1718 Fluorescent In Situ Hybridization (FISH) Is an Effective Tool To Detect Unsuspected Trisomies in Hydropic Gestation.

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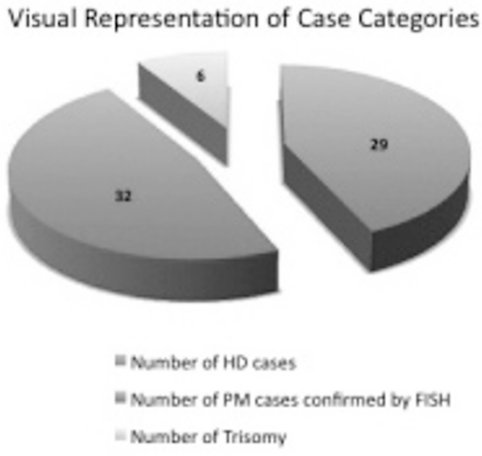
Background: Histological classification of products of conception (POC) with hydropic changes has always been challenging because there is considerable morphological overlap between gestations with hydropic degeneration (HD), partial moles (PM) and hydropic changes due to trisomies. Ancillary studies are essential in establishing diagnosis and flow cytometry has been considered the gold standard to identify triploidy confirming the diagnosis of PM. Fluorescent In situ Hybridization (FISH) is a molecular cytogenetic technique that has been used more recently to identify triploidy in POCs and due to the use of chromosome-specific probes, FISH can also detect specific trisomies. The aim of our study was to assess the efficacy of FISH as a novel ancillary technique in identifying trisomies in POCs with hydropic changes.

Design: The study cohort consists of 67 cases of POC accessionned between 2007 and 2010 for which the differential diagnoses included PM and HD and for which FISH had been requested. FISH was performed on 4-µm formalin-fixed paraffin-embedded tissue sections using the Vysis AneuVysion Prenatal Test specific for chromosomes X, Y, 13, 18 and 21, and a probe specific for chromosome 16 (Abbott-Molecular).

Results: FISH revealed an unsuspected trisomy in 6/67 cases (9%). There were four cases of trisomy 16, one trisomy 18 and one trisomy 21. FISH revealed triploidy confirming a diagnosis of PM in 32/67 cases. In the remaining cases, the FISH results were within normal range and a diagnosis of HD was rendered.



Conclusions: Thus FISH has emerged as an effective tool in not only the definitive diagnosis of PM, but also detection of trisomies in HD cases and the discernment of the cause of the miscarriage. In our study, an unsuspected trisomy was identified in a significant proportion (9%) of POCs with hydropic changes submitted for FISH analysis. A diagnosis of gestation with a trisomy can have significant clinical implications. Thus, FISH, rather than flow cytometry, should be considered as the new gold standard for the evaluation of hydropic POCs.

Pulmonary

1719 The Impact of Pleural Invasion and Its Subdivision on T Staging of Non-Small Cell Lung Cancer.

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Background: Visceral pleural invasion (VPI) is a factor that upstages T1 tumours to T2 in the 7th TNM for non-small cell lung carcinoma (NSCLC). However. the value of further subdivision of VPI is controversial. This study assesses the significance of VPI on survival and the value of splitting pleural invasion into further subgroups.

Design: Histopathological data was prospectively collected on 728 resected NSCLCs between 1999 and 2006, according to the RCPathology Minimum Data Set, of which 465 had overall survival data (OS) and 455 had disease free survival data (DFS). Pleural invasion was assessed by using haematoxylin and eosin (H&E) staining and Elastin van Gieson (EVG) staining to highlight the pleura. Pleural invasion was further subclassified according to proposed criteria for PL0 (no VPI), PL1 (VPI not reaching the surface), PL2 (VPI reaching the surface), PL3. (parietal pleural invasion). To assess VPI as an independent variable, T category based purely on size was also documented without contribution of VPI. Actuarial survival was estimated using the Kaplan-Meier method and compared using the Log-rank test. Cox proportional hazards regression was used to ascertain the individual contribution of factors associated with survival and to compare adjusted survival between the groups.

Results: The presence of visceral pleural invasion was associated with a hazard ratio of 1.57 (95%CI 1.19 to 2.11; P=0.001) and 1.49 (1.11 to 2.01; P=0.007), adding 56% and 49% to the mortality at each stage, for the 6th and 7th edition of the TNM respectively, in relation to OS. There was no evidence to suggest any difference in overall (P=0.262) or disease free survival (P=0.183) between the PL1-3 categories.

Conclusions: We confirm the importance of tumour breaching the outer elastin layer of the visceral pleura (VPI) as a signifcant factor in T staging, independent of tumour size. However our data do not support further subdivision of pleural invasion into PL1-3 subcategories.

1720 Detection and Clinicopathologic Features of ALK Rearranged Lung Adenocarcinoma.

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Background: The *EML4-ALK* fusion in lung adenocarcinomas (AD) is mutually exclusive with *EGFR* and *KRAS* mutations and is associated with striking responses to ALK kinase inhibitors. The optimal detection strategy and morphologic features of this genetic subset of lung AD remain poorly defined. We compared *ALK* fusion detection by fluorescence in situ hybridization (FISH), immunohistochemistry (IHC), and reverse transcriptase-polymerase chain reaction (RT-PCR), and then used this highly validated set of cases to examine the clinicopathologic features of ALK-rearranged (ALK-R) tumors.

Design: We screened 622 *EGFR* and *KRAS* wild type lung AD for *ALK* rearrangement by dual color split signal FISH assay (Abbott-Vysis; positivity defined as presence of split signals or loss of 5' ALK probe in >5% of tumor cells); 32 *ALK*-R and 99 *ALK*-germline (ALK-G) tumors were then tested by IHC [ALK D5F3 monoclonal antibody, Cell Signaling; scored as negative (0-1+) and positive (2-3+)], and RT-PCR (using 6 *EML4* forward primers; 1 *ALK* reverse primer).

Results: Of the 622 cases tested by FISH, 48 (8%) cases were *ALK*-R and 571 (92%) were *ALK-G*. As *EGFR*- and *KRAS*-germline cases make up about 55% of lung AD in our population, we estimate a 4% overall prevalence of *ALK* fusion. The most common predominant histology was solid (54%), followed by acinar (17%) and papillary (Pap 8%). Signet ring cells were identified in 7 cases (15%) including solid (n=5), Pap (n=1) and Micropapillary (MP, n=1) subtypes. *ALK*-R tumors were strongly associated with non-smokers, solid-predominant histology and high stage (IIIB-IV) at presentation. The concordance rate of IHC and RT-PCR assay with FISH were 97% (77/79; