistently underexpressed gene in *EGFR* mutant cases compared to *EGFR* non-mutant cases was a MAPK phosphatase gene at 8p21, DUSP4 (MKP-2). Single copy genomic loss at *DUSP4* was strongly associated with *EGFR* mutant status (p<0.0001). The remaining copy of *DUSP4* was often silenced by promoter methylation. Functional studies showed that DUSP4 has growth suppressive effects in *EGFR* mutant lung adenocarcinoma lines.

Conclusions: *EGFR* mutations in lung adenocarcinomas are strongly associated with genomic loss and low expression of *DUSP4*. DUSPs are known to be transcriptionally upregulated by MAPK signaling as a negative feedback mechanism and DUSP family members are emerging as putative tumor suppressors in other cancers. Loss of negative feedback regulation by DUSP4 may be an additional step in the development or progression of some *EGFR*-mutant lung adenocarcinomas. Our data highlight the value of large, integrated, highly annotated genomic datasets in generating novel insights into complex common cancers.

1542 p16/CDKN2A Deletion as a Diagnostic Marker To Distinguish Benign from Malignant Mesothelial Proliferations

CT-S Chung, GC Santos, DM Hwang, O Ludkovski, J Squire, M-S Tsao. University Health Network, Toronto, ON, Canada; University of Toronto, Toronto, ON, Canada.

Background: The distinction between benign reactive mesothelial proliferations and malignant mesothelioma can be very challenging. Homozygous deletion of 9p21, targeting the CDKN2A (p16) gene, has been reported in up to 74% of malignant mesotheliomas. One group has suggested the use of FISH-based detection of p16 deletion on cytologic preparations as a diagnostic marker to distinguish benign from malignant mesothelial proliferations. The purpose of this project was to develop an ancillary test to distinguish benign from malignant mesothelial lesions based on detecting p16 deletion using paraffin-embedded sections.

Design: Dual-colour FISH for p16 and chromosome 9 was performed on paraffin-embedded sections in 56 biopsy or resected malignant mesothelioma cases (43 epithelioid, one sarcomatoid, 12 biphasic), 11 reactive mesothelial proliferations, and eight equivocal biopsy cases for which a histopathological distinction between a benign and malignant process was uncertain.

Results: Homozygous and/or hemizygous deletion of p16 was detected in 34/56 (61%) cases of malignant mesothelioma. All eight equivocal biopsies were subsequently confirmed clinically to be malignant mesothelioma; two of which were desmoplastic mesotheliomas. For the latter, FISH was difficult to interpret due to low cellularity and difficulty in distinguishing mesothelial cells from fibroblasts. For the remaining six cases, one had insufficient cells to evaluate the p16 signals, while the remaining five cases showed homozygous and/or hemizygous p16 deletion.

Conclusions: Our study showed a high prevalence of p16 deletion in malignant mesothelioma, which is consistent with findings reported by others. We are the first to document the use of FISH for p16 deletion in paraffin-embedded sections of malignant mesothelioma, and to develop an assay that is a useful and feasible ancillary test to help distinguish benign from malignant mesothelial lesions in difficult biopsy cases, with the exception of the desmoplastic histologic subtype. The finding of p16 homozygous or hemizygous deletion by FISH adds further support for a malignant diagnosis and thus, has the potential to prevent delay in diagnosis and treatment.

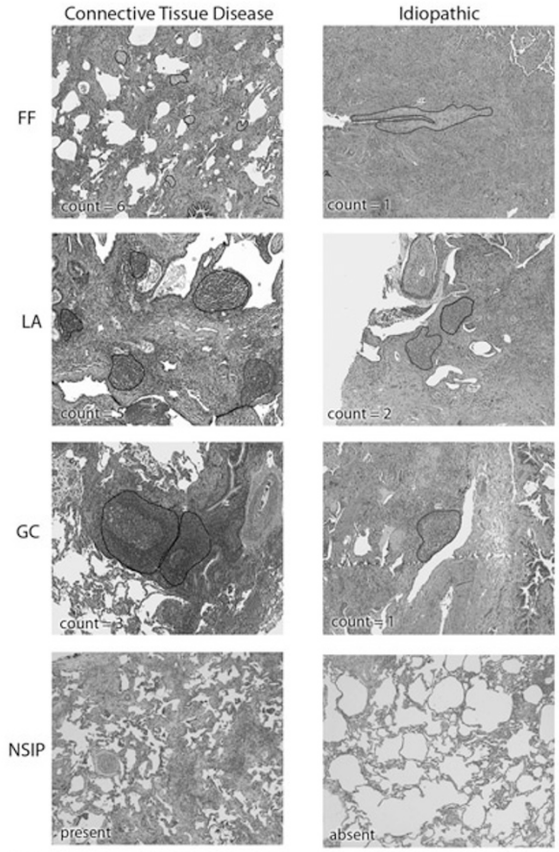
1543 How Usual Is Usual Interstitial Pneumonia (UIP)? Connective Tissue Disease-Associated Versus Idiopathic

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

Background: UIP can occur idiopathically (I-UIP) or in the setting of connective tissue disease (CTD-UIP) such as rheumatoid arthritis (RA-UIP). Patients with CTD have a better prognosis than those without. Prior qualitative studies have demonstrated differences in the histologic characteristics of CTD-UIP and I-UIP. The purpose of this retrospective study was to evaluate a quantitative scoring system to distinguish histologically between CTD-UIP and I-UIP.

Design: Lower lobe lung wedge biopsies from 10 patients (5 CTD-UIP and 5 I-UIP) were evaluated: The number of fibroblastic foci (FF), lymphoid aggregates (LA) and germinal centers (GC) per total tissue area and the ratios of FF area, LA area, and GC area to total tissue area were determined. The presence or absence of the nonspecific interstitial pneumonia (NSIP) pattern in less-involved lung was recorded (Figure 1).

Figure 1: Measurement of Fibroblastic Foci, Lymphoid Aggregates & Germinal Centers and Evaluation of NSIP

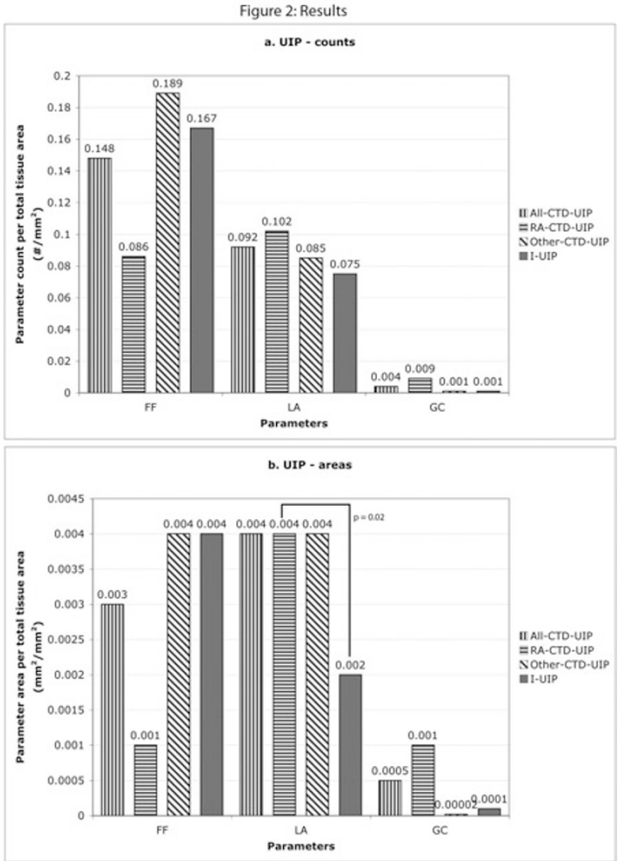


LEGEND: magnification = 40x
all areas were outlined with Automated Cellular Imaging System (ACIS) Review software

Groups were compared using the Student's t- and Wilcoxon rank-sum tests.

Results: The CTD group had a lower FF count and FF area, but a higher LA count, LA area, GC count, GC area, and prevalence of NSIP than I-UIP. These differences did not attain statistical significance. Compared to I-UIP, RA-UIP was associated with a significantly higher LA area per total tissue area (X vs Y, p=0.02; Figure 2).

Figure 2: Results



LEGEND: all parameters were averaged per patient, per total tissue area (mm²)

Conclusions: This study revealed a trend toward fewer, smaller fibroblastic foci, more, larger lymphoid aggregates and germinal centers, and a higher prevalence of NSIP in CTD-UIP vs I-UIP. RA patients had larger lymphoid aggregates than idiopathic patients. This parameter might help identify patients with RA, which has important prognostic and therapeutic implications.

1544 Clinical and Pathologic Features of Non-Tuberculous Mycobacterial Respiratory Isolates

D Cohen, M Boos, G Hall, W Tomford, CF Farver. Cleveland Clinic, Cleveland, OH.

Background: Non-tuberculous mycobacterial (NTM) pulmonary infections are responsible for increasing morbidity and mortality. Over the past 20 years, the type of organisms isolated and the patient population at risk for these infections have changed. Studies correlating culture results with the clinical and pathologic features of these patients have been limited.

Design: The pathology files of the Cleveland Clinic were reviewed for patients with positive mycobacterial cultures from respiratory isolates between 1989-1998 with tissue biopsies. Histologic slides prepared from the specimens and clinical data, including treatment and recurrence of disease, were reviewed.

Results: There were 1181 mycobacterial cultures of which 678 (57%) were respiratory specimens. These included 423 sputums, 194 bronchoalveolar lavages, 30 open lung biopsies, 19 tracheal aspirates and 12 pleural fluids. NTM were isolated in 570 (84%) and *M. tuberculosis* in 108 (16%). *M. avium* complex (MAC) was the most common organism (275). The 79 histology specimens derived from 56 bronchoscopic and 23 surgical biopsies. Patients were grouped into seven clinicopathologic groups: 23 NTM in setting of neoplasm; 2 solitary pulmonary nodule; 12 chronic bronchiolitis/bronchiectasis; 1 hypersensitivity pneumonitis; 18 chronic pulmonary disease; 7 disseminated NTM; and 16 NTM with another infectious process. Sixty-nine patients had clinical follow-up ≥ one month (mean: 39 mos). Twenty-five patients received antibiotics only, 15 received surgery only, 5 received both and 24 received no therapy. Nine patients had disease recurrence, of which 5 received antibiotics only and 4 received no therapy. Average time to recurrence was 55.3 months. Six of the patients with recurrence had chronic bronchiolitis/bronchiectasis. Granulomas were present in 20 of 79 pathologic specimens. Other patterns of injury included: 3 chronic interstitial pneumonitis, 4 acute and organizing pneumonia/BOOP, 4 chronic bronchiolitis/bronchiectasis, 10 neoplasm, 2 diffuse alveolar damage, 11 other infectious etiology, 25 no diagnostic abnormality. Four of the 79 specimens had organisms present by tissue acid fast stains.

Conclusions: NTM organisms represent a majority of mycobacterial respiratory pathogens in a large referral center. MAC is the most common pathogen. Granulomas were found in approximately 25% of the histology specimens. Tissue organismal stains were not helpful in identifying organisms. Recurrence after disease resolution is unusual.

1545 Integrative Genomic Microarray Analyses Reveal Novel Molecular Targets in Non-Small Cell Lung Carcinoma

KJ Craddock, D Strumpf, W Xie, TPH Buys, B Chi, WL Lam, I Jurisica, MS Tsao. University Health Network, University of Toronto, Toronto, ON, Canada; University of Toronto, Toronto, ON, Canada; British Columbia Cancer Research Centre, Vancouver, BC, Canada.

Background: Non-small cell carcinomas (NSCLC) typically have complex karyotypes with multiple chromosomal aberrations that result in net gains and losses of genetic material. Although recurring areas of amplification have been described involving genes thought to be important in the carcinogenesis, the list of identified potential oncogenes remains limited.

Design: High-resolution comparative genomic analysis was performed on DNA from 115 archived NSCLC specimens using a whole genome tiling path bacterial artificial chromosome array with 26,363 overlapping clones. Circular binary segmentation (DNAcopy) was employed to define the segmental DNA gains and losses in each tumor genome. Minimal common regions (MCRs) of amplification were then identified for the entire tumor panel. A subset of salient alteration features were determined by integrating genomic data with results from gene expression microarray experiments: expression levels for key genes within MCRs were identified through significance analysis of microarray (SAM) analysis of Affymetrix U133A array for 178 fresh frozen NSCLC samples and analysis of two similar publicly available datasets.

Results: Observed segmental alterations were in concordance with previous studies, including frequent gains at chromosome 1q, 3q, 5p, and 8q, and frequent losses at 3p, 5q, 6q, 8p, 9p, 13q, and 17p. MCRs of amplification encompassed *MYCC*, *MYCL1*, *hTERT*, *KRAS*, multiple *RAS*-associated genes, cyclins and cyclin-dependent kinases. Refined analysis of key genes within MCRs using multiple expression array datasets revealed 16 high-confidence genes, six of which have previously been identified as amplified in NSCLC. The ten novel genes include molecules involved in the DNA replication apparatus, integrin-mediated signaling pathway, prostaglandin metabolism, and glycolysis. An interaction network analysis of the 16 genes showed a statistically significant functional relationship, with enrichment of cell-cycle-related molecules.

Conclusions: Integrative genomic analysis on a large panel of lung tumours has revealed potentially important players in the pathogenesis and malignant progression of NSCLC. Further investigation of these genes and the pathways involved could unveil new prognostic biomarkers or novel therapeutic targets in this disease.

1546 Immunohistochemistry for Phosphorylated EGF Receptor Predicts Survival and Phosphorylated ERK Predicts Tumor Response to Chemo-Radiotherapy in Unresectable, Locally Advanced Non-Small Cell Lung Cancer Patients

J-C Cutz, TS Tsakaridis, J Wright, H Hirte, G Singh, M-S Tsao. McMaster University, Hamilton, ON, Canada; Juravinski Cancer Center, Hamilton, ON, Canada; University of Toronto, Toronto, ON, Canada.

Background: Epidermal growth factor receptor (EGFR) and downstream effectors such as extracellular signal-regulated kinase (ERK), are predicted to play major roles in lung cancer cell proliferation. However, studies fail to show that immunohistochemical evaluation of total EGFR protein holds prognostic and predictive value in non-small cell lung cancers (NSCLC) with respect to overall survival (OS) or radiographic response (RR) to chemo-radiotherapy (CRT). The current study aims to refine the evalution of this important pathway by assessing the relative immunoreactivity of phosphorylated (activated) EGFR (phospho-EGFR, p-EGFR) and related signalling molecules in cellular sub-compartments (nuclear, cytoplasm, membrane).

Design: Immunohistochemistry (IHC) for total EGFR, p-EGFR, p-ERK, p-Akt and p-Stat3 and the proliferation marker Ki-67 was performed on formalin-fixed, paraffin-embedded tumor tissue samples from patients with locally advanced NSCLC enrolled in a prospective study undergoing CRT. Correlation analysis of IHC scores for plasma membrane, cytoplasm and nuclear compartments with tumor RR and patient OS was performed.

Results: Tumors from 44 patients who received curative CRT were evaluated by IHC. RR was 50.15±15% (mean±SD) at 6 weeks post-therapy with an OS of 14.13±9.6 months. We observed a statistically significant negative correlation between membrane and cytoplasmic p-EGFR and OS (p=0.020). Multivariate COX PH model analysis showed that high IHC scores of membrane p-EGFR is an independent poor prognostic factor (p=0.018). Cytoplasmic p-Erk levels correlate negatively with RR (p=0.022) and univariate regression analysis showed a strong tendency of cytoplasmic p-Erk to predict a lower RR (p=0.059).

Conclusions: Our results suggest that p-EGFR levels, but not total-EGFR, as measured by IHC serves as an independent poor prognostic factor in this subset of patients. In addition, IHC scores of cytoplasmic p-Erk appear to predict poor RR to curative chemo-radiotherapy in locally advanced NSCLC.

1547 Prognostic Significance of p16/CDKN2A Deletion in Pleural Mesotheliomas

S Dacic, HH Popper, H Kothmaier, I Halbwedl, P Morbini, B Murer, CE Comin, F Galateau-Salle, F Demirag, H Zeren, R Attanoos, A Gibbs, PT Cagle. University of Pittsburgh, Pittsburgh; Institute of Pathology, Graz, Austria; Instituto di Anatomia Pathologica, Pavia, Italy; Ospedale Umberto, Mestre-Venezia, Italy; University of Firenze, Firenze, Italy; CHU Cote de Nacre, Caen, France; Ataturk Chest Disease Hospital, Ankara, Turkey; Adana University, Adana, Turkey; Cardiff and Vale NHS Trust Llandough Hospital, Cardiff, United Kingdom; The Methodist Hospital, Houston.

Background: Pleural mesothelioma is usually diagnosed at an advanced stage with a short survival. Recent studies suggested that p16/CDKN2A homozygous deletion is associated with aggressive behavior using one year survival as a cutoff for long survival. The aim of this study was to determine prognostic significance of p16/CDKN2A deletion in pleural mesotheliomas with true long-term survival of three or more years.

Design: High-density tissue microarrays were constructed from formalin-fixed, paraffin-embedded samples of 48 mesotheliomas collected from 9 institutions in USA, Austria, Italy, France, Turkey and UK. Long survival (LS) was defined as > 3 years, and short survival (SS) as <3 years. Dual-color FISH analysis was performed using a Spectrum Green- labeled chromosome 9 centromeric probe and a Spectrum-Orange labeled, locus specific CDKN2A (p16) probe (Vysis, Downers Grove, IL). Each tumor was assessed by the average and the maximum numbers of copies of p16 gene per cell and the average ratio of p16 gene to chromosome 9 copy numbers. Homozygous deletion was defined if both 9p21 signals were lost in at least 20% of nuclei.
Results: Hybridization was successful in 34 cases (71%) (18 LS; 16 SS). Homozygous deletion of p16/CDKN2A was seen in 56% cases, more frequently in SS group. Of 18 cases with LS, homozygous deletion was identified in 6 cases (33%), while 12 cases (67%) were negative. Of 16 cases with SS, homozygous deletion was identified in 13 cases (81%) and 3 cases (19%) were negative. The difference in frequency of p16 homozygous deletion between LS and SS was statistically significant (p=0.04).
Conclusions: To our knowledge, our study is the first to demonstrate decreased frequency of p16 homozygous deletion in mesotheliomas from patients with long term survival of greater than 3 years in contrast to patients with rapidly fatal mesotheliomas. This observation suggests that restoration of p16/CDKN2A gene function by targeted gene therapy may prolong survival in patients with mesothelioma.

1548 Evidence Based Pathology: Are EGFR Tests Valid Predictors of Treatment Response to Erlotinib or Gefitinib in Patients with Pulmonary Adenocarcinoma?

AM Dastane, R Gupta, RJ Mckenna, A Marchevsky. Cedars Sinai Medical Center, LA, CA.

Background: Detection of EGFR overexpression (EGFR-O) by immunohistochemistry (IHC) or fluorescent in-situ hybridization (FISH) and of EGFR mutation (EGFR-M) by PCR have been proposed as predictive tests of response to erlotinib (Tarceva^{R}) and gefitinib (Iressa^{R}) in patients with lung cancer. It is controversial whether these tests should be used for the routine evaluation of these neoplasms.
Design: A systematic review of the English language literature using the search terms "EGFR" and "lung neoplasms" was performed to query current best evidence regarding the predictive value of tests for EGFR-O and EGFR-M. The studies that correlated EGFR expression with tumor response to erlotinib or gefitinib were selected and classified according to the testing method. Meta analysis was performed using Comprehensive meta-analysis software (Biostat Inc Englewood, N.J.).
Results: 144 studies of lung cancer patients studied for EGFR expression have been published since 2002, but only 11 studies compared EGFR-O or EGFR-M with response to getifinib therapy. Three of these used IHC, PCR and FISH, 3 used PCR and FISH, 3 used only PCR, and one each used FISH and IHC, respectively to evaluate for EGFR-O or EGFR-M. Two of 4 studies evaluating EGFR-O by IHC reported significant correlation with response rate to gefitinib. Five of 7 studies evaluating EGFR-O by FISH reported a significant correlation with response to gefitinib and proposed that this method is superior to PCR for predicting reponse to treatment. Three of the 9 studies reporting EGFR-M by PCR showed a significant response to gefitinib. Meta-analysis showed significant correlations between EGFR-M detected by PCR and/or EGFR-O detected by FISH and response to getifinib therapy. No data were found to evaluate correlations between EGFR expression and response to erlotinib therapy.
Conclusions: Review of currently available "best evidence" raises questions regarding the validity of EGFR testing in patients with lung AC. Reported data include the evaluation of patient cohorts with variable characteristics and using different end-points to assess response to treatment. Meta-analysis of heterogenous data suggests that EGFR testing by PCR and FISH but not by IHC probably correlates with response to getifinib therapy. Additional studies are needed to evaluate whether there is a significant correlation between EGFR-O and/or EGFR-M and response to erlotinib therapy in patients with pulmonary neoplasms.

1549 Problems with the Diagnosis of Carcinoid Tumor on Pulmonary Frozen Sections

AM Dastane, R Gupta, RJ Mckenna, AM Marchevsky. Cedars Sinai Medical Center, LA, CA.

Background: The diagnosis of carcinoids and other neuroendocrine tumors (NET) can be difficult, particularly by lung frozen section (FS). These neoplasms can be confused for non-small cell carcinoma (NSCLC), metastatic breast cancer, lymphoma and other neoplasms, resulting in unnecessary lobectomies or second thoracotomies.
Design: 2405 patients underwent FS at Cedars-Sinai Medical Center from 2002-2007; 77 cases were NET, including 53 typical carcinoids, 11 atypical carcinoids, 11 large cell neuroendocrine carcinomas (LCNEC) and 2 small carcinomas (SCLC). The diagnosis of carcinoid was suggested on FS in 4 lymphomas. All FS and the corresponding permanent sections were reviewed. The deferral rate (DR) and error rate (ER) were calculated and compared with our overall FS experience. The presence of 15 diagnostic features were evaluated in the correctly and incorrectly diagnosed cases and analyzed with descriptive statistics.
Results: DR and ER for all pulmonary frozen sections during the 5-year period were 4.32% and 1.45%. In contrast, the DR for NET was 9.09 % (7/77) and the ER was 18.51% (15/81). Errors resulted in 4 unnecessary lobectomies and 2 second thoracotomies. Eight of 53 typical carcinoids were misdiagnosed: 4 were diagnosed as NSCLC, 2 as organizing pneumonia, and 1 each as carcinoma NOS, and LCNEC. Two of 11 atypical carcinoids were misclassified on frozen sections as LCNEC and NSCLC. One of 2 SCLC was classified as LCNEC. Ten of 12 LCNEC were reported as NSCLC; they were not used to estimate the ER. Features that were visible in the permanent sections of carcinoids but apparently absent in the FS were nesting and trabecular growth patterns, cellular uniformity, and salt and pepper nuclear chromatin. Presence of necrosis and mitoses can contribute to the misdiagnoses of carcinoma. Touch preparations (TP) were available in 34.6% of the correctly diagnosed cases and 28.5% of misdiagnosed and deferred cases.
Conclusions: Carcinoid tumors can be difficult to diagnose on FS resulting in increased DR, ER and unnecessary surgeries. The quality of FS is particularly important as the tumor cells can appear larger, more pleomorphic and lack visible nesting or trabecular pattern in inadequate preparations. The extent of necrosis and mitotic activity should be carefully evaluated to avoid the confusion between carcinoid tumor and carcinomas. TP can be helpful to avoid these pitfalls.

1550 Expression of Alpha-Methylacyl-COA Racemase (AMACR) in Tissue Microarray of 39 Cases of Diffuse Malignant Mesothelioma (DMM)

DW Davis, QJ Zhai, W Li, D Tan, TC Allen, HH Popper, PT Cagle. University of Texas Health Science Center, Houston, TX; The Methodist Hospital/Weill Cornell Medical School, Houston, TX; University of Texas M.D.Anderson Cancer Center, Houston, TX; University of Texas Health Center, Tyler, TX; Institute of Pathology, Medical University of Graz, Graz, Austria.

Background: AMACR is a mitochondrial and peroxisomal enzyme involved in the metabolism of branched-chain fatty acid and bile acid intermediates. AMACR has recently been shown to be a biomarker that is expressed in a number of neoplasms, including prostate, breast, and colorectal adenocarcinomas, and in some gastrointestinal epithelial dysplasias. Many proteins shown to be highly upregulated in pathological states by immunohistochemistry are potential targets for therapies utilizing monoclonal antibodies. To our knowledge, AMACR expression in DMM has never been investigated. Given the need for better treatment modalities against DMM and AMACR's potential as a therapeutic target, the aim of this study was to evaluate AMACR expression in DMM.
Design: Tissue microarrays from 39 archival cases of DMM and controls were immunostained with Racemase (Anti-Racemase, DakoCytomation, Carpinteria, CA; 1:100). Male:female ratio is 9:1 and average age is 72.3 years (range 47 to 97). Histologic types include: 25 epithelioid, 11 sarcomatous, 3 biphasic. Cytoplasmic staining intensity was interpreted as follows: 0 = no expression, 1= weak, 2 = intermediate and 3 = strong expression.
Results: 29/39 (74.4%) DMM showed expression of AMACR. Of those, 24 showed weak expression (82.8%), 4 intermediate expression (13.8%), and 1 strong expression (3.4%). Intermediate and strong AMACR expression occurred only in epithelial histologic types.
Conclusions: AMACR is often expressed in DMM and could be a potential target for future therapeutic intervention.

1551 Utility of S100P in Differentiating Adenocarcinoma of the Lung from Papillary Carcinoma and Follicular Carcinoma of the Thyroid

P Dorion, J Shi, K Zhang, C Schuerch, F Lin. Geisinger Medical Center, Danville, PA.

Background: Adenocarcinoma of the lung (ACL) and papillary carcinoma of the thyroid (PCT) and follicular carcinoma of the thyroid (FCT) share several important immunohistochemical markers, such as positive staining for cytokeratin 7 and TTF-1. The distinction between ACL and carcinoma of the thyroid largely relies on positive immunoreactivity for thyroglobulin in thyroid carcinoma. Interpretation of immunostaining for thyroglobulin can be problematic because of the frequent background staining. S100P belongs to the family of S100 calcium binding proteins. Overexpression of S100P has been shown in pancreatic carcinoma and urothelial carcinoma and associated with a poor clinical outcome in some human carcinomas. Our recent studies demonstrated that antibody to S100P protein (AJSP 2007; in press) is a useful marker in distinguishing adenocarcinoma of the pancreas from reactive/benign pancreatic ducts. In this study, we investigate the utility of S100P in differentiating ACL from PCT and FCT.
Design: We immunohistochemically evaluated the diagnostic value of S100P on conventional tissue sections from 39 cases of ACL, 20 cases of PCT, and 20 cases of FCT. The staining intensity was graded as weak or strong. The distribution was recorded as negative (less than 5% of tumor cells stained), 1+ (5-25% of tumor cells stained), 2+ (26-50% of tumor cells stained), 3+ (51-75% of tumor cells stained), or 4+ (more than 75% of tumor cells stained).
Results: The results demonstrated a nuclear and cytoplasmic staining pattern of S100P in 31 of 39 cases of ACL (79.4%), with 3+ or 4+ staining in 20 cases (51%); whereas all cases of PCT, FCT and normal thyroid tissue were negative. The alveolar lining epithelial cells were negative for S100P in all cases. Focal S100P positivity in normal bronchial epithelium adjacent to the adenocarcinoma was observed in only 10 of 39 cases (25.6%).
Conclusions: The results demonstrate that S100P is a useful marker in differentiating ACL from PCT and FCT. In addition, S100P may play a role in the carcinogenesis of adenocarcinoma of the lung because of the loss of or reduced expression of S100P in normal lung tissue and bronchial epithelia.

1552 Incidence and Significance of Benign Diagnoses on Core Needle Biopsies of Lung

EE Doxtader, S Mukhopadhyay, A-LA Katzenstein. SUNY Upstate Medical University, Syracuse, NY.

Background: Core needle biopsy (CNB) is a safe and reliable method for diagnosing peripheral lung nodules, and is generally considered superior to fine needle aspiration in establishing specific benign diagnoses. The purpose of this study was to review our experience with CNB of the lung to further assess the incidence and significance of benign diagnoses.

Design: All CNBs of the lung performed at our institution between January 2005 and August 2007 were reviewed. Benign and malignant diagnoses were recorded and the clinicopathologic characteristics of benign diagnoses were further evaluated, including histologic features, size, number, radiographic characteristics, uptake on position emission tomography (PET), clinical impression, and follow up.
Results: 421 core needle biopsies of lung were identified. Benign diagnoses were made in 147 (35%), while the remaining 274 (65%) were malignant. Patients with benign diagnoses ranged from 3 to 86 years old (mean 58, median 59), and 15 (10%) were 40 or younger. There were 79 men and 68 women (M:F=1.2:1). Of the benign diagnoses, 106/147 (72%) were specific and included necrotizing granulomas (33), non-necrotizing granulomas (19), scars (15), organizing pneumonia (12), hamartoma (9), and a number of miscellaneous entities (18). Organisms were detected in 11 of 33 necrotizing granulomas (7 histoplasma, 2 cryptococcus, 1 coccidioides, 1 fungal hyphae). Radiographically, 7 non-necrotizing granuloma lesions were spiculated, and 5 were PET positive. Most hamartomas were well defined radiographically, and 2 were PET positive. 34/147 (23%) benign diagnoses were non-specific and included mainly fibrosis and chronic inflammation (28), while 7/147 (5%) were considered non-representative (normal lung-5, pleura/skeletal muscle-2). Repeat biopsy yielded a malignant diagnosis in 2 cases, including 1 with fibrosis and chronic inflammation and 1 scar which contained atypical cells in the initial biopsy.
Conclusions: CNB is an accurate method of diagnosing benign lung lesions, and it yields specific diagnoses in the majority of the cases. The technique is especially helpful when radiographic findings are suspicious for malignancy, since a specific diagnosis obviates the need for re-biopsy. Even non-specific diagnoses such as fibrosis and chronic inflammation rarely lead to re-biopsy, probably because the lesion is well sampled by this technique.

1553 Prognostic Markers in Low Stage Lung Cancer Using Real Time RT-PCR from Paraffin-Embedded Tissue

E Duncavage, J Clark, B Goodgame, R Govindan, J Pfeifer. Washington University, St. 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 and its side effects. We aim to develop a prognostic test for LS-NSCLC using ubiquitous paraffin-embedded tissue.
Design: Starting from a previously defined set of 64 genes shown to predict outcome for LS-NSCLC using fresh frozen tissue and Affymetrix ™arrays (Lu Y *et al*. PLoS Med. 2006), we created a real time RT-PCR based assay optimized for paraffin-embedded tissue. Fourteen patients were randomly selected from a group of over 1000 patients with surgically resected LS-NSCLC and follow-up data. The average length of follow-up was 7 years among the selected patients. Of the 14, seven patients had no recurrence, 4 had a recurrence, and 3 had second lung primaries. The expression levels of twenty genes, representing the 10 most significantly associated with good or bad prognoses, were measured. RNA was extracted from 10µm sections of paraffin blocks and verified with Agilent™ chips. Primers were designed *in silico* from referenced GenBank sequences to yield an amplicon length of 80-120bp. Gene expression was measured in duplicate using SYBR green chemistry on an ABI™ 7300 analyzer, and amplicon identity was confirmed by melting curve analysis. Individual samples were normalized to β-*actin* expression.
Results: A total of 1,500 RT-PCR reactions were analyzed and the results from replicate samples averaged. Significance was determined using a Wilcoxon rank-sum test within the SAS™ statistical package. Of the 20 selected genes 3 were shown to be significant predictors of recurrence ($p<0.05$) including *BN2P1, CDH8a*, and *PSEN1a*. At least one gene, *CDH8*, has been implicated as a prognostic factor in other cancers.
Conclusions: We were able to demonstrate significant correlation between gene expression and outcome in archival paraffin-embedded tissue. Expansion of the number of genes in the RT-PCR panel and analysis of a larger patient population may provide a molecular test that can be used to predict prognosis for LS-NSCLC using routine surgical pathology material.

1554 Multiple Lung Nodules, Synchronous Primaries Versus Synchronous Metastases: Does It Matter?

DK Durnick, C Couture, JA Wampfler, SD Cassivi, P Yang, MC Aubry. Mayo Clinic, Rochester, MN; Mayo Clinic, Rochester; Laval University, Quebec, Canada.
Background: Multiple synchronous lung cancers comprise ~2% of all lung cancers. Distinguishing multiple synchronous primaries from pulmonary metastases remains challenging. Although this distinction affects staging, and potentially treatment, it is not clear that it affects outcome. Therefore our aim was to assess the importance of separating multiple primaries from metastases as a prognostic feature.
Design: Clinical and histologic parameters of Mayo Clinic patients with synchronous lung cancers diagnosed between 1997 and 2003 were reviewed. Groups based on histologic similarity and location were compared.
Results: 79 women and 72 men were diagnosed with synchronous lung cancers. 14% were never smokers. 106 patients had 2 nodules and 45 had ≥3, ranging from 0.3-15 cm. The histologic subtypes include adenocarcinoma (66%) including 15% BAC, squamous cell carcinoma (18%) and large cell carcinoma (5%). Overall median survival was 2.2 years and 5-year survival 25%. Survival was not affected by location (same lobe, different lobe or different lung) or similarity in histology. In univariate analysis, older age at diagnosis, non-BAC, grade, number of nodules, primary vs metastatic, N2 disease and surgical treatment affected survival. However, in multivariate analysis, only age ($p=0.003$), non-BAC ($p=0.013$) and N2 disease ($p=0.0002$) were predictors of poor outcome.
Conclusions: Distinguishing synchronous primaries from metastasis did not impact survival or treatment. Older age, non-BAC histology and N2 disease were significant predictors of outcome.

	Same lobe, same histo N=38	Same lobe, diff histo N=9	Same lung, diff lobe, same histo N=44	Same lung, diff lobe, diff histo N=7	Diff lung, same histo N=8	Undetermined N=43	p-value
Stage (%)							<0.0001
IA	0	34	2	0	0	0	
IB	0	11	0	0	38	0	
IIB	0	0	0	14	12	0	
IIIA	0	22	0	14	0	2	
IIIB	90	33	5	0	0	14	
IV	10	0	93	72	50	84	
Favor (%)							<0.0001
Primary	11	100	28	86	25	0	
Metastasis	81	0	67	0	75	17	
Both	3	0	0	14	0	0	
Undetermined	5	0	5	0	0	83	
Surgery (%)							<0.0001
No Surgery	0	0	0	0	0	44	
1 Resection	74	78	27	0	12	37	
>1 Resection	26	22	73	100	88	19	

1555 Proliferative Activity in Fibrosing Lung Diseases: A Comparative Study of Ki-67 Immunoreactivity in Usual Interstitial Pneumonia (UIP), Bronchiolitis Obliterans-Organizing Pneumonia (BOOP), and Diffuse Alveolar Damage (DAD)

OA El-Zammar, A-LA Katzenstein. SUNY Upstate Medical University, Syracuse, NY.
Background: UIP, BOOP and DAD all contain fibroblasts in differing numbers and distribution that are thought to be related to acute lung injury. Although the clinical course and prognosis for the three conditions are different, we postulated that the fibroblasts and overlying epithelium would have similar proliferative activity.
Design: Ki-67 labeling indices were measured in 16 UIP, 8 BOOP, and 7 DAD cases using a commercial antibody (Biogenex). Counting was facilitated by utilizing dual stains (Ki-67/CK7, Ki-67/CD3, Ki-67/CD68) in most cases. Staining was recorded as follows: for UIP, fibroblasts in fibroblast foci, epithelial cells overlying fibroblast foci, and intraalveolar macrophages; for BOOP, intraluminal fibroblast plugs, epithelial cells overlying plugs, and intraalveolar macrophages; for DAD, interstitial fibroblasts, overlying epithelial cells, and intraalveolar macrophages. For comparison, staining of fibroblasts in 5 ordinary skin scars and 5 keloids was also examined.
Results: The greatest proliferative activity was found in DAD in fibroblasts (23.4%, range 10-33) and overlying epithelium (45.5%, range 19-90), while proliferative activity was considerably lower in both UIP (fibroblasts 2%, range 0.4-4.6; epithelium 2.8%, range 0-6.8) and BOOP (fibroblasts 4.3%, range 0.9-8.6; epithelium 1.75%, range 0.9-4.0). Fibroblasts in skin scars showed brisk activity (17%, range 11-26) while those in keloids were less active (4.9%, range 1-8). UIP macrophages showed an unexpectedly high proliferative rate (19.5%, range 9-35) compared to BOOP (6.0%, range 2-10) and DAD (8.7%, range 4-15).
Conclusions: The low proliferative activity of fibroblasts and overlying epithelium in fibroblast foci of UIP and fibroblast plugs of BOOP compared to the high activity in DAD suggests different reactions to acute injury in these conditions. Since keloids are thought to represent abnormal wound healing, the similar low proliferative activity of fibroblasts in both UIP and keloids supports the hypothesis of abnormal wound healing in UIP. However, the low activity in BOOP cannot be explained on this basis. The unexpectedly high proliferative activity of macrophages in UIP suggests a pathogenic role for these cells.

1556 The Accuracy of Pretreatment Biopsy of Pleural Malignant Mesothelioma in Predicting Histopathologic Type in Extrapleural Pneumonectomy

WC Foo, MD Hofer, DJ Sugarbaker, JM Corson, LR Chirieac. Brigham and Women's Hospital, Boston, MA.
Background: Pathologic classification of diffuse malignant mesothelioma (DMM) into epithelioid, sarcomatoid, and biphasic types is an important predictor of survival. The diagnosis of DMM is usually based on histopathologic examination of an adequate thoracopic or open biopsy. Since DMM is often heterogeneous, a biopsy may not be representative of the entire tumor. The goal of this study was to determine the accuracy of pretreatment biopsy in establishing the histopathologic type of DMM.
Design: We examined 151 consecutive patients with pleural DMM treated from 1988 to 1997 at Brigham and Women's Hospital by extrapleural pneumonectomy (EPP) followed by heated chemotherapy who had a pretreatment biopsy available for review. We characterized the presence of epithelioid and sarcomatoid histology in the resection and pretreatment biopsy specimens. In the 40 EPP specimens with mixed histology in the biopsy, the heterogenous distribution of epithelioid and sarcomatoid patterns were recorded. Associations between the histology in pre- and post-treatment specimens were investigated.
Results: The histology type of DMM in pretreatment biopsies were epithelioid in 120 patients (79%), mixed in 21 patients (14%), sarcomatoid in 8 patients (5%), and indeterminate in two patients (1%). The histology type of DMM in resection specimens was epithelioid in 93 patients (62%), mixed in 51 patients (34%), and sarcomatoid in 7 patients (4%). Biopsy findings were concordant with resection findings in 116 patients (Spearman $r=0.64$, $p<0.0001$). The 34 discordant and 117 concordant cases are listed in the following table.

Histopathologic Type of DMM

Pretreatment Biopsy	Posttreatment EPP
Epithelioid 120 (79%)	90 epithelioid, 30 mixed
Mixed 21 (14%)	20 mixed, 1 epithelioid
Sarcomatoid 8 (5%)	7 sarcomatoid, 1 mixed
Indeterminate 2 (1%)	2 epithelioid

Conclusions: Our data suggests that a diagnosis of mixed or sarcomatoid DMM in the pretreatment biopsy is highly predictive of the histology in the resection specimen. A diagnosis of epithelioid DMM in the pretreatment biopsy is less accurate, and it changed to a less favorable one in a significant proportion. The results of our study emphasize the importance of accurate biopsy sampling in patients with malignant mesothelioma and the value of resection specimens for accurate diagnosis.

1557 Nuclear Localization of Maspin Is Associated with an Increased Response Rate and Favorable Pathological Features in Resected Lung Adenocarcinoma and, in Stage I, Constitutes an Independent Predictor of Improved Survival

AB Frey, S Sheng, H Pass, F Lonardo. Wayne State University, Harper Hospital and The Karmanos Cancer Institute, Detroit, MI; New York University, New York, NY.

Background: Maspin (Ma) is a Mammary Serine Protease Inhibitor with tumor inhibitor properties, yet expression in non-small cell lung cancer (NSCLC) has been described to portend both a favorable and an unfavorable outcome. Although these reported variations may be partly due to methodological differences, other important features of Ma may explain this paradox, yet have not been consistently considered in previous studies: 1) Ma shows a histotype-specific cellular expression pattern: in squamous cell carcinoma, Ma almost exclusively stains the nucleus and cytoplasm; in Adenocarcinoma (Aca) Ma stains the nucleus in most cases and 2) *in vitro* and *in vivo* evidence links Ma's tumor inhibitor activity to its nuclear localization, and tumor progression to reduced nuclear/cytoplasmic levels. These data justify the hypothesis that nuclear/cytoplasmic Ma levels may influence clinical and biological features of Aca, yet this remains untested in well-controlled clinical series.

Design: To test this hypothesis, we studied a tissue bank of well-characterized, resected Aca, comparing the distribution of select pathological and clinical variables between cases with nuclear only (N) stain vs. cases with nucleo-cytoplasmic or negative expression. Histologic grade, immunohistochemical (IHC) expression of Ma, Ki-67, p53, VEGF-A, VEGF-C, and VEGFR3 were determined and correlated with the tumor's clinical features including stage, clinical response, survival, and mean SUV value on PET scan in 80 resected Aca (n=80; Stage I 46, Stage II 10, Stage III 20, Stage IV 4) with prolonged follow-up (mean 41.5 months).

Results: The (N) Ma group (n=47), compared with the groups of [nucleo/cytoplasmic (28) and negative (5)] cases showed ($p \leq 0.05$): higher clinical response rates; lower histological grade, proliferative rate (mean values of 34.5% vs. 64%), p53 expression, and VEGF-A levels. Kaplan-Meyer analysis showed that, in stage I Aca (N), Ma predicts improved survival and, by multivariate analysis, constitutes an independent prognostic parameter.

Conclusions: N Ma selects Aca with distinct histological and biological features, supporting the hypothesis that in this malignancy, Ma's biological activity may be influenced by its sub-cellular localization; particularly, its tumor inhibitor properties are linked to its nuclear localization.

1558 Expression of Matrix Metalloproteinase-7 (MMP-7) in 45 Diffuse Malignant Mesotheliomas (MM): Potential Target for Therapy

JM Gardner, TC Allen, J Jagirdar, HH Popper, PT Cagle. Weill College of Medicine Cornell University, The Methodist Hospital, Houston, TX; University of Texas Health Center, Tyler, TX; University of Texas Health Science Center, San Antonio, TX; Medical University of Graz, Graz, Austria.

Background: MMP-7 (matrilysin) is a multifunctional protease that degrades extracellular matrix. The role of MMP-7 has been investigated in GI tract cancers, where it is believed to facilitate invasion, metastasis, resistance to apoptosis, and activation of EGF receptors. Targeting of MMPs has been investigated as a method of anticancer chemotherapy (Oncogene, 2000). Additionally, cancer cells expressing MMP-7 are reportedly more susceptible to treatment with an EGFR tyrosine kinase inhibitor (gefitinib). MM is a fatal cancer of the serosal lining for which no therapeutic options that produce prolonged disease-free survival exist. One gene expression profile has shown upregulation of MMP-7 in a small sample of MM (Int J Cancer, 2001). We investigated MMP-7 expression by immunohistochemistry in a series of MM to further document the potential for MMP-7 as a target to improve response to chemotherapy in this fatal disease.

Design: We utilized a tissue microarray composed of paraffin embedded tissue sections from 45 cases of MM. The microarray was immunohistochemically stained for MMP-7 (1:50, Abcam), and tissue sections from each case were evaluated for staining intensity (weak, moderate, or strong), extent (percentage of tumor cells stained), and pattern (nuclear, cytoplasmic, or both).

Results: Positive staining for MMP-7 was seen in 43 of 45 cases of MM. Of these positive cases, 84% (36/43) exhibited strong staining, 5% (2/43) exhibited moderate staining, and 11% (5/43) exhibited weak staining. All positive cases stained diffusely, with 50-100% of tumor cells staining for MMP-7. The staining pattern was cytoplasmic in 77% (33/43), nuclear in 2% (1/43), and both nuclear and cytoplasmic in 21% (9/43).

Conclusions: The frequent strong expression of MMP-7 in our series indicates that MMP-7 likely plays a role in the progression of MM. This finding suggests that MMP-7 may be a target for chemotherapeutic intervention in many MM and that further investigations of this potential are warranted.

1559 The Etiology of Granulomatous Inflammation in Thoracic Biopsies: A Frequently Unresolved Diagnostic Problem

RC Guintu, NA Wylie, MA Morgan, AM Marchevsky. Cedars-Sinai Medical Center, Los Angeles, CA; Specialty Laboratories, Santa Clarita, CA.

Background: The etiology of granulomatous inflammation (GRI) in thoracic biopsies frequently remains unknown. Intraoperative cultures are considered as the "gold standard" but are frequently negative. Ziehl-Neelsen (ZN), Gomori's methenamine silver (GMS) and other stains have limited sensitivity. It is controversial whether Auramine-Rhodamine (AR) is more sensitive and specific than ZN. The role of molecular tests in routine practice is yet to be determined.

Design: We reviewed 362 consecutive GRI cases including 263 lung wedges, 94 intrathoracic lymph nodes and 5 pleural biopsies. All cases underwent intraoperative mycobacterial and fungal cultures and were stained with ZN, AR and GMS. Paraffin sections from 32 cases were also tested with the Artus Real ART ™ Mycobacterium Differential Lightcycler PCR kit. The sensitivity and specificity of each method was calculated. The clinical history was reviewed to determine the diagnosis of patients with biopsies that were negative with all 5 tests.

Results: An infectious etiology was detected by at least one test in only 138 biopsies (38.1 %). Mycobacterial culture was positive in 79 biopsies, including 21 cases of *Mycobacterium tuberculosis* and 58 with other mycobacteria. Only 34 and 35 of the 79 culture positive cases were positive with ZN and AR stains, yielding sensitivities relative to culture of 43% and 44.3 %. Only 5 of 19 cases (26.3%) with positive mycobacterial cultures were positive with the molecular test. Three of 13 cases with negative mycobacterial cultures were positive with the molecular test. Fungal culture was positive in 11 biopsies. GMS stain was positive in 31 cases but yielded a sensitivity of 84.6% for cases with positive fungal cultures. The 224 cases negative with all 5 tests included patients with sarcoidosis (n=45), Wegener's granulomatosis (n=2), rheumatoid nodule (n=1) and undetermined etiology (n=176).

Conclusions: The etiology of 48.6 % of thoracic biopsies with GRI remained unresolved. AR stain added only marginal sensitivity over ZN for the detection of mycobacteria. The molecular method applied to paraffin embedded tissues yielded low sensitivity for the detection of mycobacteria. The results raised questions about the sensitivity of intraoperative cultures. The value of culture and molecular studies to determine the etiology of GRI is discussed.

1560 Lessons Learnt from Mistakes and Deferrals in Frozen Section Diagnosis of Pulmonary Lesions: An Evidence Based Pathology Approach

R Gupta, AS Khuranna, RJ McKenna, AM Marchevsky. Cedars Sinai Medical Center, LA, CA.

Background: The diagnosis of lung lesions by frozen section (FS) diagnosis can be difficult as inflammatory atypia and artifacts introduced by the procedure may closely simulate a malignancy. Deferred diagnoses or mistakes may result in second thoracotomies or unnecessary lobectomies.

Design: We reviewed our experience with 2,405 patients that underwent FS from 2002-2007. In 143 of these cases, including 65 benign conditions, 35 adenocarcinomas, 22 carcinoids, 15 lymphomas and 6 other neoplasms the diagnosis was deferred or erroneous. Deferral (DR) and error rates (ER) for the FS diagnoses of reactive atypia (RA) and adenocarcinoma (AC) were calculated. We reviewed all FS slides and corresponding permanent sections and compared the presence of 23 pathological parameters in AC and RA using descriptive statistics. Each pathological parameter found to be significant was assessed for its diagnostic applicability using odds ratio (OR).

Results: Overall DR and ER were 4.36% and 1.58% respectively. DR and ER for the diagnoses of lymphoma and carcinoid tumor were considerably higher at 10.38% and 18.18%. Thirteen of 23 pathological parameters, including grossly visible nodule, glandular/papillary growth patterns, granulomas, atypical mitoses, and others were significant at a $p<0.05$ level. However, the diagnostic applicability of individual parameters was found to be variable, somewhat limited and independent of their chi-square p-value, as shown by OR ranging from 0.16 to 13.

Conclusions: Pathological parameters that can be helpful to distinguish RA, AC and other conditions on pulmonary FS are discussed. Recognition that lymphomas and carcinoids can be particularly difficult to diagnose by FS may reduce ER and DR.An evidence-based approach that distinguishes between "statistically significant" and really useful diagnostic parameters in daily pathology practice is emphasized.

1561 Lack of E-Cadherin and Pan-Cadherin Immunoexpression Is Associated with Metastasis in Small Cell Lung Cancer

AK Haque, N Singhal, T Allen, R Barrios, A Sienko, PT Cagle. The Methodist Hospital, Houston, TX; University of Texas Tyler, Tyler, TX.

Background: Decreased cell adhesion is a cardinal event in carcinogenesis and tumor metastasis. Cadherins are calcium dependent adhesion molecules responsible for cell recognition and tissue integrity. Loss of Cadherins is reported in cancers of different sites. Soluble E-Cadherin in serum of patients with lung cancer could be used as a tumor marker. We investigated immunoexpression of E-Cadherin and Pan-Cadherin in 43 small cell lung cancer (SCLC), and determined correlation with metastasis.

Design: Tissue microarray constructed from 43 SCLC were immunostained with E-Cadherin antibody (1:25) and PAN-Cadherin antibody (1:100); Cell Signalling, Beverly MA. Three punches from each case were used, and each punch scored for percentage of cells stained and intensity of staining using a scale of 0-100 and 1-3 respectively; each value was averaged. Appropriate negative and positive controls were used. Data were analyzed using Spearman rank correlation, Mann-Whitney U test and Kruskal-Wallis test.

Results: E-Cadherin and Pan-Cadherin expression was seen in 40% of tumors. Significant inverse correlation was seen between E-Cadherin and Pan-Cadherin expression and presence of metastasis at diagnosis (P=0.03).

Conclusions: Reduced expression or lack of expression of E-Cadherin amd Pan-Cadherin in SCLC is associated with metastasis, and consequently a higher stage and poorer prognosis. Regulation of Cadherin mediated adhesion may be a potential therapeutic target for control of SCLC metastasis.

1562 Expression of Activated AKT (And Two Other Related Proteins) in Unresectable NSCLC Does Not Correlate with Radiologic Chemoresponse

ML Hearp, ZS Ahmed, PS Talwar, M Wright, K Kaiser, JS Coon, A Tyner, O David. University of Illinois, Chicago; Rush University Medical Center, Chicago.

Background: Akt has been shown to mediate chemoresistance in numerous in vitro studies of lung cancer. There are conflicting reports concerning its role as a prognostic marker. This is the first attempt to correlate overexpression of activated Akt (pAkt) with radiologic chemoresponse in patients diagnosed with and treated for unresectable NSCLC. PTEN, a tumor suppressor that blocks Akt activation, and PTK6, a regulatory tyrosine kinase that promotes epithelial cell differentiation and also antagonizes Akt activation, were also evaluated.

Design: 211 patients with a pathologic diagnosis of NSCLC were identified in the registry of the UIC Cancer Center between 1999 and 2005. 140 of these were classified as Stage 3A or higher and thus eligible for the study. Of these only 26 met all eligibility criteria, which included the availability of diagnostic tissue and pre and post treatment CT scans. Archival material was reviewed to verify the presence and type of tumor. Additional sections were stained with pAkt(ser), PTEN and PTK6 and scored independently by 3 pathologists who were blinded to the chemoresponse outcome. A final score of positive or negative based on staining frequency and intensity was assigned for each marker. Response to chemotherapy was measured using the RECIST (Response Evaluation Criteria in Solid Tumors) guidelines. The measurements were done by a pulmonologist and a radiologist who were blinded to tissue biomarker status. Responders were defined as patients with Partial or Complete Response. Nonresponders were those with Stable Disease or Progression of Disease.

Results: There were 7 responders and 19 nonresponders. Of the responders 2 were positive and 5 were negative for pAkt overexpression. Of the nonresponders 3 were positive and 16 were negative. Of the 7 responders, 6 were positive and 1 was negative for PTEN. Of the nonresponders, 15 were positive and 4 were negative for PTEN. All responders were negative for PTK6. Of the nonresponders, 4 were positive, 14 were negative, and 1 had no remaining tissue in the section stained with PTK6. There were no significant relationships between chemoresponse and pAkt (p-value = 0.59), PTEN (p-value = 0.99), or PTK6 (p-value = 0.29) by Fishers Exact Test.

Conclusions: Although this study is limited by small sample size, the results suggest that Akt is likely not a suitable marker of chemoresponse. Larger studies of alternate biomarkers might be warranted.

1563 Evidence-Based Criteria To Distinguish Metastatic Breast Cancer from Primary Lung Neoplasms on Frozen Section

JB Herbst, RA Jenders, RJ McKenna, AM Marchevsky. Cedars-Sinai Medical Center, LA, CA; Cedars-Sinai Medical Center and University of California, Los Angeles.

Background: The distinction between primary lung carcinoma (PLC) from metastatic breast carcinoma (MBC) in patients with a prior history of breast cancer is very difficult at frozen section (FS). This information is used by thoracic surgeons to guide the extent of resection. Inaccurate diagnoses of PLC can lead to unnecessary lobectomies, while false negative diagnoses of MBC may require a second thoracotomy to complete a lobectomy after initial wedge resection.

Design: We performed a systematic review of the literature and reviewed our experience with 129 FS performed at our institution from patients with a lung nodule and a history of breast cancer from 1989-2006. They included 62 PLC and 38 MBC patients. The pre-test odds ratio (OR) of PLC to MBC was estimated and compared with those of other institutions. The presence of 12 histopathological features was assessed. The post-test OR of each feature present in PLC or MBC was estimated. Histopathological features that favored PLC or MBC were selected and post-test OR were calculated for each feature and combination of multiple features. The validity of significant evidence-based diagnostic criteria (EBDC) was tested with a group of 19 pathologists, including faculty, fellows and residents by administering two tests; the second test was performed after the participants learned the EBDC.

Results: The pre-test OR of PLC to MBC was 2.3 (OR range at other institutions:0-6.33). EBDC that favor the diagnosis of PLC include the presence of acini, lepidic growth pattern, nuclear pseudoinclusions and a central scar, with individual post-test OR of 34, 17, 14, and 11 respectively. EBDC that favor the diagnosis of MBC include the presence of comedonecrosis, solid nests, cribriform and trabecular architecture, with individual post-test OR (PLC/MBC) of 0.115, 0.0087, 0 and 0 respectively. The sensitivity and specificity of the EBDC was 85% and 100% respectively. The validity test showed a relative 15% improvement in diagnostic accuracy (p=0.0001) after the participants learned the EBDC.

Conclusions: The formal process of using an evidence-based probabilistic approach for the selection of diagnostic criteria and estimating the odds of favoring each diagnosis is helpful to identify EBDC that distinguish PLC from MBC by FS. These criteria are mostly helpful to distinguish MBC from patients with primary adenocarcinoma of the lung.

1564 Acquisition of Invasion in Lung Adenocarcinoma: Using Laser Capture Microdissection and Comparative Genomic Hybridization To Analyze Segmental Copy Number Changes of Chromosome 7

LC Herlitz, B Levy, O Nahum, JL Chen, CA Powell, AC Borczuk. Columbia Univ. Medical Center, New York, NY.

Background: The main histologic feature differentiating bronchioloalveolar carcinoma (BAC) from mixed subtype adenocarcinoma is the presence of tissue invasion. Acquisition of an invasive phenotype is critical in the progression of lung AdCa. Previous expression profiling of lung AdCa showed overabundance of differentially expressed genes located on chromosome 7q when comparing BAC and mixed AdCa (tumors containing both invasive and BAC components). We hypothesize that an important mechanism for gene expression changes in invasive tumors is copy number alterations of chromosome 7q loci.

Design: Frozen sections were used for PALM Zeiss laser-capture microdissection (LCM) to capture tumor cells, and Whole Genome Amplification (WGA) using the GenomePlex ® Whole Genome Amplification (Sigma Aldrich, St. Louis, MO) to obtain DNA. Comparative Genomic Hybridization (CGH) was performed on individual cases of 7 BAC and 5 mixed AdCa hybridized against normal diploid DNA. To confirm results from the individual CGH data, LCM obtained DNA from 9 BAC tumors were pooled and compared to 7 pooled mixed subtype AdCa. Pooled DNA samples were compared to normal diploid DNA and against each other. TaqMan RQ PCR for copy number was used to confirm the CGH results for chromosome 7 by targeting EGFR, TRRAP, FAM3C, and MET.

Results: CGH on 5 mixed subtype tumors showed segmental gains of chromosome 7q. None of the 7 BAC cases showed increase in 7q by individual CGH. In both the mixed AdCa and BAC tumors, gains of chromosome 7p were seen. Comparison of pooled BAC and mixed AdCa to diploid DNA showed similar gains in 7p in both, but a gain of 7q material in mixed AdCa and no change in 7q in the BAC pool. When compared head-to-head, the pooled BAC cases and pooled mixed AdCa cases showed no difference in 7p, while 7q regions were relatively increased in the mixed AdCa tumors. RQ-PCR confirmed the CGH findings.

Conclusions: Both BAC and mixed AdCa show increases in 7p; however, regions of 7q are increased in mixed AdCa but not in BAC tumors. Acquisition of an invasive phenotype correlates with the increase of genetic material on 7q. This confirms our hypothesis that the pattern of increased expression of 7q genes by gene expression profiling in mixed AdCa is accounted for by chromosomal changes. Detection of these chromosomal changes was aided by the use of LCM.

1565 The Extent of Sarcomatoid Component Is an Independent Predictor of Survival in Malignant Mesothelioma

MD Hofer, WC Foo, DJ Sugarbaker, JM Corson, LR Chirieac. Brigham and Women's Hospital, Boston, MA.

Background: Diffuse malignant mesothelioma (MM) is classified into epithelioid, sarcomatoid, and biphasic types which have prognostic value. The biphasic type has a mixture of epithelioid and sarcomatoid components, but the predictive value of their proportions has not been documented. We investigated the clinical significance of the percentage of the sarcomatoid component in biphasic MM.

Design: We evaluated 153 consecutive patients with epithelioid (n=106), biphasic (n=40), and sarcomatoid (n=7) diffuse MM of the pleura treated with extrapleural pneumonectomy from 1988 to 1997. The mean follow-up period after surgery was 25.4 months. The percentage of sarcomatoid component by light microscopy of the 40 biphasic MM specimens (average of 25.3 tumor-containing slides, range 11-79) was recorded by two pathologists. The average of the two readings of each case and the pathologic stage (TNM, Sugarbaker and Bouchard) were correlated with overall survival.

Results: The biphasic MM had a bimodal distribution: predominantly sarcomatoid (more than 50% sarcomatoid component, n=19) or predominantly epithelioid (less than 50% sarcomatoid component, n=21). The extent of sarcomatoid component was significantly associated with overall survival ($p<0.0001$). Patients with predominantly sarcomatoid biphasic MM have a similar survival as patients with monophasic sarcomatoid MM (10.4 and 9.1 months). Patients with predominantly epithelioid biphasic MM had a better survival than patients with predominantly sarcomatoid biphasic MM (17.5 months). Patients with monophasic epithelioid MM performed best with a median survival of 30.5 months. The difference in survival was statistically significant ($p<0.0001$). In a multivariate analysis, which included sex, age, and stage, the sarcomatoid component ($p<0.0001$), and age ($p=0.001$), remained as independent prognostic indicators of survival.

Conclusions: Our results indicate that the extent of the sarcomatoid component predicts overall survival in patients with biphasic MM. Patients with a sarcomatoid component greater than 50% have a similar survival to patients with monophasic sarcomatoid MM. In contrast, patients with predominantly epithelioid biphasic MM have a survival that is intermediate between monophasic epithelioid MM and biphasic sarcomatoid MM. Our data emphasize the importance of accurate histopathologic assessment of resected specimens in patients with MM treated with extrapleural pneumonectomy.

1566 Downregulation of GADD45α Protein Expression Is Associated with Advanced Tumor Stage and Decreased Survival in Squamous Cell Carcinoma of the Lung

SM Homan, CE Sheehan, JS Ross, TA Jennings. Albany Medical College, Albany, NY.

Background: Growth arrest and DNA damage-inducible protein a (GADD45α) has been shown to have a regulatory role in cell cycle control and genomic stability. In some solid tumors, its altered expression has been shown to correlate with poor prognosis

and resistance to DNA damaging agents such as radiation and platinin drugs. The expression pattern and prognostic significance of GADD45α in non-small cell lung cancers (NSCLC) has not been previously studied.
Design: Formalin-fixed, paraffin embedded sections from 115 NSCLC, including 44 squamous cell carcinomas (SCC), 43 adenocarcinomas (AC), and 28 bronchoalveolar carcinomas (BAC) were immunostained by automated methods (Ventana Medical Systems, Inc, Tucson, AZ) using rabbit polyclonal GADD45α antibody (sc-792; Santa Cruz Biotechnology, Santa Cruz, CA). Cytoplasmic immunoreactivity was semiquantitatively assessed based on staining intensity and distribution in all tumors and the results were correlated with histologic and prognostic variables.
Results: Overall, intense cytoplasmic GADD45α immunoreactivity was noted in the adjacent benign bronchial epithelium, while the benign alveolar epithelium was more variable. Decreased expression of GADD45α was observed in 69/115 (60%) NSCLC, including 66% SCC, 51% AC, and 64% BAC. Within SCCs, decreased cytoplasmic expression correlated with advanced tumor stage, with 100% advanced stage vs. 61% early stage tumors showing loss of GADD45α immunoreactivity (p=0.045). In addition, downregulation was noted in 100% of cases in which death occurred within one year of diagnosis and 78% within 5 years vs. 44% of cases of death beyond 5 years (p=0.021). In ACs, while immunoreactivity was decreased in 100% Stage IV tumors, correlation with tumor stage did not reach statistical significance. Cytoplasmic immunoreactivity was not significantly associated with other prognostic factors investigated. On multivariate analysis, only tumor stage independently predicted disease related death.
Conclusions: Expression of GADD45α is significantly decreased in NSCLC compared with adjacent non-neoplastic bronchial epithelium. Those SCCs of higher stage as well as those with decreased survival show a significant downregulation of GADD45α protein expression. These results provide evidence for an association between altered GADD45α expression and tumorigenesis and suggest a correlation between decreased cytoplasmic expression and poor prognosis.

1567 Expression of Phosphorylated AKT in Non Small Cell Lung Cancers

SM Homan, RA Patel, CE Sheehan, JS Ross, TA Jennings. Albany Medical College, Albany, NY.
Background: Akt is a serine/threonine protein kinase that plays a critical role in the regulation of cell growth and survival. When activated by a variety of signal factors, phosphorylated Akt (p-Akt) stimulates cell growth and antagonizes apoptotic pathways through subsequent phosphorylation of multiple downstream targets. Altered p-Akt expression has been described in a variety of human cancers, but its potential roles in NSCLC development and progression have not been previously studied.
Design: Formalin-fixed, paraffin embedded sections from 122 NSCLC, including 45 squamous cell carcinomas (SCC), 46 adenocarcinomas (AC), and 31 bronchioloalveolar carcinomas (BAC) including both pure BACs and adenocarcinomas with BAC features, were immunostained by automated methods (Ventana Medical Systems, Inc, Tucson, AZ) with a monoclonal antibody to phosphorylated Akt (Phospho-Akt (Ser 473), Cell Signaling Technology, Inc, Danvers, MA). The staining pattern was semiquantitatively assessed in both the tumor and adjacent benign lung tissue and increased expression of p-Akt was defined as greater than 25 % staining. Expression was correlated with histologic and prognostic variables, including gender, tumor stage, tumor grade, tumor size, lymph node status, recurrence rate, and survival.
Results: Cytoplasmic staining for p-Akt was increased in 33% of the NSCLCs as compared to adjacent benign lung which showed less than or equal to 25% staining in 98% of cases. There was a significant difference in p-Akt expression between tumor types with increased p-Akt expression in 42% of SCCs, 20% of ACs, and 39% of BACs (p=0.05). Of the cases of SCC, 100% of the low grade tumors showed an increase in p-Akt staining as compared to 38% of the high grade tumors (p=0.036). Furthermore, 100% of the ACs with increased p-Akt staining were of low clinical stage (p=0.039). Increased p-Akt was not significantly associated with other prognostic factors investigated.
Conclusions: Phosphorylated-Akt expression is significantly increased in NSCLC as compared with normal lung tissue and is associated with the NSCLC tumor subtype. Interestingly, tumors that showed an elevation in cytoplasmic p-Akt, were most consistently low grade tumors. The differential expression of activated Akt between low and high grade/stage tumors indicates a potential role for p-Akt in NSCLC tumorigenesis and prognosis and warrants further study.

1568 Deletion of Gene for Surfactant Protein A Correlates with Over-Expression of EGFR, C-MYC, 6p11-q11and Polysomy 10/10q22-23 in Non-Small Cell Lung Cancer (NSCLC). A FISH Based Study of 45 Patients

RL Katz, T Zaidi, R Fernandez, A Khanna, M Spitz, A Vaporciyan, NP Caraway. UT M.D. Anderson Cancer Center, Houston, TX.
Background: Thyroid transcription factor 1 (TTF1) is implicated in the regulation of surfactant gene expression. Loss of TTF1 is associated with an aggressive subtype of adenocarcinoma (AC) of lung. Non-small cell lung cancers (NSCLC) with deletion of 10q22-23 which codes for surfactant A protein (SP-A), are associated with high stage and poor survival. We investigated loss of SP-A expression in relationship to aberrations of centromeres of chromosome 6,10, and locus specific probes for EGFR (7p), 5p, and c-MYC (8q24) and 10q22-23.
Design: From 45 resected NSCLCs, histologically classified as adenocarcinoma (30) and squamous carcinoma (15), touch imprints were prepared. Patients were staged as: Stages 1, (23),II, (7), III (8), IV, (7). Fluorescence in situ hybridization (FISH) was performed for centromeric 10/10q22-23 using in-house probes and a commercial 4-color probe set comprising 5p15, 6p11-q11, 7p12 and 8q24.(LaVysion, Downer's Grove, Il) on 100 representative tumor cells. Deletions and polysomies of 10q were scored relative to centromeric 10, while the average number of FISH signals per probe per cell was scored for the 4-color probe set on 100 tumor cells. Pearson's correlations and T-test for adeno-versus squamous carcinoma were performed.
Results: Considering all histologies deletion of SP-A was significantly correlated with polysomies of centromeric 10, 10q22-23, 6p11-q11, EGFR and c-MYC. (p= 0.000, 0.000, 0.000, 0.014, 0.001 and 0.009). Adenocarcinoma demonstrated higher percentages of deletions of SP-A and polysomies of 10 and 10q22-23 compared to squamous carcinoma however these values were not significant. There was no significant difference between values for 5p15, 6p11-q11, 7p12 and8q24 for adeno- and squamous carcinoma.
Conclusions: Loss of the gene for SP-A is strongly correlated with overexpression of a number of genes and chromosomes, including c-MYC, and EGFR, which drive cell cycle events and proliferation. Thus SP-A may be function as a tumor suppressor gene.

1569 D2-40, Mesothelin, Podoplanin and Other Mesothelioma-Related Markers in Thymic Tumors: A Tissue Microarray Analysis of 57 Cases

T Khoury, A Arshad, M Arnouk, D Tan. Roswell Park Cancer Institute, Buffalo, NY; MD Anderson, Houston, TX.
Background: Thymic tumors are anterior mediastinal neoplasms. Although the histology of mesothelioma and thymic tumors are quite different, there are occasions when such differential could be raised, particularly in thymic carcinoma (type C) and thymoma type B3. Although previous studies have examined mesothelioma-related markers in thymic tumors, recent markers have emerged and need to be examined as well.
Design: We examined the expression of D2-40, Mesothelin, Podoplanin, Calretinin, CK5/6, WT1, Thrombomodulin, and HBME-1 in 57 thymic tumors. The tumors were classified according to the World Health Organization scheme. Tumor representatives along with 5 normal thymuses were constructed in 2 TMA blocks. The stains were scored based on staining intensity from 0 to 3. Score of 1 and above was considered positive.
Results: Histologically, there were 5 type A, 13 type AB, 7 type B1, 5 type B2, 19 type B3, and 8 type C tumors. For type C, all cases were of squamous cell carcinoma type. The above listed antibodies showed expression in 17 (29.84%), 10 (17.54%), 4 (7.01%), 1 (1.75%), 42 (73.68%), 0 (0%), 7 (12.28%), and 11 (19.29%) tumors, respectively. Only Mesothelin had differential expression when compared to histologic type. While it was negative in thymomas type A, AB, B1, B2 and B3, Mesothelin was positive in 4 of 8 type C thymic tumors (p<0.001). D2-40, Podoplanin and HBME-1 were expressed in the epithelial component of all 5 normal thymuses.
Conclusions: Mesothelioma markers including D2-40 are variably expressed in thymic tumors. Therefore, when a question of differential diagnosis between mesothelioma and thymic tumor is raised, these stains should be interpreted with caution.

	Total (n=57)	A, AB, B1, B2 (n=30)	B3 (n=19)	C (n=8)
D2-40	17 (29.84%)	8 (26.7%)	8 (42.1%)	1 (12.5%)
Podoplanin	10 (17.54%)	4 (13.3%)	4 (21.05%)	2 (25%)
Mesothelin	4 (7.01%)	0 (0%)	0 (0%)	4 (50%)
Calretinin	1 (1.75%)	0 (0%)	0 (0%)	1 (12.5%)
CK 5/6	42 (73.68%)	21 (70.0%)	18 (94.73%)	3 (37.5%)
WT1	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Thrombomodulin	7 (12.28%)	3 (10.0%)	2 (10.52%)	2 (25%)
HBME-1	11 (19.29%)	8 (26.7%)	2 (10.52%)	1 (12.5%)

1570 Mutation and Intron 1 Polymorphism of the Epidermal Growth Factor Receptor, and Its Relationship with Gefitinib Sensitivity in Korean Patients with Non-Small Cell Lung Cancer

MJ Kim, SH Seok, KC Shin, KH Lee. Yeungnam University Hospital, Daegu, Republic of Korea.
Background: The epidermal growth factor receptor (EGFR) mutations are predictors of response to treatment with EGFR tyrosine kinase inhibitors (TKI). Despite initial responses, some patients with EGFR TKIs eventually develop recurrent disease resistant to further TKI therapy. Therefore, it has been suggested that other genetic alteration may occur independently of EGFR mutations. Currently, EFGR intron 1 polymorphism has been found to have therapeutic implications. However, its clinical significance related to gifitinib responsiveness is still controvertial, at present. We examined EGFR CA repeat polymorphism in intron 1 of EGFR gene and its relationship to EGFR gene mutation in non-samll cell lung cancer (NSCLC) patients treated with gefitinib.
Design: EGFR mutation was analyzed for 240 samples of NSCLC including squamous cell carcinoma (n=116), adenocarcinomas (n=111), and other non-small cell carcinoma (n=7). All specimens were taken by bronchoscopic or needle biopsy. For 85 patients treated with gefitinib, 56 cases were analyzed for EGFR mutations and its correlation with CA repeat polymorphism in intron 1 of EGFR and clinical outcome including responsiveness, time to progression (TTP).
Results: Total 49 patients (20%) harbored EGFR mutations including 36 cases (32%) of adenocarcinomas, 13 cases of squamous cell carcinomas (11%), and 2 (29%) of other carcinomas. EGFR mutation was significantly associated with adenocarcinoma (p=0.003), female sex (p=0.005), and non-smokers (p=0.038) and correlated with longer TTP (p=0.047). In a subgroup of 85 patients treated with gefitinib, CA repeat performed in 56 cases was low in 22 (40%) and high in 33 (60%) patients. EFGR intron 1 polymorphism had no relationship with EGFR mutational status (32% (7/22) in low vs. 30% (10/33) in high, p=0.905). Low repeat patients showed a trend of longer TTP than high CA repeat, but its relationship was not significant (p>0.05).
Conclusions: Our data further support the importance of EGFR mutation with regard to a distinct clinical profile and prognostic implication for NSCLC patients. This study suggests that intron1 CA repeat polymorphism of EGFR gene confers no significant clinical outcome on NSCLC patients treated with gefinitib. In addition, CA repeat status was not correlated with EGFR mutation.

1571 Immunohistochemical Expression of Signaling Molecules Erb-B3, MKP-3, and Stat1 in Non-Small Cell Lung Carcinoma

SM Kulich, PT Cagle, EM Cozza, SR Land, D Lenzner, L Brcic, S Dacic. VA Pittsburgh Healthcare System, Pittsburgh; University of Pittsburgh Medical Center, Pittsburgh; The Methodist Hospital, Houston; University of Pittsburgh Graduate School of Public Health, Pittsburgh; University of Zagreb School of Medicine, Zagreb, Croatia.

Background: Despite improved treatment strategies, lung cancer is still the leading cause of cancer death worldwide. Recently, a gene expression profiling study of lung carcinoma in an Asian population identified a 5-gene signature associated with survival. The aim of this study was to characterize the expression of a subset of these gene products using immunohistochemistry in a large series of non-small cell lung carcinoma (NSCLC) arising in Western patients in an attempt to validate the gene expression data at an immunohistochemical level.

Design: High density tissue microarrays were constructed from paraffin-embedded, formalin fixed 347 NSCLC (187 adenocarcinomas, 90 squamous cell carcinomas, 70 large cell carcinomas). AJCC stages included in the study included 226 stage 1, 57 stage 2, 42 stage 3 and 22 stage 4. Standard immunohistochemistry for Erb-B3, MKP-3, and Stat1 was performed. Each core was evaluated independently by 2 pathologists for percentage of cells (in 10% increments) and intensity of staining (four point scale, 0-3) in tumor cells. Similarly, expression of Stat1 was evaluated in tumor stromal cells.

Results: Percentage and intensity of expression of Erb-B3 ($p<0.001$), MKP-3 ($p<0.0001$) and Stat1 ($p=0.027$) was associated with tumor histology with the expression of all 3 markers being more frequently associated with adenocarcinoma. Percentage and intensity of expression of Erb-B3 and percentage of tumor cells expressing MKP-3 significantly correlated with advanced tumor stage. The percentage of stromal cells expressing Stat1 significantly correlated with adenocarcinoma histology and advanced stage.

Conclusions: Our preliminary data confirms the previously reported prognostic significance of expression of Erb-B3 and MKP-3 signaling molecules at an immunohistochemical level in cases of NSCLC arising within the Western hemisphere. In addition, our observation that expression of Stat1 in both the malignant epithelial and non-neoplastic stromal component of NSCLC appears to be of prognostic significance and highlights the importance of studying tumor-stromal interactions in understanding the biology of NSCLC.

1572 Alpha-methylacyl-co-a-racemase (AMACR) Expression Is a Biomarker of Poor Prognosis in Stage 1 and 2 Large Cell Carcinomas of the Lung

UR Kundu, W Li, D Tan, TC Allen, QJ Zhai, PT Cagle. University of Texas, Houston; MD Anderson Cancer Center, Houston; University of Texas, Tyler; Methodist Hospital/ Weill College of Medicine Cornell University, Houston.

Background: Alpha-methylacyl-co-a-racemase (AMACR) is an enzyme with increased expression in certain cancers where it is proposed to predict prognosis. Biomarkers of this type are potential targets for diagnostic/therapeutic purposes and may stratify patients into predicted outcomes prior to entry into new clinical trials. Lung cancer is the number one cause of cancer deaths in the industrialized world with little improvement in survival. Early stage non-small cell lung cancers (NSCLC) are presumably cured by resection, yet half of these patients will die. We describe AMACR expression in the first large series of NSCLC and its utility in predicting prognosis of patients with early stage NSCLC for use as a biomarker in clinical trials.

Design: Sections of tissue microarrays with three 1 mm punches of 240 NSCLCs (113 adenocarcinomas, 76 squamous cell carcinomas, and 51 large cell carcinomas) were immunostained with an anti-racemase antibody from DAKO (dilution 1:100). AMACR nuclear/cytoplasmic expression was graded 0 to 3 and averaged in each case. A score of 0 or 1 was weak expression and a score of 2 or 3 was strong expression. 5-year survival data for patients with stage 1 and 2 disease was obtained from medical records. AMACR expression was correlated to 5-year survival rates using Winstat software for Kaplan-Meier curves.

Results: Nuclear and/or cytoplasmic AMACR expression was seen in 69/76 squamous cell carcinomas (91%), 72/113 adenocarcinomas (64%), and 28/51 large cell carcinomas (55%). Strong AMACR nuclear expression was associated with decreased 5-year survival in large cell carcinomas and correlated with higher tumor grade ($p<0.05$). Significant correlation with cytoplasmic staining was not seen. Correlation of AMACR nuclear or cytoplasmic expression with survival was not seen in other tumor types.

Conclusions: AMACR expression in poorly differentiated large cell carcinomas of the lung is statistically associated with poor 5-year survival in early stage patients with prior presumably curative resection. In the future, AMACR expression may serve in stratifying these patients into a category of increased risk of death from their cancers despite resection. Furthermore, increased AMACR expression in lung cancers may serve as a target for molecular radiology and therapy.

1573 Pulmonary Pathology Following Bone Marrow Transplantation

AR Laury, JS Truell, CK Lai, MC Fishbein. Brigham and Women's Hospital, Boston, MA; David Geffen School of Medicine at UCLA, Los Angeles, CA.

Background: Autologous or allogeneic stem cell transplantation (SCT) is used to treat malignancies, and congenital immunodeficiencies. Respiratory failure is a common, serious, and often fatal consequence, due to neoplasm, infection, radiation injury and/or drug toxicity. Each of these complications requires different treatment.

Design: We reviewed medical records and all microscopic slides of lung tissue from 87 patients who had an autopsy performed and 46 patients who had lung biopsies from 1995 to 2007 after undergoing SCT. Death was 1-1500 days after SCT (mean=105, median=56). Lung biopsies were obtained 19-912 days after SCT (mean=274, median=158). Of the autopsied patients, 79 were transplanted for lymphoma, leukemia, or myelodysplasia; 5 for solid organ malignancies, and 3 for severe combined immunodeficiency. Of those that were biopsied 40 were transplanted for lymphoma, leukemia, or myelodysplasia, 5 for solid organ malignancies, and 1 for severe combined immunodeficiency. The autopsy group ranged from 6 months to 64 years old (mean=33, median=37); the biopsy group from 6 months to 69 years (mean=40, median=44).

Results: The most common findings in biopsies were interstitial fibrosis/ pneumonitis, probably related to chemotherapy and/or irradiation (32%), followed by normal lung (30%), organizing pneumonia (20%), and diffuse alveolar damage (DAD)(16%). At autopsy 56% of the patients had alveolar hemorrhage, 50% had DAD, and 33% had pneumonia, with an identifiable organism present in 84%. Interstitial fibrosis/ pneumonitis was seen in 10%. Thirty-two percent of patients had clinically unexpected diagnoses at the time of biopsy; 35% at autopsy. Six patients had recurrent malignancy in the lung tissue, and one patient had a new, unexpected malignancy. In autopsy specimens, similar pathologic findings with similar incidence were found early (<60days) and late (>60 days); however, DAD, alveolar hemorrhage and infectious diagnoses were more common in biopsies within 60 days of SCT.

Conclusions: Significant pulmonary disease is common after SCT. In the majority of cases, examination of lung tissue provides diagnostic information that can guide therapy. Moreover, given the frequency of unexpected findings, early surgical biopsy could direct therapy to prolong survival in SCT patients.

1574 Epidermal Growth Factor Receptor Expression and Gene Amplification in Non-Small-Cell Lung Carcinomas: Correlation between Gene Copy Number and Protein Expression

HJ Lee, JH Chung, G Choe, S Jheon, SW Sung, CT Lee. Seoul National University College of Medicine, Seoul National University Bundang Hospital, Seongnam, Gyeonggi-do, Republic of Korea.

Background: Progress in lung cancer biology led to the identification of the epidermal growth factor receptor (EGFR) signaling pathway as a therapeutic target leading to the development of tyrosine kinase inhibitors (TKIs).

Design: We determined EGFR protein overexpression by immunohistochemical analysis and EGFR gene amplification by fluorescence in situ hybridization (FISH) in 132 formalin-fixed, paraffin-embedded lung tumors including squamous cell carcinoma (SCC; 68 patients), adenocarcinoma (ADC; 50 patients) and other non-small cell carcinoma (14 patients). Protein expression was assessed by immunohistochemistry and gene copy number was evaluated by fluorescent in situ hybridization (FISH).

Results: EGFR protein overexpression was observed in 67% of the NSCLC, more frequently in SCC than ADC (75% vs 44% $p<.001$). There were no statistical diffrences in EGFR expression with respect to sex, smoking history, BAC feature, nodal status or stage. EGFR FISH-positivity as represented by high polysomy and gene amplification was observed in 33.2% of the NSCLC patients. The FISH patterns were disomy (29.6%) and low polysomy (37.2%), high polysomy (21.9%) and gene amplification (11.4%). Protein expression levels significantly correlated with the gene copy number per tumor cell ($p=0.000$). The proportions of EGFR-expressing tumors according to the gene copy status were: 90% of gene amplified cases, 75% of high polysomy, 40% of low polysomy, and 25% of disomy cases.

Conclusions: EGFR overexpression or high gene copy numbers had no significant influence on tumor recurrence. EGFR overexpression is frequent in NSCLC, is most prominent in SCC, and correlates with increased gene copy number per cell. Additional studies are needed to determine whether EGFR gene amplification or protein expression bears any informative value in predicting response to EGFR inhibitor therapy.

1575 Overexpression of NRF2 Is Correlated with Elevated p53 Levels in Pulmonary Papillary Adenocarcinoma by Tissue Microarray Analysis

QK Li, E Tully, FB Askin, E Gabrielson. Johns Hopkins Hospitals, Baltimore, MD.

Background: NRF2 is a transcription factor that regulates genes encoding antioxidants, xenobiotic detoxification enzymes, and drug efflux pumps. Elevated level of NRF2 are related to cancer cell survival and potential protection against chemotherapeutic agents in non-small cell lung cancers (NSCLC). Loss of KEAP1 activity leads to overexpression of NRF2 in some the cancer cells. The function and mechanism of NRF2 expression are not well understood. True Papillary Adenocarcinoma (PA) is a recently recognized subtype of pulmonary adenocarcinoma with poor prognosis and response to chemotherapeutic agents. We investigated the expression of NRF2 and its potential role in progression of PA. Furthermore, loss of p53 pathway function commonly occurs in NSCLC and contributes to aggressive tumor behavior. We also investigated the potential relationship between NRF2 and p53 expression in PA.

Design: We searched the files of the Johns Hopkins Hospital for True Pulmonary Papillary Adenocarcinoma from 1981 to 2007. The diagnostic criteria were established by Silver and Askin. A total of 58 cases were found. Immunohistochemistry (IHC) studies on tissue microarrays used a goat antibody against human p53 peptide at 1:50 dilution; and a rabbit antibody against human NRF2 peptide at 1:250 dilution. IHC was scored in both nuclei and cytoplasm as follows: 0, undetectable; 1+, weakly positive; 2+, moderately positive; and 3+, intensely positive.

Results: Patients ranged in age from 45 to 84 years old. Among them, 72.4% were smokers. The M:F ratio was 1:0.8. Tumors ranged from 1.2 to 9 cm and pathologic stage ranged from T1 to T4. IHC demonstrated: (1) prominent NRF2 and p53 staining (3+) in both the nucleus and cytoplasm of all cancer cells; (2) expression of NRF2 was independent of tumor size and stage; (3) overexpression of NRF2 correlates with expression of p53.

Conclusions: Both NRF2 and p53 are strongly expressed in PA cancer cells. Overexpression of NRF2 correlates with p53 expression and is independent of tumor size and stage. Overexpression of NRF2 by virtually all the cancer cells in PA differs from results in other NSCLC and may relate to poor prognosis and chemotherapeutic resistance in PA patients. NRF2 expression in PA may be regulated by KEAP1-independent pathway, in contrast to previous studies. p53 pathway may play an important role. The mechanism of regulation of NRF2 may involve several different signaling pathways and further studies may help us to understand chemotherapeutic resistance in cancer patients.

1576 Primary Mediastinal Germ Cell Tumors with Somatic Transformation to Angiosarcoma: A Report of 9 Cases

A Luiña Contreras, P Tamboli, BA Czerniak, C Guo. UT MD Anderson Cancer Center, Houston, TX.

Background: Germ cell tumors (GCT) occasionally undergo somatic transformation to a sarcoma. Angiosarcoma is a rarely observed phenotype in these transformations. Here we report the clinicopathological features of primary mediastinal GCT with transformation to angiosarcoma.

Design: We identified 9 patients treated at our center (between 1987 and 2002), who had GCT with transformation to angiosarcoma. All relevant clinicopathological data, including follow-up were reviewed. Immunohistochemical stains were performed in 6 of 9 tumors.

Results: All 9 patients were men, with a mean age of 36.3 years (range: 26-43 years). All patients had primary GCT of the mediastinum. Secondary mediastinal involvement from a testicular GCT was excluded clinically. 3/9 cases had angiosarcoma transformation in a pure teratoma. The other 6 cases were mixed GCT with teratoma, and other GCT types including seminoma (3), mixture of embryonal carcinoma and yolk sac tumor (2) and embryonal carcinoma (1). 3/9 tumors also showed other types of somatic transformation, including rhabdomyosarcoma (n=2) and leiomyosarcoma (n=1). The angiosarcoma component was characterized by irregular vascular spaces lined by highly atypical cells. In 6/9 tumors, the vascular nature of these atypical cells was confirmed using immunohistochemical stains for CD31, CD34 or Factor VIII. Follow-up was available for 8 patients with a mean follow up of 33.9 months (median: 28.5; range: 20-64). All 8 patients received chemotherapy, and 3 received additional radiation therapy. Five patients died of disease (mean disease specific survival: 31.4 months, median: 27; range: 21-49). Of the three remaining patients, one patient developed distant metastasis 20 months after diagnosis, and two were alive with no evidence of disease at 30 and 64 months, respectively.

Conclusions: Angiosarcoma arising in a teratoma is a rare occurrence and is associated with a poor prognosis. It is essential for pathologists to be aware of this transformation as it has important ramifications for patient management. Angiosarcoma may not respond to conventional chemotherapy regimens used against GCT, and requires a sarcoma specific chemotherapy regimen.

1577 Prognostic Value of Aspartyl (Asparaginyl) $^{b-}$ Hydroxylase (AAH) in Lung Carcinomas: Correlation with Survival

M Luu, E Sabo, M Resnick, W Greaves, S de la Monte, JY Wang, R Tavares, L Simao, J Wands, LJ Wang. Rhode Island Hospital and The Warren Alpert Medical School of Brown University, Providence, RI; Liver Research Center, Rhode Island Hospital and The Warren Alpert Medical School of Brown University, Providence, RI; Rhode Island Hospital and The Warren Alpert Medical School of Brown University.

Background: Aspartyl (asparaginyl) $^{b-}$ hydroxylase (AAH) is a dioxygenase that catalyzes asparaginyl residues present in epidermal growth factor like domains of certain proteins. AAH is over-expressed in various malignant neoplasms including those of the liver, stomach and pancreas. Studies have demonstrated that AAH plays an important role in regulating invasion and metastasis of these neoplasms and that the levels of AAH expression correlate with an aggressive tumor phenotype and poor prognosis. The present study characterizes the prognostic significance of AAH expression in different sub-types of lung carcinomas.

Design: Tissue microarrays (TMAs) were created from archival paraffin embedded tissue samples from 375 patients with lung carcinomas, including 213 adenocarcinomas (176 adenocarcinomas, and 37 bronchioloalveolar carcinomas), 113 squamous cell carcinomas and 49 large cell carcinomas. Sections were stained immunohistochemically with a mouse monoclonal antibody (FB-50) directed against the human AAH protein and the results were subjected to uni and multivariate survival analysis with adjustments for age, stage and treatment status.

Results: Strong FB50 staining intensity was more common in adenocarcinomas (bronchioloalveolar 31% and non-bronchioloalveolar 28%), as compared to squamous cell carcinomas (10%),(p=0.002, and p=0.001, respectively) and to large cell carcinomas (10%),(p=0.008, and p=0.007, respectively). Univariate survival analysis revealed that FB50 expression inversely correlated with patient survival in bronchioloalveolar carcinoma (p=0.02) and squamous cell carcinoma (p=0.004) groups, with a strong trend in the large cell carcinoma group (p=0.057). In squamous cell carcinomas, FB50 expression was an independent predictor of survival, along with the stage and the tumor size (p=0.025, p=0.029 and p=0.0001, respectively by Cox test).

Conclusions: Our results indicate that among lung carcinomas, expression of FB-50 is associated with a poor prognosis for bronchioloalveolar and squamous cell carcinoma sub-types, with a strong trend for the large cell carcinoma type.

1578 RON Overexpression and Activation in Primary and Metastatic Lung Carcinoma

A Malik, S Krishnaswamy, C Gong, AN Husain, R Salgia, M Tretiakova. University of Chicago, Chicago, IL.

Background: RON is a member of MET proto-oncogene family of tyrosine kinase receptors. RON is involved in cell motility, adhesion, invasion, and inhibition of apoptosis. In vitro and mRNA studies of RON gene indicated its role in progression and metastasis of various epithelial tumors including lung cancer. However, this data has not been confirmed at the protein level. The purpose of the present study was to characterize RON expression and phosphorylation by immunohistochemistry in lung cancer.

Design: 175 cases of primary and metastatic lung cancer were studied with institutional IRB approval on tissue microarrays which were immunostained using RON and phospho-RON (p-RON) antibodies. TMA layout was designed to include tumor center, advancing edge and surrounding normal tissue for every case. Each marker was evaluated by two pathologists for staining localization and intensity using 3-grade system (negative, weak, or strong), and correlated with stage.

Results: RON and p-RON tumor overexpression was observed in the majority of lung cancer cases across all subtypes. Ron exhibited cytoplasmic positivity, whereas p-RON was found mainly in the nucleus. Predominance of strong staining for RON among all positive cases was found in small cell, large cell, and metastatic lung cancers. From cases with high RON positivity 61.5-67% were from high stage tumors. There was a substantial positive correlation between RON and p-RON expression in all tumor types (table).

Histologic type	RON expression			p-Ron expression			Correlation
	0	1+	2+	0	1+	2+	r
Squamous cell carcinoma (N=25)	8%	54%	38%	22%	48%	30%	0.55
Small cell carcinoma (N=30)	10%	19%	71%	14%	38%	48%	0.75
Large cell carcinoma (N=35)	15%	26%	59%	33%	36%	31%	0.73
Adenocarcinoma (N=46)	27%	38%	35%	39%	44%	17%	0.72
Metastatic lung cancer to brain (N=21)	20%	10%	70%	52%	43%	5%	0.66
Metastatic lung cancer to LN (N=18)	15%	27%	58%	45%	41%	14%	0.75

Conclusions: RON is widely expressed and constitutively phosphorylated in all histotypes of lung cancer with a potential role as a novel therapeutic target. We found positive correlation between RON/p-RON expression levels, higher tumor stage and more regional spread of the tumor suggesting that RON is likely involved with a more malignant state through its proliferative, motile and morphological effects. It therefore provides prognostic information and helps to identify patients who require adjunctive therapy for small cell cancer and more extensive resection for non small cell cancer patients.

1579 Evidence-Based Pathology and the Pathologic Evaluation of Thymomas: The World Health Organization Classification Can Be Simplified into Only 3 Categories Other Than Thymic Carcinoma

A Marchevsky, R Gupta, RJ McKenna, M Wick, C Moran, MF Zakowski, S Suster. Cedars-Sinai Medical Center, Los Angeles, CA; Virginia Medical Health System, Charlottesville, VA; Pathology MD Anderson Cancer Center, Houston, TX; Memorial Sloan-Kettering Cancer Center, New York, NY; Wisconsin Medical Center, Madison, WI.

Background: The clinical validity and applicability of the WHO histopathological classification of thymomas ("classification") has been questioned. Evidence based pathology promotes the use of systematic reviews and analysis of data with meta-analysis rather than subjective reviews of the literature.

Design: We performed a review of the English literature from 1999-present to identify "best evidence" regarding the use of the "classification". The data was analyzed with meta-analysis software (Biostat Inc Englewood, N.J.).

Results: Only level-three evidence published in retrospective cases series is currently available regarding the use of the "classification". Meta-analysis demonstrates that only 3 WHO categories of thymomas are associated with significant survival differences: A/AB/B1, B2 and B3. It also shows significant heterogeneity in the results published in different studies. There is no current evidence to determine whether thymoma types are significant prognostic features for patients previously stratified by stage.

Conclusions: There is a lack of randomized clinical trials evaluating the prognosis of patients with thymoma and the effects of various treatment modalities. The W.H.O classification of thymomas needs revision and could probably be simplified into fewer classes with significant prognostic value. Future studies are needed to evaluate the prognostic and/or predictive value for thymoma patients previously stratified by stage. The latter information is important to help select patients that may benefit from neo-adjuvant chemotherapy or post-operative radiation therapy and other modalities.

1580 EGFR Molecular and Phenotypic Evaluation in Patients with Pulmonary Adenocarcinoma: A Comparison of Different Methodologies and a Proposal for an Algorithmic Approach to EGFR Testing

AM Marchevsky, R Gupta, AM Dastane, F Forozan, C Riley, J Lopategui. Cedars Sinai Medical Center, LA, CA.

Background: Detection of EGFR overexpression (EGFR-O) by immunohistochemistry (IHC), EGFR-O or polysomy (EGFR-P) by fluorescent in-situ hybridization (FISH) and EGFR point mutations and deletions (EGFR-M) by polymerase chain reaction (PCR) have been proposed as predictive tests of response to gefitinib (Iressa^{R}) or erlotinib (Tarceva^{R}) therapy in patients with pulmonary adenocarcinoma (AC). It is controversial whether all of these tests predict a clinical response to therapy. There have been few studies comparing the results of EGFR testing with different methodologies and there are no guidelines regarding which test is valuable in clinical practice.

Design: We reviewed our experience in 67 patients with AC tested for EGFR-O, EGFR-P and EGFR-M with IHC, FISH and/or PCR. Membrane immunoreactivity in greater than 20% of tumor cells was scored as a positive reaction. FISH was interpreted as amplified when the ratio of positive signal:CEP was greater than 2 and polysomy was identified when more than 40% nuclei had more than 4 gene copies. PCR was performed for deletion in exon 19 and PCR and restriction fragment length polymorphism (RFLP) were used to detect point mutations in exon 21 (L858R). Thirty-two of the cases were tested with all 3 methods, while the other 35 tumors were tested only with IHC and PCR. We compared the percentage of positive test results by the 3 tests and calculated the negative predictive value of each test using FISH results as "true positive or negative".

Results: The following table summarizes the percentage of cases that were positive and negative with each methodology.

Results

	IHC	PCR	FISH
% positive cases	66.2	19.1	43.8
Negative predictive value of positive FISH test	94.7	66.7	NA

IHC yielded the best negative predictive value (94.7%) of all 3 tests. Only 1 of 14 FISH positive and none of the 13 PCR positive cases was negative by IHC. Five of the 6 cases that expressed EGFR-M by PCR were FISH positive.

Conclusions: IHC provides an excellent negative predictive value for EGFR-O detection. None of the IHC negative cases expressed EGFR- M by PCR. An algorithm

that uses IHC to screen cases for further EGFR phenotypic and molecular analysis with FISH and PCR is proposed. The need for future studies that compare the results of EGFR testing with the 3 methods in larger number of cases and with response to therapy rates is discussed.

1581 Differences of Fibroblastic Foci of Usual Interstitial Pneumonia (UIP) and Intraalveolar Buds of Chronic Organizing Pneumonia (COP)/ Bronchiolitis Obliterans Organizing Pneumonia (BOOP): Comparison of the Expression of Matrix Metalloproteinases (MMPs) and Tissue Inhibitors of Matrix Metalloproteinases (TIMPs)

O Matsubara, N Imazeki, H Tsuda, Y Nakatani, EJ Mark. National Defense Medical College, Tokorozawa, Saitama, Japan; Chiba University, Chiba, Japan; Massachusetts General Hospital and Harvard Medical School, Boston, MA.

Background: Fibroblastic foci and profusion of fibroblastic foci contribute to irreversible fibrosis in usual interstitial pneumonia (UIP) and are correlated with increased mortality. On the other hand, the polypoid granulation tissue plug (intraalveolar bud or Masson's body) is one of the pathologic characteristics in chronic organizing pneumonia (COP)/ bronchiolitis obliterans organizing pneumonia (BOOP) but is not related to UIP. We compared the quantitative expression of various matrix metalloproteinases (MMPs) and tissue inhibitors of matrix metalloproteinases (TIMPs) in UIP and COP/BOOP.

Design: Immunostaining for MMP-1, MMP-2, MMP-3, MMP-7, MMP-9, TIMP-1, TIMP-2, transforming growth factor (TGF) beta-1, connective tissue growth factor (CTGF) and Ki-67 was carried out in paraffin embedded sections of lung from 12 video-assisted thoracoscopic surgery (VATS) biopsies with UIP and from 10 VATS biopsies with COP/BOOP using a standard indirect avidin-biotin horseradish peroxidase method with various antigen retrieval methods.

Results: MMPs and TIMPs expression was observed in regenerating pneumocytes, bronchiolar epithelium and inflammatory cells including macrophages, and rarely in the myofibroblastic cells in UIP and COP/BOOP, and was rarely observed in controls. MMP-1, MMP-3, MMP-9, TIMP-1 and TIMP-2 expression in the fibroblastic foci in UIP was greater than in the intraalveolar buds in COP/BOOP. Ki-67 expression was almost similar in both groups. TGF beta-1 and CTGF were expressed strongly in fibroblastic foci but faintly in the intraalveolar buds.

Conclusions: Differences in expression of MMPs and TIMPs in the fibroblastic foci in UIP may suggest metabolically higher activity when compared to the intraalveolar buds in COP/BOOP and the difference in the natural history of the two diseases.

1582 Idiopathic Tracheal Stenosis: A Clinicopathological Study of 63 Cases and Comparison of the Pathology to Chondromalacia

F Meng, EJ Mark, O Matsubara. Massachusetts General Hospital, Boston, MA.

Background: Tracheal stenosis in adults usually is the result of mechanical injuries either from direct trauma or intubation. Rarely cases develop in patients without such a precedent history, but there are few reports of the pathology of in such cases.

Design: We reviewed 63 cases of patients who came to the Massachusetts General Hospital for tracheal resection between1988-2007 and had no antecedent explanation for their tracheal stenosis. We studied the clinical facets, the topographical location of the stenosis, the pathology, serological evidence for ANCA, and histopathological findings including semi-quantitation of scarring and inflammation. We categorized forms of cartilage damage. We contrasted these 63 cases with 34 cases of patients who had tracheal stenosis after mechanical injury. We performed ancillary stains for elastic tissue, collagen and micro-organisms. We evaluated estrogen receptor (ER) and progestogen receptor (PR) by immunochemistry in some cases.

Results: All 63 cases of ITS occurred in females, with a mean age of 49 years. The most common symptom was shortness of breath or dyspnea on exertion. The average duration of symptoms was greater than 2 years, which was considerably longer than the average duration of symptoms in patients with CM. Nine of 23 patients gave a history of gastroesophageal reflux. All but one of the cases of ITS occurred in the subglottic region and/or upper one-third of the trachea. The lumen was narrowed to between 3 and 6 mm in most cases. Pathologically, most cases of ITS showed extensive keloidal fibrosis and dilation of mucus glands, a finding that was not present in most cases of CM. Distinctive was relatively normal cartilage with smooth inner and outer perichondrium, whereas in CM there was extensive degeneration of cartilage with irregular border of inner perichondrium observable at shirt sleeve magnification. ANCA was negative in all 52 cases in which it was measured. Immunohistochemical staining for ER and PR was positive in mesenchymal cells in most cases.

Conclusions: ITS is a rare disease and restricted to females. The frequency of gastroesophageal reflux and the positive staining for ER and PR are possible clues to etiology. Some form of fibromatosis is also possible. ITS can be distinguished histologically from CM in tracheal resection specimen in most cases.

1583 Persistence and Progression of Bronchial Dysplasia: Relationship to Histologic Grade, Overall Airway Damage and Angiogenic Change

DT Merrick, YE Miller, RL Keith, S Braudrick, WA Franklin. Denver VAMC & Univ. of Colorado HSC, Denver.

Background: Patients at high-risk for lung cancer frequently harbor bronchial dysplasia. These lesions are believed to be precursors of invasive lung cancer and the degree of atypia is thought to indicate increased risk for subsequent progression to malignancy. An analysis of patients enrolled in Colorado SPORE bronchoscopy protocols who had undergone multiple bronchoscopies was undertaken to characterize the long-term outcome associated with these lesions.

Design: Diagnosis, bronchoscopy date and biopsy site data for 121 patients undergoing multiple bronchoscopy and 14 cases with a biopsy within the lobe of a subsequent carcinoma were collected (1,354 biopsies from 551 unique sites). 48 total subjects had associated NSCLC. Within subjects, biopsy sites from different bronchoscopies were matched and classified into progression, persistent or regression groups. Diagnoses were assigned according to WHO classification criteria: non-dysplastic (normal or basal cell hyperplasia), squamous metaplasia, mild, moderate and severe dysplasia, and carcinoma-in-situ (CIS) with some analyses using high grade (HGD - moderate/severe) and low grade (LGD - mild) dysplasia groups. Persistent/progressive and regressive change were tracked. Persistence was also correlated with dysplasia index (DI - #dysplastic/#total biopsies) and presence of angiogenic squamous dysplasia (ASD - lesions with projections of stromal-vascular structures into overlying bronchial epithelium).

Results: Severe dysplasia was more frequently persistent than mild dysplasia (61.7% vs 46.0%, $p = 0.02$). Persistent lesions were associated with higher DI than regressive lesions for both HGD and LGD groups ($p < 0.01$). In addition, for all sites that were followed for at least three years, lesions demonstrating ASD morphology were associated with more frequent persistence than those lesions without changes of ASD (71.0% vs 44.8%, $p = 0.04$). Squamous cell carcinoma (SCC) associated cases showed increased DI (0.508 vs 0.353, $p = 0.04$), and sites of HGD from SCC cases showed increased frequency of persistence (77.8% vs. 50.0%, $p = 0.03$) versus cases without a history of invasive carcinoma.

Conclusions: Increased persistence of bronchial dysplasia is directly related to severity of atypia and overall airway damage (DI), and increased duration of persistence is associated with ASD. Squamous cell carcinoma cases are associated with high DI and persistence of HGD. These data support the role of bronchial dysplasia as pre-malignant lesions in NSCLC.

1584 The Role of Molecular Analysis in Differentiating Invasive Adenocarcinoma of the Lung with a Predominant Lepidic, Bronchioalveolar Pattern from a Bronchioloalveolar Carcinoma with Focal Invasion

A Mohanty, SD Finkelstein, TC Pereira, JF Silverman. Allegheny General Hospital, Pittsburgh, PA; RedPath Integrated Pathology Inc, Pittsburgh, PA.

Background: Invasive lung adenocarcinomas often demonstrate a peripheral lepidic spread / bronchioloalveolar (BA) pattern that can occasionally represent the majority of the tumor. In contrast, bronchioloalveolar carcinoma (BAC) can develop focal invasion. Since histologic differentiation between these two processes is very difficult, we evaluated the efficacy of molecular analysis for this distinction.

Design: A representative paraffin block was retrieved from 9 cases of invasive adenocarcinomas that had a significant lepidic / BA pattern component. Four to five microdissection targets were removed under stereoscopic guidance from foci of invasive adenocarcinoma, areas of BA pattern, and normal controls. DNA was obtained and analyzed by PCR for LOH at 1p, 3p, 5q, 9p, 10q, 17p, 17q, 18q, 21q, 22q and point mutation in K-ras-2. These markers were chosen based on the literature and on preliminary data from our laboratory. The concordance between the areas of invasive adenocarcinoma and the areas of lepidic spread/BA pattern were evaluated using two criteria: (1) Comparing the fractional mutation rates (FMR), calculated for each tumor area as the sum of all the high amplitude mutations (mutations present in at least 75% of the cells in a given tumor) /total number of informative markers, for each of the aforementioned areas and (2) The similarity in the gene copy affected.

Results: In 4/9 cases, the FMR was equal in the invasive components and the BA pattern foci, indicating that these represent invasive adenocarcinoma with lepidic spread. In 5/9 cases the FMR was higher in the invasive area, indicating that these were BAC with foci of invasion based on the premise that additional mutations were acquired in the process of developing invasion.

Conclusions: Molecular analysis can classify pulmonary adenocarcinomas with a significant BA pattern into two different neoplastic processes. The first is an invasive adenocarcinoma spreading predominantly in a lepidic pattern. The second is a tumor that most likely originated as BAC, with subsequent mutations acquired by a clone that resulted in focal invasion. We believe that the morphologic and prognostic differences between these 2 types of adenocarcinomas should be further studied in an effort to better manage and treat patients in each group, and molecular analysis can help provide the fundamental etiologic distinction.

1585 Topoisomerase II-alpha, Minichromosome Maintenance Protein 2 (MCM2), and X-Linked Mammalian Inhibitor of Apoptosis Protein (XIAP) Expression in Pleural Diffuse Malignant Mesothelioma (PDMM): Possible Role for Chemotherapeutic Intervention

BH Moore, PT Cagle, TC Allen, A Sienko, R Barrios, A Haque, QJ Zhai. Weill College of Medicine Cornell University, The Methodist Hospital, Houston, TX; University of Texas Health Center, Tyler, TX.

Background: PDMM have a poor prognosis, and current therapy is not curative. Agents against new targets are the only hope for significant increase in survival. Topoisomerase II relaxes supercoiled DNA and is required for DNA replication. MCM2 is a member of the minichromosome maintenance protein family, which regulates DNA elongation. Topoisomerase II-alpha and MCM proteins are upregulated in aberrant S-phase induction, which is caused by malfunction at the G1/S cell-cycle checkpoint. Since most cancers occur due to loss of cell-cycle regulation, this is a logical molecular target for therapy. Another target, XIAP, is a member of inhibitor of apoptosis proteins (IAP) that can cause resistance to apoptosis and possibly resistance to treatment. These proteins are under investigation as targets for new cancer therapies (Mol Cancer Ther. 2007;6:1501-8.; Oncologist. 2007;12:397-405; Mol Cancer Ther. 2007;6:957-66). We immunostained PDMM cases with ProExC (antibody cocktail against Topoisomerase II-alpha and MCM2) and XIAP to determine if these proteins are potential targets for therapy.

Design: Sections of a tissue microarray with 3 punches each from 43 PDMM were immunostained with ProExC (TriPath Imaging; prediluted) and XIAP (anti-hILP/XIAP, BD Biosciences; 1:100). Only nuclear staining for ProExC and cytoplasmic staining for XIAP was considered positive.

Results: See Table 1.

Results

	ProEx™C		XIAP	
	Positive	Negative	Positive	Negative
All	74% (31/42)	26% (11/42)	49% (21/43)	51% (22/43)
Epithelioid	82% (23/28)	12% (5/28)	46% (13/28)	54% (15/28)
Sarcomatous	58% (7/12)	42% (5/12)	17% (2/12)	83% (10/12)
Desmoplastic	0% (0/2)	100% (2/2)	0% (0/2)	100% (2/2)
Biphasic	100% (3/3)	0% (0/3)	66% (3/3)	33% (1/3)
Male	76% (25/33)	24% (8/33)	51% (18/35)	49% (17/35)
Female	75% (3/4)	25% (1/4)	25% (1/4)	75% (3/4)

Table 1

Conclusions: We demonstrated increased expression of ProExC and XIAP in a substantial percentage of PDMM with a higher positive rate in the epithelioid versus the sarcomatous subtype. Since these proteins are under investigation as targets of new cancer therapies, further study of ProExC and XIAP in PDMM is warranted for potential therapeutic implications.

1586 Stem Cell Marker Expression in Pulmonary Carcinomas Is Associated with Histological Type and Differentiation

AL Moreira, M Moore, T Akhurst, RJ Downey. Memorial Sloan Kettering Cancer Center, New York, NY; MSKCC, NY, NY.

Background: Lung regeneration involves a reserve stem cell population that proliferates and differentiates after injury. It has been hypothesized that pulmonary epithelial cancers may arise from a disorder of this process, so that a malignant cell represents failure to fully attain the characteristics of differentiated tissue and to shed the characteristics of a stem cell. If so, cancer cells may be expected to share the characteristics of both stem and differentiated cells, with poorly differentiated tumors being more likely to express stem cell markers.

Design: Sections of resected untreated pulmonary carcinomas including adenocarcinoma (ACA) (n=27), squamous cell carcinoma (SQCC)(n=10), large cell carcinoma (LCLC)(n=11), and small cell carcinoma (SCLC)(n=10), were studied by immunohistology for putative stem cells markers (Musashi-1, CD34, CD21, CD10, c-kit, Thy-1 and OCT-4) as well as for progenitor cells committed to the squamous lineage (p63) or Clara cell lineage (DC-LAMP and CC-10).

Results: Musashi-1, a RNA-binding protein involved in the regulation of precursors cell division and differentiation, was diffusely positive in 100% of SCLC, 30% of LCLC, in 4% of ACA (primarily in solid type ACA), and 0% of SQCC. An inverse relationship between CD21 and musashi-1 staining was observed. Diffuse positivity for c-kit was seen in 25% of SCLC, 9% in LCLC, 11% of ACA (primarily in solid type), and in 20% of SQCC. Diffuse positivity for CD10 was seen in 40% of SQCC, 30% of LCLC, 25% of SCLC, and 0% of ACA. DC-lamp and CC-10 are seen only in 30% of adenocarcinomas (primarily in BAC type). P63 marks 100% of SQCC. There is weak stain in 30% of ACA, mostly in BAC and acinar types, 40% in LCLC, and 60% of SCLC. No staining for OCT-4 and Thy-1 (embryonic stem cell markers) is seen in any of the tumor types studied.The expression of more than three stem cell markers is seen in 100% of SCLC, 37% of ACA, 54% of LCLC, and in only 20% of SQCC.

Conclusions: Pulmonary carcinomas can express stem cell markers, and this expression varies with histological type and degree of differentiation. SCLC has diffuse expression of multiple markers and is closer to stem cells. In ACA, there is heterogenous expression of stem cell markers, which is higher in solid type carcinoma. The simultaneous expression of multiple stem cell markers correlates with a low level of histological differentiation.

1587 Pulmonary Meningothelial-Like Nodules: New Insights into a Common but Poorly Understood Entity

S Mukhopadhyay, OA El-Zammar, A-LA Katzenstein. State University of New York Upstate Medical University, Syracuse, NY.

Background: Although pulmonary meningothelial-like nodules (MLNs) have been recognized for decades, their nature and significance remain uncertain. Most previous data are based on autopsies or lobectomies for tumor. This study was undertaken to evaluate MLNs in surgical lung biopsies, lobectomies and pediatric autopsies to clarify their incidence, distribution and relation to age and underlying disease, and to shed light on their origin.

Design: 400 consecutive surgical lung biopsies, 22 extensively sampled (27 sections) lobectomy specimens, 20 resections for pneumothoraces in patients under 30, and 9 pediatric autopsies were examined. Location and distribution of MLNs were noted, and associated diagnoses were recorded. Stains for progesterone receptor (PR), CD56, EMA, TTF-1, CD99, CD34, CD31 and Ki-67 were performed in selected cases.

Results: 131 MLNs were identified in 60 cases, including 51/400 surgical biopsies (12.8%) and 9/22 lobectomies (41%). They were solitary in 34 and multiple in 26. Patients ranged from 22 to 84 (mean, 63), with only 3 younger than 40. No MLNs were found in pneumothorax resections or in pediatric autopsies. There were 41 women and 19 men (F: M=2.1: 1). There was no association with underlying diseases, including various types of interstitial fibrosis, organizing pneumonia, granulomatous disease and malignancies. Although an increased frequency was found in thromboembolic disease (2/5; 40%), vasculitis (4/12; 33%) and respiratory bronchiolitis (6/26; 23%), the number of cases was too small to be significant. MLNs were randomly distributed in alveolar septa and were rarely present in scars. There was no relation to venules. Although small vessels were common in the nodules, they appeared to be non-specifically entrapped. Immunostains were positive for PR (14/14), CD56 (14/14) and EMA (10/10), and negative for TTF-1 (0/4), CD99 (0/11), CD34 (0/6) and CD31 (0/6). Ki-67 was focally positive in 2/12.

Conclusions: The incidence of MLNs in our study is higher than previously appreciated. Their presence in nearly half of extensively sampled lobectomies suggests that they may be present in all lungs if sufficiently sampled. Their absence in patients under 20, however, suggests that they are not congenital rests. The positive staining for CD56 is novel. CD56 has been reported in meningiomas, supporting the concept that MLNs are of meningothelial origin. A more appropriate term may therefore be "meningothelial nodules". The lesions appear to have no clinical significance.

1588 Basal Cell Differentiation in Lung Adenocarcinoma

D Nonaka, L Chiriboga. New York University School of Medicine, New York, NY.

Background: Lung carcinomas are a heterogeneous group of tumors at the cytologic, histologic, immunophenotypic and molecular genetic levels, and this heterogeneity is particularly prominent in the adenocarcinoma category. Histopathologically adenocarcinoma often contains a variety of morphologic patterns and cell types, the latter of which often show features of clara cells, type II pneumocytes, mucinous cells and squamous cells. Basal cells are a normal component of airways, and their number is greater in the large airways than in the small airways. Two types of basal cells were described; type A (CK14+/CK17+) predominantly found in the large airways and type B (CK14-/CK17+) found in the small airways. Basal cells have been rarely investigated in lung adenocarcinoma as a neoplastic component.

Design: A total of 108 cases of stage I primary lung adenocarcinomas were retrieved, and immunostains were performed by using tissue microarrays for p63, TTF-1, CK7, CK20, HMW-CK (34βE12), CK14, CK17, surfactant apoprotein-A (SPA), surfactant apoprotein-C (SPC), clara cell protein-16 (CC16), and CD208 (DC-LAMP). The extent of staining was graded as 1+, 1-25%; 2+, 25-50%; 3+, 50-75%; 4+, >75%.

Results: There were 62 mixed adenocarcinomas, 48 cases of which contained bronchioloalveolar (BAC) component, 9 BAC, 11 acinar, 4 papillary, 20 solid, and 2 mucinous carcinomas. 40/108 cases (37%) contained p63-positive neoplastic cells to a variable extent with 4+ reaction being seen in 3 cases (2.8%). Among the tissue cores with p63-positive carcinoma components, 87% were generally diffusely positive for TTF-1, 85 and 80% were variably positive for CK17 and 34βE12 while CK14 was focally expressed only in 7% of the cores. 92.5% showed CK7+/CK20- pattern. Markers for clara cells and type II pneumocytes, CC16, DC-LAMP, SPA, and SPC were found in 41, 23, 80, and 43% of the tumors to a variable extent. p63-positive neoplastic components were histologically as heterogeneous as p63-negative tumors although 65% showed pure BAC (12.5%) or mixed tumor with BAC areas (52.5%), and 52.5% had a significant amount of cells similar to clara cell morphology.

Conclusions: p63-positive cells are not uncommon components in lung adenocarcinomas, and most of them show a CK14-/CK17+ immunophenotype, which corresponds to a basal cell (reserve cell) of the distal airway (type B basal cell). Presence of p63 and/or 34βE12 positive cells in lung carcinoma does not always indicate squamous cell differentiation, and the possibility of tumor with a basal cell phenotype should be considered.

1589 Mechanisms of Epithelial-Mesenchymal Transition in Pulmonary Sarcomatoid Carcinomas (PSC)

G Pelosi, B Oprandi, A Sonzogni, D Galetta, G Veronesi, B Del Curto, G Rossi, A Cavazza, GL Arrigoni, M Papotti, E Dessy, A Tironi, L Bercich, G Viale. European Institute of Oncology, Milan, Italy; University of Modena, Modena, Italy; Reggio Emilia Hospital, Reggio Emilia, Italy; San Raffaele Hospital, Milan, Italy; University of Turin, Turin, Italy; University of Brescia, Brescia, Italy.

Background: Pulmonary sarcomatoid carcinomas (PSC) are poorly differentiated NSCLC containing a sarcomatous or sarcoma-like component, characterized by the occurrence of epithelial-mesenchymal transition (EMT). Loss of E-cadherin expression has been related to EMT in different carcinoma types, but little is known on its regulatory mechanisms in PSC patients.

Design: Ninety-two PSC according to 2004 WHO classification entered the study, including 70 pleomorphic carcinomas, 6 spindle cell carcinomas, 9 carcinosarcomas and 7 blastomas. Immunoreactivity for the E-cadherin/beta-catenin adhesion system was evaluated on paraffin sections, as well as for several gene products involved in EMT induction in experimental models, such as Snail, Slug, c-Jun, c-MET, EGFR, p38/p44, tenascin, fascin, S-100A4, matrilysin, laminin-5 and caveolin.

Results: There were 17 females and 75 males (range 38-85 years), the majority of which being current or former smokers. While tumor diameter ranged from 0.6 to 19 cm, the diverse histologic subtypes did not differ for tumor stage (32 tumors were in stage I, the remaining cases in stage II to IV) or patients' survival (5-year life expectancy was less 40%). The expression of E-cadherin appeared to be downregulated in the sarcomatoid component of all members of the tumor family along with vimentin and fascin overexpression, whereas membrane or cytoplasmic decoration of beta-catenin was observed in the same sarcomatoid cells. An inverse relationship was observed in the same cells between E-cadherin and c-Jun ($p<0.05$) and, marginally, caveolin ($p<0.10$) immunoreactivity, whereas a direct relationship was noted with EGFR expression ($p<0.05$). Beta-catenin nuclear accumulation was limited to the epithelial component of blastomas. Loss of E-cadherin expression in PSC correlated with shorter patients survival ($p=0.013$).

Conclusions: Sarcomatoid component of PSC undergo E-cadherin loss-depending EMT, likely induced by c-Jun and caveolin accumulation, and with corresponding expression of vimentin and fascin. In this model, EGFR may modulate EMT in an E-cadherin-independent manner. Restoring E-cadherin expression or targeting EGFR might have potential therapeutic implications in these life-threatining tumors.

1590 *TTF1* Amplification Defines TTF1 Overexpression in Lung Adenocarcinomas

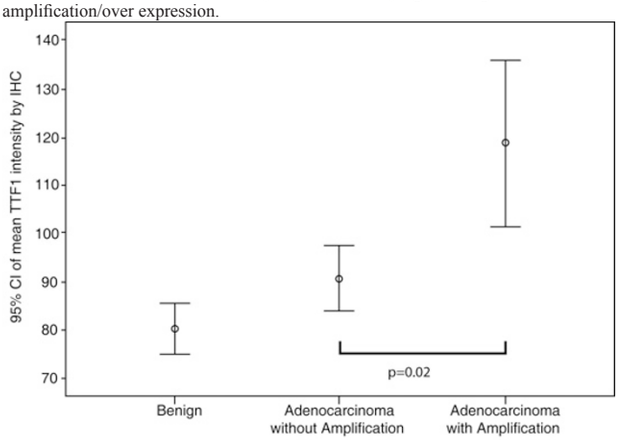
S Perner, F Demichelis, L Johnson, B Weir, J Barletta, M Meyerson, L Chireac, MA Rubin. Brigham and Women's Hospital, Boston, MA; The Broad Institute of Harvard and MIT, Cambridge, MA.

Background: Lung cancer is the leading cause of cancer death worldwide. We reported a high-level amplification of 14q13.3 as the most common recurrent event (~12% of tumors) in lung adenocarcinomas. Within this interval, we identified TTF1 (thyroid transcription factor 1) as lineage-specific transcription factor and novel proto-oncogene. Although TTF1 is already known to be expressed in virtually all lung adenocarcinomas and thus used as a diagnostic marker, we asked whether *TTF1* amplification was associated with the degree of protein expression.

Design: We interrogated 198 lung adenocarcinomas for TTF1 protein expression by immunohistochemistry and for *TTF1* amplification status by FISH. Kaplan-Meier survival analysis was performed on 70 patients to assess if *TTF1* amplification status is associated with outcome.

Results: There is a significantly higher TTF1 expression in adenocarcinomas with *TTF1* amplification as compared to adenocarcinomas without amplification (p=0.02; Wilcoxon test) (see Figure). Also, there is a trend that patients with *TTF1* amplification have worse outcome than patients without (p=0.15).

Conclusions: This study demonstrates for the first time that the routinely used diagnostic lung cancer marker TTF1 may also be a prognostic biomarker for lung adenocarcinomas. Future work attempts to confirm the prognostic significance of TTF1 amplification/over expression.



1591 Angiotensin II Antagonism Inhibits Collagen Deposition in Idiopathic Pulmonary Fibrosis

J Ramirez, A Xaubet, J Pereda, BD Uhal, X Li, M Molina-Molina. Hospital Clinic. CIBER. IDIBAPS. University of Barcelona, Barcelona, Spain; Hospital Clinic. CIBER Enfermedades Respoiratorias. IDIBAPS. University of Barcelona, Barcelona, Spain; Michigan State University, East Lansing, MI.

Background: Angiotensin II (ANGII) is a tissular growth factor that has been implicated in the pathogenesis of lung fibrosis. ANGII expression is increased in the lungs of patients with Idiopathic Pulmonary Fibrosis (IPF). The aim of our study was to investigate the effects of ANGII antagonism through losartan in human lung tissues and cell cultures.

Design: The lung tissue samples were obtained from surgical lung biopsy. Non-smokers subjects with pneumothorax that required surgical treatment were used as a control group (n = 6). IPF patients (n = 6) were diagnosed following the American Thoracic and European Thoracic Consensus Statement and all the cases showed a Usual Interstitial Pneumonia (UIP) histological pattern. Lung tissue explants were incubated in 24 wall plates during 20 hours; half of them were treated with losartan and the remaining without any additional treatment. The tissue explants were processed for PCR and immunohistochemistry. Fibroblast cultures were obtained from the surgical lung biopsy. Every fibroblast cell line was frozen after the 3rd passage until the experiment procedure. The experimental study was performed inducing control fibroblasts with ANGII and treating IPF fibroblasts with losartan.

Results: There was an increase in lung collagen synthesis when lung explants and fibroblasts were stimulated with ANGII. IPF explants and fibroblasts treated with losartan showed a reduction in collagen deposition.

Conclusions: In this preliminary study we have got evidence of ANGII relationship with collagen deposition in interstitial pulmonary fibrosis as well as the possibility of collagen reduction with ANGII antagonist treatment. Supported by FIS-PIO60064, FIS-IDIBAPS, CM05/00118, SEPAR-Fundacio respira, SOCAP, FUCAP.

1592 Gene Expression Profiling of Pulmonary Neuroendocrine Tumors: Identifying Clinically Useful Tumor Markers for Diagnosis and Treatment

MS Roh, WD Travis, WL Gerald. Dong-A University College of Medicine, Busan, Korea; Memorial Sloan-Kettering Cancer Center, New York, NY.

Background: Pulmonary neuroendocrine (NE) tumors include a spectrum of low-grade typical carcinoid (TC), intermediate-grade atypical carcinoid (AC) and high-grade large cell neuroendocrine carcinoma (LCNEC) and small cell carcinoma (SCLC). Historically these tumors have been a difficult challenge for pathologists to diagnose and for clinicians to treat. Comprehensive molecular analysis may reveal distinct subset and provide insight to their biology for identifying new diagnostic and therapeutic targets.

Design: We performed gene expression profiling analysis (Affymetrix U133A) of 65 pulmonary NE tumors including 35 TCs, 7 ACs, 15 LCNECs and 8 SCLCs. We used two-way unsupervised hierarchical clustering and supervised analysis to identify markedly differentially expressed genes between groups. We used two different measures; fold change (ratio) between the normalized means of each group of samples and a Student's t-test.

Results: Unsupervised clustering analysis distinguished high-grade NE tumors (LCNEC and SCLC) from carcinoid tumors (TC and AC). Among the top ranked 200 genes markedly differentially expressed (threefold ratio and t-test of $p<0.0001$), genes related to the antigen/nucleotide-binding, motor activity, transcription and cell cycle were up-regulated, and genes related to the ion/electron transport, development and cell adhesion were down-regulated in high-grade NE tumors. High-grade NE tumors were characterized by increased expression of genes implicated in tumor invasion and metastasis, such as FOXM1, CTHRC1, ANLN, MCM6, NEK2, MLF1IP and KNTC1. TC and AC were mostly in Clusters 3 (54.3% and 42.9%) and 2 (34.3% and 57.1%), respectively; LCNEC were mostly in Cluster 1 (93.3%) and all 8 SCLC were in Cluster 1 ($p<0.001$). Cluster 1 compared to Cluster 2 and 3, correlated significantly with older age ($p=0.001$), more smoking ($p<0.001$), and worse survival ($p<0.001$). Four of 16 carcinoids (25.0%) in Cluster 2 were Stage 3 or 4 compared to all the remaining 38 carcinoids that were in Stage 1 and 2 ($p=0.034$).

Conclusions: Our findings suggest that carcinoid tumors and high-grade NE tumors have distinct molecular features, despite sharing certain morphological characteristics. Why approximately half of carcinoids are in two clusters is not clear, although all the advanced stage cases were in Cluster 2. These gene products likely regulate the distinctive biology of these tumors and may serve as potential diagnostic and therapeutic targets.

1593 Rate of Concordance/Discordance between Bronchial Biopsy and Cytology When Specimens Are Taken on the Same Bronchoscopic Procedure: An Institutional Experience from 2000 to 2005

MK Sackett, DR Salomao, E Yi, M-C Aubry. Mayo Clinic, Rochester, MN.

Background: Often biopsy and cytology specimens are taken during the same diagnostic bronchoscopic procedure. At the Mayo Clinic, Rochester, biopsy and cytology specimens are read separately by 2 different groups of pathologists. The aim of this study was to evaluate the rate of concordance/ discordance between the diagnoses of these 2 specimens.

Design: A computer search was performed to identify all cases for which biopsy and cytology specimens were obtained during the same procedure, from January 1, 2000 to December 31, 2005. Cases were divided in categories according to biopsy and cytology diagnoses. For the purpose of this study the cytological diagnosis of suspicious for carcinoma was considered positive result. Atypical cytologies were considered negative. Positive cases were further evaluated as for concordance related to the histological subtype of small cell (SCLC) versus non small cell carcinoma (NSCLC). Cases with different histological subtypes were considered discordant.

Results: Specimens from 888 patients were identified. 352 (40%) had at least a positive cytology or biopsy. This group was comprised of 216 (61%) men and 136 women, with mean age of 67 years. Table 1 details the cytology and biopsy results for all patients. Of the positive cases, the majority had both positive cytology and biopsy (216/352, 61%). Only 5 cases (2%) were discordant (Table 2). Re-review of cases showed that discordance was the result of either prominent necrosis and artifact in the biopsy specimen or a combined histology with second component unrecognized in the cytology.

Table 1

	Results of Cytology/Biopsy			
	Positive/Positive N (%)	Negative/Positive N (%)	Positive/Negative N (%)	Negative/Negative N (%)
Total Cases	216 (24)	97 (11)	39 (5)	536 (60)
Discordant histology	5 (2)			

N=Number of cases

Table 2 - Discordant Cases

Cytology Results	**Biopsy Results**
SCLC	Combined SCLC + NSCLC
NSCLC	SCLC
SCLC	Combined NSCLC + SCLC
NSCLC	SCLC
NSCLC	SCLC

Conclusions: At our institution, patients who underwent bronchoscopic procedures most often had negative results for both cytology and biopsy specimens. For patients diagnosed with cancer, usually cytology and biopsy were both positive. Even though the cytology and biopsy specimens were reviewed by two different groups of pathologists, the concordance rate for the histological subtype was high. Discordance was due to necrosis, artifact or an unrecognized component of a combined carcinoma.

1594 Napsin A: A New Marker for Lung Adenocarcinoma Is Complementary and More Sensitive Than TTF-1 (Thyroid Transcription Factor-1): Evaluation of 967 Cases by Tissue Microarray

IM Sainz, J Fukuoka, PT Cagle, J Jagirdar. University of Texas Health Science Center at San Antonio, San Antonio, TX; Toyama University Hospital, Toyama, Japan; Weill College of Medicine Cornell University, The Methodist Hospital, Houston, TX.

Background: Napsin A (Nap-A) is a functional *aspartic proteinase* which is expressed in the normal lung parenchyma in type II pneumocytes proximal and convoluted renal tubules and in the lysosomal granules of alveolar macrophages and to a lesser degree in pancreatic acini and ducts. Nap-A is involved in maturation of the biologically

active Surfactant Protein-B peptide. Thyroid transcription factor (TTF-1) is identified as a *nuclear* tissue specific protein with DNA-binding activity that interacts with the thyroglobulin gene in the rat. TTF-1 regulates gene expression in the thyroid, lungs and diencephalon during embryogenesis. In the lung, TTF-1 regulates the expression of surfactant proteins A, B, and C, and Clara cell secretory protein genes. TTF-1 expression is highly specific for thyroid carcinomas and lung tumors, particularly lung adenocarcinomas (ACA). We propose to compare the value of TTF-1 vs Nap-A in the differential diagnosis of lung ACA.

Design: Immunohistochemistry was performed on paraffin embedded sections for Nap-A (IBL,CO., LTD.Japan, 1:100 dil.) and TTF-1 on tissue microarray of **967** cases containing **94** Lung ACA, **91** Lung Squamous Cell CA (SQCA), **90** Renal Cell CA, **95** Hepatocellular CA, **91** Colon CA, **96** Thyroid CA, **85** Bile Duct CA, **78** Ovarian CA, **48** Uterine CA, **36** Pancreatic CA, **29** Lung Small Cell CA (LSCC) and **83** Breast CA. Cases were evaluated as negative (no staining to minimal light brown-dust) and positive (moderate to intense brown cytoplasmic for Nap-A, and Nuclear for TTF-1).

Results: Less than **5 %** of carcinomas of the bladder, pancreas, breast, liver, biliary tract, colon, ovary, uterus and lung SQCA and LSCC were positive for Nap-A. ~10% of kidney and thyroid CA were positive for Nap-A. **74%** of Lung ACA were positive for **Nap-A** vs. **63%** were positive for **TTF-1**. **11%** of lung ACA detected by Nap-A were missed on TTF-1 staining. Staining for Nap-A, unlike that of TTF-1, was easier to interpret because when it was positive it was strong and diffuse.

Conclusions: Napsin A is a valuable marker for detecting lung ACA vs other ACAs such as those from the breast, colon, biliary tract, pancreas, urinary bladder and ovary. It is less useful in distinguishing lung ACA from thyroid and renal carcinomas.

1595 Presence of Alveolar Macrophages Correlates with Lower Stage and Increased Survival in Non-Small Cell Lung Carcinoma

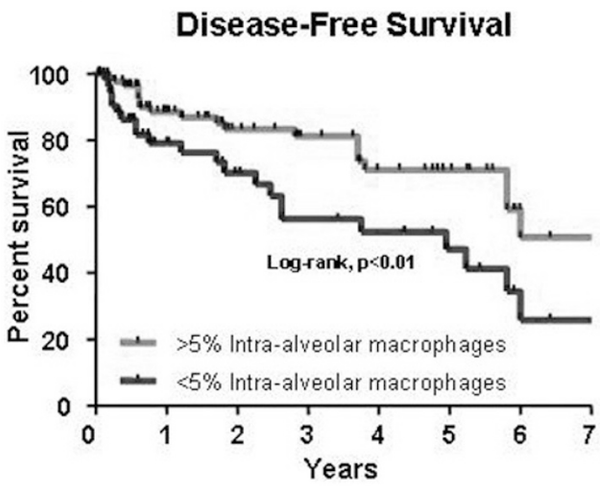
A Saqi, P Wagner, N Altorki, P Lee, S Paul, J Port, M Vazquez. Weill Medical College of Cornell University, New York, NY; New York Hospital/Weill Cornell Medical Center, New York, NY.

Background: The significance of tumor-infiltrating inflammatory cells has been assessed in various tumors-- including colorectal carcinoma, ovarian carcinoma and melanoma-- and their abundance has been correlated with a survival advantage. There are limited data on the role of the immune response in the prognosis of non-small cell lung carcinoma (NSCLC). The aim of this study was to determine if there is a correlation between the presence of lymphoid cells or intra-alveolar macrophages in NSCLCs and tumor stage or outcome.

Design: Hematoxylin-and-eosin-stained slides of 153 primary lung carcinomas from 120 patients were examined for the presence of intra-alveolar macrophages (<5% vs. >5%), tumor-infiltrating lymphocytes (<5% vs. >5% of tumor stroma) and lymphoid aggregates (present vs. absent) within the tumor. These histologic findings were compared with standard AJCC staging parameters (TNM), as well as disease-free survival. Correlation was determined using a two-tailed Fisher's exact test (significance, $p<0.05$). Kaplan-Meier curves were generated to analyze disease-free survival, and curves were compared using a log-rank test.

Results: The presence of intra-alveolar macrophages within a tumor was strongly associated with low T score (T1: 46/92, 50% vs. T2-T4: 18/61, 30%, $p=0.01$) and node-negative status (N0: 76/92, 83% vs. N1-N2: 34/61, 56%, $p<0.001$). Disease-free survival was significantly longer among lesions containing intra-alveolar macrophages than those without ($p<0.01$, see figure). No association was observed between the presence of lymphocytes or lymphoid aggregates and staging parameters or survival.

Conclusions: Intra-alveolar macrophages are associated with lower tumor stage and longer survival among NSCLCs. Although further studies will be necessary to elucidate the mechanisms accounting for this association, we can hypothesize that macrophages migrate via chemotaxis into a subset of tumors, where they play a role in the antitumor immune response.



1596 The Presence of a CD21+ Follicular Dendritic Cell (FDC) Network Is Useful in Distinguishing Bronchus Associated Lymphoid Tissue (BALT) from Pulmonary Acute Cellular Rejection

HA Sattar, T Krausz, S Bhorade, AN Husain. University of Chicago, Chicago, IL.

Background: Bronchus associated lymphoid tissue (BALT), especially when crushed or not associated with bronchial wall, can be confused with pulmonary acute cellular rejection. Rarely present in normal adult lungs, BALT generally indicates chronic antigenic stimulation and likely arises through a process termed lymphoid neogenesis. Given that lymphoid neogenesis retains the architecture of primary lymph node tissue, we postulated that, as in lymph node, the lymphoid tissue of BALT would be organized by an underlying follicular dendritic cell (FDC) network, and that this FDC network would be helpful in distinguishing BALT from acute cellular rejection.

Design: 12 transbronchial biopsies taken from patients in our nascent lung transplantation program during the first 9 months of 2007 and 2 cases of chronic rejection (1 explanted lung and one wedge biopsy) and were collected from our Pathology archives. Each case was reviewed by conventional H and E staining to determine the presence/grade of rejection and to determine whether or not BALT was present. Using unstained slides cut at the time of initial diagnosis, we investigated the presence of follicular dendritic cells by immunohistochemical staining for CD21. Normal human tonsil served as a positive control.

Results: Of the twelve transbronchial biopsies, a CD21+ FDC network was detected in 3 of the 7 cases (43%) that showed lymphoid aggregates of BALT, and was absent in all foci of acute cellular rejection (n=3 each showing A1,B0 rejection). When present, the follicular dendritic cells were in the center of the lesion and the number of positive cells appeared proportional to the size of the lesion. Both cases of chronic rejection (1 inactive and 1 active) revealed a dense CD21+ FDC network present in areas of BALT. Such a network was not detected in areas of ongoing acute or established chronic rejection.

Conclusions: A CD21-positive FDC network, when present, reliably distinguishes BALT from acute cellular rejection (sensitivity = 43%; specificity = 100%). Use of CD21 antibody would be beneficial in reducing the rate of over-diagnosis of BALT alleviating unnecessary immunosuppression and its associated risks. The usefulness of this marker should be further substantiated through larger studies.

1597 ProEx C Expression in Non-Small Cell Lung Cancers (NSCLC) Is Associated with Longer Patient Survival

M Shahjahan, QJ Zhai, TC Allen, AE Sienko, A Haque, R Barrios, S Chakraborty, DR Mody, PT Cagle. The Methodist Hospital, Houston, TX; The University of Texas Health Center at Tyler, Tyler, TX.

Background: NSCLC patients have a poor prognosis, with half of patients with stage 1 and 2 cancers dying of their disease despite tumor resection that should cure their disease. ProEx C is a biomarker reagent containing antibodies to minichromosome maintenance protein 2 (MCM2) and topoisomerase II A (TOP2A) used to detect the presence of aberrant S-Phase induction in cells. Increased expression of MCM and TOP2A proteins has been observed in cervical, esophageal, skin, prostate, ovarian, endometrial, colon and lung cancers. Except for cervical cancer, data on the frequency of ProEx C over-expression in human cancers are limited. In this study, we evaluated the prognostic significance of ProEx C expression on survival of patients with NSCLC.

Design: 289 patients with NSCLC were included. Immunohistochemistry was utilized to measure the expression of ProEx C (Tripath Imaging; prediluted) in sections of tissue microarray with 3 punches from each case. Immunopositivity in tumor cells was graded on a scale from 0 to 3 and averaged for the 3 punches from each tumor. Average score of 0 or 1 was classified as negative/weak expression, while average score of 2 or 3 was classified as strong expression. ProEx C expression was compared to 5-year survival using Kaplan-Meier analyses. Survival data was analyzed according to cell type (adenocarcinoma, adenocarcinomas with >50% bronchioloalveolar component, squamous cell carcinoma, large cell carcinoma) and tumor stage.

Results: 199/289 (69%) of the NSCLC expressed ProEx C. Specifically, 36% of adenocarcinomas, 7% of adeno/bronchioloalveolar carcinomas, 36% of squamous cell carcinomas and 21% of large cell carcinomas had strong ProEx C expression. In patients with stage 1 and 2 disease, overall, strong expression of ProEx C was statistically significantly associated with longer survival ($p=0.01$). This statistically significant association held true for adenocarcinoma with >50% bronchioloalveolar component ($p=0.04$) and large cell carcinoma type ($p=0.03$) but not for the other cell types.

Conclusions: Our study is the largest series to investigate ProEx C expression in NSCLC. ProEx C is expressed in more than 2/3 of NSCLC and strong expression is associated with longer 5-year survival in certain cellular subtypes. This suggests a role in tumor progression of these cell types and may provide a potential basis for targeted therapy.

1598 Loss of Expression of von Hipple-Lindau Gene Product (pVHL) in Moderately and Poorly Differentiated Neuroendocrine Carcinoma of the Lung – A Possible Role in Carcinogenesis

J Shi, H Liu, W Jin, F Lin. Geisinger Medical Center, Danville, PA; Fujian Medical University, Fuzhou, Fijian, China.

Background: The von Hipple-Lindau gene product (pVHL) has been linked to the carcinogenesis of both hereditary and sporadic clear cell renal cell carcinomas. Our recent study showed 1) that pVHL was expressed in normal pancreatic tissue; and 2) that loss of expression of pVHL was observed in pancreatic intraepithelial neoplasia (PanIN) and invasive ductal adenocarcinoma of the pancreas (Lin et al. AJSP 2007; in press). In this study, we investigate the expression of pVHL in neuroendocrine neoplasms from various organs.

Design: We immunohistochemically evaluated the expression of pVHL in 82 cases of neuroendocrine neoplasms on conventional tissue sections. They included small cell undifferentiated carcinoma of the lung (N=11); large cell neuroendocrine carcinoma of the lung (N=13); Merkel cell carcinoma of the skin (N=8); metastatic neuroendocrine

carcinoma of the liver (N=11); well-differentiated neuroendocrine carcinoma (carcinoid tumor) of the lung (N=10); moderately differentiated neuroendocrine carcinoma (atypical carcinoid) of the lung (N=3); and well- and moderately differentiated neuroendocrine carcinomas from the ileum (N=7), colon (N=13), and stomach (N=6). The staining intensity was graded as weak or strong. The distribution was recorded as negative (less than 5% of tumor cells stained), 1+ (5-25% of tumor cells stained), 2+ (26-50% of tumor cells stained), 3+ (51-75% of tumor cells stained), or 4+ (more than 75% of tumor cells stained).

Results: The results demonstrated membranous and cytoplasmic staining for pVHL in 10 of 82 cases, including 7 of 10 carcinoid tumors, 2 of 7 cases from the ileum, and 1 of 11 cases from the liver. Most of the positive cases showed 2+ or 3+ positivity for pVHL. The remaining cases were negative for pVHL.

Conclusions: The results demonstrate that loss of or reduced expression pVHL is a frequent finding in moderately and poorly differentiated neuroendocrine carcinomas, which suggests that pVHL may play a role in the carcinogenesis of high-grade neuroendocrine carcinomas. In addition, pVHL may have some diagnostic value in differentiating atypical carcinoid tumor from carcinoid tumor.

1599 Genotype-Phenotype Correlation in Non-Small Cell Lung Carcinoma

LM Sholl, AJ Iafrate, B Yeap, A Holmes, Y Chou, M Wu, Y Goan, L Su, PA Jänne, DC Christiani, LR Chirieac. Brigham and Women's Hospital, Boston; Massachusetts General Hospital, Boston; Kaohsiung Veteran General Hospital, Kaohsiung, Taiwan; Dana Farber Cancer Institute, Boston; Harvard School of Public Health, Boston.

Background: Epidermal growth factor receptor (EGFR) activation via kinase domain mutation and/or gene amplification occurs in a subset of non-small cell lung carcinomas (NSCLC). To date, there are no well-established histologic criteria to predict the presence of EGFR activation. We hypothesized that NSCLC with EGFR activation has a characteristic histologic phenotype.

Design: Paraffin-embedded NSCLC specimens from 79 Chinese non-smoking women in Taiwan treated with surgery alone were characterized per the WHO criteria as adenocarcinoma (ACA) with papillary, acinar, solid or BAC (mucinous or nonmucinous) features, or squamous cell carcinoma (SCC). All specimens were examined for EGFR, KRAS and HER2 mutations by PCR-capillary sequencing, EGFR gene copy number by FISH and protein expression by EGFR immunohistochemistry (IHC). Associations between the dominant histology patterns, EGFR activation, KRAS and HER2 were investigated.

Results: The results of the histologic and molecular analyses are as follows:

Dominant Histology	# of cases (% of total)	# EGFR mutated (%)	# EGFR gene amplified (%)	# EGFR IHC positive (%)
Acinar	20(25)	16(80)	3(15)	14(70)
BAC	3(4)	3(100)	0	1(33)
Mucinous BAC	4(5)	1(25)	0	0
Papillary	29(37)	26(90)	2(7)	24(83)
Solid	12(15)	9(75)	4(33)	8(67)
SCC	11(14)	5(45)	0	8(73)
Total	79(100)	60(76)	9(11)	55(70)

KRAS mutations were present in 2 cases, both mucinous BAC. HER2 mutation was present in one case, an EGFR mutation-negative ACA, acinar type. Of the 60 mutated cases, 44 (73%) showed positive (>10% cells staining) EGFR IHC, compared to 11 of 19 (58%) WT cases (p=0.255). All 9 cases containing EGFR gene amplification had an EGFR mutation and expressed the EGFR protein in >50% of tumor cells. There was a trend toward increased representation of solid-type ACA in the gene amplified category vs overall (p=0.05).

Conclusions: Our genotype-phenotype correlation study demonstrates that EGFR gene mutations occur frequently in all ACA subtypes. We identify a subset of non-BAC-predominant ACA with coexisting EGFR gene amplification, mutation, and protein expression. Our results more clearly define a NSCLC phenotype that predicts for the presence of multiple EGFR molecular abnormalities.

1600 EGFR Gene Amplification Is Invariably Associated with Exon 19 Deletion in Non-Small Cell Lung Carcinoma

LM Sholl, AJ Iafrate, B Yeap, A Holmes, YP Chou, MT Wu, YG Goan, L Su, PA Jänne, DC Christiani, LR Chirieac. Brigham and Women's Hospital, Boston; Massachusetts General Hospital, Boston; Dana Farber Cancer Institute, Boston; Kaohsiung Veteran General Hospital, Kaohsiung, Taiwan; Harvard School of Public Health, Boston.

Background: A subset of non-small cell lung carcinomas (NSCLC) contain an activated form of the epidermal growth factor receptor (EGFR) secondary to mutations in the kinase domain (exons 18 to 21) and/or gene amplification. Deletions in exon 19 and the L858R substitution in exon 21 each account for 40-50% of mutations. Gene amplification occurs in approximately 15% of cases. To date, no studies have examined the correlation between mutation type and amplification status. We hypothesized that there is a preferential association of EGFR gene amplification with selected types of EGFR mutations.

Design: Paraffin-embedded NSCLC specimens from 79 non-smoking Chinese women in Taiwan treated by surgery alone were examined for gene copy number by FISH and scored according to published criteria as gene amplified, high polysomy, low polysomy, or disomic. EGFR kinase domain mutations were detected by PCR-capillary sequencing. Mutant and wild type alleles were quantified in a subset of exon 19 deletion cases (n=37) and L858R mutation cases (n=9) by QPCR.

Results: FISH analysis revealed that 9 cases (11%) were gene amplified, 25 cases (32%) had high polysomy, 20 cases (25%) had low polysomy, and 25 cases (32%) were disomic. The exon 19 deletion mutation was present in 100% of cases with gene amplification; the exon 19 deletion was found in 56%, 55%, and 44% of high polysomy, low polysomy, and disomic cases, respectively (Fisher's exact test; p=0.009). The non-amplified categories contained a heterogenous mix of WT, missense- and insertion-mutation cases. Preferential amplification of the mutant allele occurred exclusively in the cases with exon 19 deletion and more frequently in the gene amplified cases (6 of 7; 86%) as compared to the non-amplified cases (6 of 39; 15%).

Conclusions: Our amplification-mutation correlation study reveals that there is a molecularly distinct subset of NSCLC that harbors EGFR gene amplification and the exon 19 deletion mutation. Amplification occurs preferentially on the mutated allele in cases with gene amplification by FISH. Our findings may have significant implications for selection of patients with NSCLC for treatment with tyrosine kinase inhibitors.

1601 Reassessment of the Histologic Spectrum of Mucinous Bronchioloalveolar Carcinomas (mBAC)

G Sica, A Yoshizawa, D Klimstra, W Gerald, W Travis. MSKCC, NY, NY.

Background: Recent focus on the pathology of bronchioloalveolar carcinomas (BAC) has emphasized the study of non-mucinous BAC (nmBAC) using the 2004 WHO strict definition of a non-invasive pattern which correlates with a 100% 5-year survival in small peripheral tumors. Mucinous BAC (mBAC) has received little attention, although it is recognized that these tumors are more associated with KRAS mutation and less responsive to tyrosine kinase inhibitors compared to nmBACs which may show EGFR mutation in 10-40% of cases. We sought to document the histologic spectrum of findings in cases where the diagnosis of mBAC had been considered.

Design: Twenty five lung adenocarcinomas with either a diagnosis of mBAC or adenocarcinoma with mBAC in the diagnostic line were evaluated for the percentage of acinar, papillary, micropapillary, solid and BAC components, grade, size and, when available, immunohistochemical (IHC) profile. The strict 2004 WHO definition of BAC was utilized.

Results: Median age was 66 (30-83) years with 13 women (52%) and 12 men (48%). Stage was IA in 13 (52%), IB in 10 (40%), and IV in 2 (8%). All tumors showed at least focal mucinous features and 4 (16%) lacked any mBAC. Most cases were mixed subtype with invasive growth. Only 1 of 25 cases (4%) showed mBAC with no invasion, which was a single focus of BAC in a patient with multicentric disease who died of tumor 3 years later. The major subtype was mBAC in 12 cases (48%), acinar in 9 (36%), papillary in 2(8%), and solid and micropapillary in 1 each (4%). Four cases with mixed mucinous and nonmucinous BAC were in tumors with major acinar (n=3) and papillary (n=1) patterns. Of the cases for which IHC stained slides were available for review, 86% were cytokeratin 7 positive (6/7), 43% were cytokeratin 20 positive (3/7), 71% were TTF-1 positive (5/7) and 29% were PE-10 positive (2/7). 5-year overall survival was 36% with no difference for the major BAC vs not BAC tumors.

Conclusions: Similar to non-mucinous BAC, most lung adenocarcinomas with a mBAC pattern are mixed subtype with invasive components. Even our single case showing a pure non-invasive mBAC pattern was from a patient with unresectable disease who died 3 years later, so we cannot exclude an invasive component in unbiopsied tumor. Despite the fact that over 90% of our cases were Stage I tumors, the overall 5-year survival of 36% indicates aggressive behavior. These data suggest that mBAC is virtually always associated with invasive adenocarcinoma. The relationship between mucinous and non-mucinous BAC needs to be reassessed with the revised 2004 WHO criteria.

1602 Expression of Galectin-4 in Non-Small Cell Lung Carcinoma (NSCLC) and Correlation with Survival

AE Sienko, TC Allen, R Barrios, S Chakraborty, PT Cagle. Weill College of Medicine Cornell University, Houston, TX; University of Texas Health Center, Tyler, TX.

Background: Lung cancer is the leading cause of cancer deaths in North America and prognosis is poor with an overall 5-year survival rate of less than 15%. New biomarkers for stratifying patients in therapeutic studies and targets of therapy are urgently needed. Galectin-4 (LGALS4) is a member of the galectin family of binding proteins (beta-galactosides) that play a major role in regulation of cell differentiation, cell proliferation and apoptosis. Galectins are also thought to play a role in immune response, cell adhesion, antigen presentation as well as cell cycle progression. Galectin-4 has been found in many malignancies, predominately colorectal carcinomas and neural tumors and has been proposed to be a potential target of anticancer therapy (Glycoconjugate Journal 20, 2004:247-255). Expression in NSCLC has not been previously documented.

Design: Tissue microarrays of 264 Stage I and II NSCLC (148 adenocarcinomas, 67 squamous cell carcinomas and 49 large cell carcinomas) were immunostained for Galectin-4 (1:100, Vector Laboratories). Nuclear and cytoplasmic staining was scored as negative (no staining or <5% of tumor cells staining), low (5-10% of cells staining), moderate (10-50% of cells staining) and high (>50% of cells staining). Nuclear and cytoplasmic staining intensity was scored as 0 = negative, 1 = weak, 2 = moderate and 3= strong. The results were analyzed by Kaplan-Meier Survival analysis by frequency and intensity of staining for tumor cell type and stage.

Results: Nuclear staining was the rule, with cytoplasmic staining present only within the subset of adenocarcinomas with mucinous features. While no significant survival differences were identified in Stage I and Stage II patients regardless of tumor cell type, frequency or intensity of staining, there was a trend of the adenocarcinomas to show increased survival with low to moderate frequency of tumor cell staining (p = 0.2) and weak to moderate nuclear staining (p = 0.1).

Conclusions: Our study showed over-expression of Galectin-4 was associated with a trend for worse prognosis in early stage NSCLC. Galactin-4 may prove useful as a prognostic marker in NSCLC, and possibly as a future therapeutic target.

1603 c-Met Expression in Pulmonary Neuroendocrine Tumors

J Song, MS Tretiakova, R Salgia, PT Cagle, AN Husain. Univ of Chicago, Chicago, IL; The Methodist Hospital, Houston, TX.

Background: Pulmonary neuroendocrine tumors include highly aggressive small cell lung carcinoma (SCLC) and large cell neuroendocrine carcinoma (LCNEC) as well as relatively indolent carcinoid. Currently, survival of patients with SCLC and

LCNEC is abysmal with limited treatment options. Novel therapies are needed. The receptor tyrosine kinase c-Met plays an important role in tumor growth, invasion, metastasis and drug resistance. c-Met is activated by its ligand hepatocyte growth factor, leading to its phosphorylation and signal transduction. c-Met overexpression has been detected in both SCLC and non-small cell lung cancers. Inhibition of c-Met by siRNA or small molecules like SU11274 decreases the viability of cancer cells, suggesting that c-Met could serve as a therapeutic target. Phase 1 trials are now underway at our institution. Here we conducted the first large-scale c-Met expression study in all three neuroendocrine lung tumor categories.

Design: Tumor tissue microarrays of 44 carcinoids, 35 SCLCs and 9 LCNECs with 3 cores from each case were immunohistochemically stained using antibodies against native c-Met and triple-phosphorylated c-Met [pY1230/1234/1235] (p-Met). The intensities of cytoplasmic and nuclear staining were separately scored in each core and averaged for each case: 0 (negative), 1+ (weak), or 2+ (strong).

Results: c-Met showed a diffuse cytoplasmic staining pattern and was positive in a majority of tumors. The intensity was strongest in carcinoid, weakest in LCNEC, with SCLC in between (p=0.009, Kruskal-Wallis test). p-Met showed predominantly nuclear staining with occasional weak cytoplasmic signals, and was also positive in a majority of tumors. There was a tendency of stronger p-Met expression in SCLC compared with carcinoid and LCNEC (p=0.06).

Conclusions: c-Met expression was frequently demonstrated in all three categories of neuroendocrine lung tumors, supporting the potential use of c-Met as a therapeutic target in these tumors, including SCLC and LCNEC for which there are currently only limited and largely unsuccessful treatment options. Nuclear translocation of p-Met was observed. Similar phenomenon has been described in other receptor tyrosine kinases. Its biological significance will be an active area of research to further understand the c-Met pathway.

Tumor Type	c-Met (cytoplasmic)			p-Met (nuclear)		
	Negative	Weak	Strong	Negative	Weak	Strong
Carcinoid	7%	27%	66%	14%	34%	52%
SCLC	8%	43%	49%	3%	31%	66%
LCNEC	11%	78%	11%	0	78%	22%

1604 Epithelial-to-Mesenchymal Transition May Be More Important for Local Invasion Than for Metastasis of Lung Carcinomas

TP Stricker, M Tretriakova, J Manaligod, R Salgia, T Krausz. University of Chicago, Chicago, IL.

Background: Epithelial to mesenchymal transition (EMT) occurs when epithelial cells undergo a phenotypic switch to a highly motile mesenchymal-like cell. EMT has recently been implicated in metastasis of models of breast and colon carcinomas. Snail, a transcriptional repressor, is a master regulator of EMT in development. If EMT is an important mechanism in lung carcinoma metastasis, we hypothesized that Snail would be expressed in lung carcinomas.

Design: Using tissue arrays, we examined the expression of Snail in archival paraffin embedded lung cancers by IHC. Immunostains for Snail were evaluated by two pathologists and graded for both percentage of cells and intensity of expression.

Results: Eleven small cell carcinomas (SCC) and 67 non-small cell carcinomas (NSCLCs) were analyzed. There was a statistically significant difference in Snail expression between SCCs (1/11, 9%) and NSCLCs (30/67, 45%) (p = 0.0001). Amongst NSCLCs, there was a statistically significant difference between adenocarcinomas (15/22, 68%) and squamous carcinomas (5/24, 21%) (p = 0.005). In tumors that were positive, Snail showed diffuse cytoplasmic staining with focal nuclear staining, which suggests that Snail may be regulated not only by expression but also by changes in localization. Interestingly, Snail was expressed with greater frequency and intensity at the leading edge of tumors. Furthermore, nuclear localization was more frequent at the leading edge and at the tumor-stroma junction. Snail expression was also analyzed in 26 lymph node and 8 brain metastases of NSCLCs. Intriguingly, Snail was only rarely expressed in the metastatic lesions (4/26 lymph nodes and 1/8 brain metastases).

Conclusions: Snail expression was significantly more frequent in NSCLCs than SCCs, suggesting that different mechanisms of invasion/metastasis may be important in these two types of tumors. Snail showed both cytoplasmic and nuclear staining, indicating that regulation of Snail localization may be important for its function. Although EMT has been hypothesized to be important for metastasis in models of breast cancer, we found that Snail was only rarely expressed in metastatic lesions. However, expression levels and nuclear localization were frequently increased at the leading edge of tumors. These result suggests that Snail expression, and EMT, may be more important for local invasion than for distant metastases in vivo.

1605 Expression of Inhibin-alpha in Primary Pulmonary Non-Small Cell Carcinomas by Immunohistochemistry

DY Tigrani, JA Weydert. University of Iowa, Iowa City, IA.

Background: Inhibin is a glycoprotein hormone that is known to be expressed in carcinomas of adrenal cortical and germ cell origins. Occasionally these tumors may be included in the morphologic differential diagnosis of a poorly differentiated carcinoma present within the lung or mediastinum. The diagnostic utility of immunohistochemical detection of inhibin in this setting is not entirely clear, as non-small cell lung cancers may express a variety of hormone products. We sought to determine the frequency of inhibin-alpha expression in non-small cell lung carcinomas.

Design: 48 cases of surgically resected non-small cell primary lung carcinomas were studied. Immunohistochemistry was performed on formalin fixed, paraffin embedded tissue sections with anti-inhibin α antibody (Dako, Carpinteria, CA) using the Dako Envision+ HRP System. Expression of inhibin in carcinoma cells was evaluated independently by two pathologists as positive (any malignant cells showing unequivocal cytoplasmic staining compared to negative control sections) or negative (malignant cells showing no staining). Extent of staining was assessed in a semi-quantitative fashion. In the event of a discrepancy, the case was re-examined at a two-headed scope and a consensus was obtained.

Results: Inhibin-alpha expression was seen in 9 of 48 cases (19%). The extent of expression was limited to less than 25% of tumor cells in all positive cases. The histologic subtype in the inhibin-positive group consisted of 8 poorly differentiated adenocarcinomas and one large cell neuroendocrine carcinoma.

Conclusions: Primary lung carcinoma is not excluded by positive staining for inhibin-alpha in the work up of a poorly differentiated carcinoma. The extent of positive staining in lung carcinomas appears to be limited to a minority of the cells in a given tumor, however, and therefore diffuse reactivity with anti-inhibin may argue against primary lung carcinoma.

1606 Tissue Microarray Study of RNA-Binding Protein IMP3 in Primary Non-Small Cell Lung Cancers: Prediction of Survival in Early Stage Squamous Cell Carcinomas

P Vohra, PT Cagle, TC Allen, K Dresser, S Chakraborty, AE Fraire. University of Massachusetts Medical School, Worcester, MA; The Methodist Hospital, Houston, TX; University of Texas Health Center, Tyler, TX.

Background: Lung cancer has a notoriously poor prognosis and kills more patients each year in the United States than the next three causes of cancer deaths (breast, colon, prostate) combined. There is a critical need for new targeted therapies for lung cancer. Biomarkers to indicate which patients are likely to have a poor outcome are needed to stratify patients for clinical trials. Expression of IMP3, an oncofetal RNA-binding protein and member of the insulin growth factor family, has been proven useful as a biomarker of disease progression in renal cell carcinoma. The potential utility of IMP 3 expression as a prognostic biomarker in non small cell lung cancers has not been previously investigated.

Design: High through-put tissue microarrays of 247 primary non-small cell lung cancers (187 adenocarcinomas, 90 squamous cell carcinomas), with 5 year or greater follow up. Three 1 mm punches per tumor, were immunostained with L523s, a mouse monoclonal antibody specific for IMP3 protein (Corixa Corporation, Seattle, WA, USA). Positive staining was defined as dark brown cytoplasmic and /or membrane staining in 30 percent or more of tumor cells. Statistical analysis was conducted by the Kaplan Meir life table with assessment of positive or negative results with tumor cell type and TNM status.

Results: Positive IMP 3 immunostaining was found in 98 (52.4%) of 187 adenocarcinomas and 69 (76.6%) of the 90 squamous cell carcinomas. By Kaplan-Meier analysis there was statistically strong trend for better survival for patients with >30% positive staining in stage 1 and 2 squamous cell carcinomas with a p value of 0.08. Adenocarcinomas showed no significant associations.

Conclusions: Based on our study, IMP3 expression in >30% of tumor cells shows a strong positive trend in predicting better 5-year survival in early stage squamous cell carcinomas of the lung. This marker may be useful for stratifying squamous cell carcinomas in studies of new therapies and as a potential target for molecular therapies.

1607 Combined Small Cell Carcinomas: Loss-of-Heterozygosity and Immunohistochemical Analysis of the Individual Components

PL Wagner, Y-T Chen, MF Vazquez, A Saqi. Weill Medical College of Cornell University, New York, NY.

Background: Combined small cell carcinomas (CSCCs) of the lung are small cell carcinomas (SCLC) that exhibit discrete areas of non-small cell carcinoma (NSCC) by morphology-- most commonly adenocarcinoma (AC), squamous cell carcinoma (SQ), large cell neuroendocrine carcinoma (LCNEC) or unspecified large cell carcinoma (LCC). Such areas can be present in up to 30% of SCLCs, and pose a dilemma in terms of treatment, since the separate components warrant radically different therapy. In order to investigate the relationship between the distinct morphologic constituents of these lesions, we compared the individual subtypes using immunohistochemistry (IHC) for a panel of markers and loss-of-heterozygosity (LOH) analysis for loci commonly deleted in SCLC (chromosome 22q13) or in both SCLC and NSCC (chromosome 3p).

Design: Seven CSCCs were evaluated. The NSCC was AC in 3 cases, LCNEC in 2, SQ in 1 and LCC in 1. Each lesion was stained for CK7, TTF-1, synaptophysin, chromogranin, CD56 and PAX-5 and the separate constituents of each tumor were assessed independently. DNA was microdissected from each morphologically distinct area and LOH analysis was performed for markers on chromosomes 3p and 22q (two loci each, designated 3A, 3B, 22A, 22B).

Results: IHC results demonstrated perfect agreement between the morphologically distinct areas in six cases (see Table); the seventh case showed near-perfect agreement with a single discrepancy in PAX-5 staining. LOH analysis (see Table) revealed identical losses in the individual components of five lesions. One lesion showed loss of chromosome 22 in the SCLC portion but not in the area of NSCC, while one lesion was uninformative at all loci tested.

CASE NUMBER	LOH	IMMUNOHISTOCHEMISTRY					
COMPONENT	LOCUS	TTF-1	CK7	SYN	CHROM	CD56	PAX-5
#1							
SCLC	22A, 22B	+	+	+	focal	+	-
LCC	22A, 22B	+	+	+	focal	+	-
#2							
SCLC	3B	+	+	+	-	+	n/a
AC	3B	+	++	++	-	+	n/a
#3							
SCLC	none	-	-	+	-	+	+
AC	none	-	-	+	-	+	+
#4							
SCLC	3A, 22B	+	+	+	+	+	weak
AC	22B	+	+	+	+	++	weak
#5							
SCLC	22A	-	+	+	-	+	+
LCNEC	none	-	+	+	-	+	+
#6							
SCLC	3A, 22B	+	n/a	+	-	+	++
LCNEC	3A, 22B	+	n/a	+	-	+	+
#7							
SCLC	3A	-	-	+	-	+	+
SQ	3A	-	-	+	-	+	-

Conclusions: We present molecular and immunohistochemical evidence that the individual elements comprising CSCCs share identity in most cases despite their distinct morphologic appearances. The prevalent expression of synaptophysin and CD56 and common loss of chromosome 22q13 indicate that these tumors are biologically closer to SCLC and should be clinically managed as such.

1608 Immunohistochemical (IHC) Expression of c-Met Receptor Tyrosine Kinase (c-Met) Has Prognostic Significance and Its Activation Is Related to Phosphorylated Protein Kinase C ß (p-PKC ß) in Malignant Mesothelioma (MM)

M Westerhoff, L Faoro, S Loganathan, R Salgia, T Krausz, AN Husain. University of Chicago, Chicago, IL.

Background: Recently, multi-targeted small molecule inhibitors have been shown to be more effective in treating some forms of cancer. Both c-Met and vascular endothelial growth factor (VEGF) upregulate angiogenesis and are over-expressed in the majority of MM. c-Met can induce increased proliferation, motility, adhesion, and invasion in cancer cells. VEGFR2/KDR, one of the known VEGF receptors, is directly able to activate downstream effectors such as SRC, FAK, and the p38-MAPK pathway. PKC ß is also downstream of VEGFR2 and is responsible for transducing signals to the MAPK pathway, making it a desirable target in treatment of malignancies, including that of MM. In prior studies we showed that overexpression of PKC ß2 is an adverse prognostic factor in MM. In this study we evaluated the correlation between survival and IHC expression of VEGFR2/KDR, c-Met, phosphorylated c-Met (p-Met), phosphorylated AKT (p-AKT), and p-PKC ß in MM, as well as the correlation of these molecules to each other.

Design: 24 MM (18 epithelioid, 3 sarcomatoid, 3 biphasic) arranged in tissue microarrays were stained by IHC for VEGFR2/KDR, c-Met, p-Met, p-PKC ß1 and ß2, and p-AKT. Reactivity was semi-quantitatively scored for intensity (0-3+). Clinical outcome data was available for 16 patients.

Results: VEGFR2/KDR had diffuse positive staining in all cases with 2+ to 3+ intensity in 84%. c-Met had diffuse membranous staining in 89% with 2+ to 3+ in 33%. p-met had diffuse nuclear immunoreactivity in 90% with 2+ to 3+ positivity in 45%. p-PKC ß2 was positive in 70% with diffuse 2+ positivity in 35%. p-PKC ß1 and p-AKT had focal weak to no reactivity. Overall survival was significantly decreased in the strong expressors (2+ and 3+) of c-Met (164 versus 604 days, p=0.008, Log Rank test). No survival differences were observed for different expression levels of VEGFR2/KDR, p-PKC ß1 or ß2, p-AKT or p-Met. There was significant correlation between expression of p-Met and p-PKC ß2 (r=0.503, p=0.024). There was no correlation between the histologic subtypes of MM and the molecular markers.

Conclusions: As shown previously, PKC ß2 expression is an adverse prognostic factor in MM. We now show that c-Met expression is also an adverse prognostic factor and that expression of p-Met correlates with the downstream target of VEGFR2/KDR, p-PKC ß2. Therefore, dual targeting of c-Met and p-PKC ß2 may be an important therapeutic strategy in MM.

1609 Immunohistochemical Analysis of KOC/IMP3 in Malignant Pleural Mesothelioma

H Xu, R Simon, PA Bourne, BO Spaulding, HL Wang. University of Rochester Medical Center, Rochester, NY; Dako North America, Carpinteria, CA; Cedars-Sinai Medical Center, Los Angeles, CA.

Background: K homology domain containing protein overexpressed in cancer (KOC), also known as IMP3 and L523S, is a member of the insulin-like growth factor II (IGF-II) mRNA-binding protein (IMP) family, which is expressed during embryogenesis and in certain malignancies. It is considered to be an oncoprotein and functions to promote tumor cell proliferation by enhancing IGF-II protein expression. KOC expression in malignant pleural mesothelioma (MPM) has been examined in only a limited number of cases.

Design: Surgically resected or biopsied MPMs (n=53) and reactive mesothelial hyperplasias (RMHs; n=12) were immunohistochemically studied using a monoclonal antibody against KOC/IMP3 (Dako). Cytoplasmic 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 p value of <0.05, as determined by two-tailed Fisher exact test, was considered statistically significant.

Results: Histologically, MPMs consisted of 46 epithelioid, 2 sarcomatoid and 5 biphasic subtypes. Three RMHs exhibited focal mild architectural and cytologic atypia without evidence of invasive growth. Immunohistochemical stains showed 34 (64%) MPMs to be moderately to strongly positive for KOC in 20% to >90% of the tumor cells. These included all non-epithelioid subtypes (2 sarcomatoid and 5 biphasic) and 27 (59%) epithelioid subtypes (p<0.01, comparing epithelioid with non-epithelioid). Interestingly, 3 RMHs with cytologic atypia showed moderate cytoplasmic staining with 5%, 10% and 60% of the cells stained, respectively, but the remaining 9 RMHs were completely negative for KOC expression (p<0.05, in comparison with MPMs).

Conclusions: KOC/IMP3 is strongly and diffusely expressed in a large proportion of MPMs and only occasionally expressed in RMHs, suggesting its usefulness as a diagnostic marker in the distinction between malignant and benign mesothelial lesions. The higher frequency of expression in sarcomatoid and biphasic MPMs suggest that KOC expression may be associated with an aggressive biological behavior.

1610 Pulmonary Inflammatory Pseudotumor (IPT), Lymphoplasmacytic Sclerosing Variant: A Distinct Pulmonary Manifestation of IgG4-Related Systemic Fibrosclerosing Disease (SFSD)

Y Yonemori, T Fujii, Y Inayama, T Kishimoto, K Kameyama, K Hara, O Matsubara, T Tanizawa, K Hiroshima, Y Nakatani. Chiba University, Chiba, Japan; Toranomon Hospital, Tokyo, Japan; Yokohama City University, Yokohama, Japan; Keio University, Tokyo, Japan; Aichi Medical University, Aichi, Japan; National Defense Medical College, Tokorozawa, Japan.

Background: IgG4-related SFSD is a multiorgan disease of presumed autoimmune etiology. In the lung, most cases manifest as IPT-like masses, but their detailed histopathological features are unknown.

Design: A total of 6 cases of SFSD with pulmonary IPT-like masses that were surgically resected were retrieved from the data base of our departments. A clinicopathological analysis was performed together with immunohistochemistry for α-smooth muscle actin (SMA), immunoglobulin light chains, IgG, IgG4, anaplastic lymphoma kinase (ALK1), and in situ hybridization for EBER. IgG+ and IgG4+ plasma cells were counted in 10 HPF and the IgG4/IgG ratio was calculated.

Results: Patients were all males, (39 to 78 y, median 49y). One patient had chest pain but the others were free from respiratory symptom. The serum IgG and CRP levels were raised in all and IgG4 level was elevated in 2 cases measured. Most pulmonary lesions were found incidentally, either synchronously with or during follow-up of extrapulmonary lesions (skin, orbit,larynx, maxilla, chest wall). 3 cases showed recurrence in the lung, which responded well to corticosteroid therapy. The resected lung tumors were typically yellowish white in color, ranging 2-12cm in size. Histopathologic features common to all cases were: Marked lymphoplasmacytic infiltration with a number of IgG4+ plasma cells, prominent lymph follicles with germinal centers, a tendency for sclerosing fibrosis, peripheral interstitial pattern, obliterative phlebitis, and pleural involvement. Myofibroblastic cells were SMA+, vimentin+, CD34-, ALK protein-, EBER-, and they were inconspicuous except for the fibrosclerosing areas. All extrapulmonary lesions were alike histopathologically. The numbers of IgG4+ plasma cells per HPF were high (mean, 58.6) and the average IgG4/IgG was 0.50.

Conclusions: Pulmonary IPT presenting as part of IgG4-related SFSD is a distinct subset of IPT with a constellation of histopathological features, for which the term "lymphoplasmacytic sclerosing variant" may be appropriate. Recognition of this entity is important to avoid unnecessary surgical resection.

1611 Expression of Inhibin-alpha in Non-Small Cell Carcinoma of the Lung with a Focus on Adenocarcinoma – A Diagnostic Pitfall in the Evaluation of Metastatic Pulmonary Carcinoma

K Zhang, J Shi, F Lin. Geisinger Health System, Danville, PA.

Background: Distinction of a primary lung non-small cell carcinoma (NSCC), particularly adenocarcinoma (AC), from a metastatic carcinoma to the lung is a practical issue and frequently encountered in diagnostic surgical pathology. Additionally, during the lung cancer staging process, an incidental finding of adrenal mass versus metastasis from the lung is frequently included in the diagnostic consideration. It has been documented that inhibin-alpha only expresses in few tumors, such as adrenocortical carcinoma, ovarian sex cord stromal tumor, and hemangioblastoma of the brain; but it does not express in AC of the lung. As the result, inhibin-alpha has been used as a highly specific marker to exclude an adrenal mass as a lung metastasis. Interestingly, we have observed the expression of inhibin-alpha in some cases of AC of the lung. In this study, we immunohistochemically evaluated the expression of inhibin-alpha in NSCC of the lung to confirm the initial finding.

Design: Fifty-six cases of primary lung NSCC classified according to the 2004 WHO classification were included in this study, including 20 cases of squamous cell carcinoma (SCC), 20 cases of adenocarcinoma in tissue microarray (TMA) sections, and 16 cases of adenocarcinoma in routine tissue sections. Using an EnVision-HRP detection kit (Dako), we evaluated the expression of inhibin-alpha on both TMA and routine sections. The majority of cases also contained normal lung tissue. The staining intensity was graded as weak, intermediate, or strong. The distribution was recorded as negative (no staining), 1+ (<25%), 2+ (26-50%), 3+ (50-75%), or 4+ (>75%). Cytoplasmic staining for inhibin-alpha was regarded as a positive result.

Results: Seven of 36 cases of AC (19.4%), including 4 of 16 cases in routine sections and 3 of 20 in TMA sections, were positive for inhibin-alpha, with 4+ positivity in one case with a solid pattern in routine section, 2+ positivity in three cases with both acinar and solid patterns, and 1+ positivity in three cases with an acinar pattern. The tumor cells showed moderate and strong staining intensity in the positive cases. Normal lung tissue, all cases of SCC, 1 case of large cell carcinoma, and 1 case of BAC were negative for inhibin-alpha.

Conclusions: Our data show that a significant percentage of adenocarcinoma of the lung expresses inhibin-alpha. Therefore, caution should be taken when using inhibin-alpha as a key antibody to exclude a lung primary in either the lung or other organs.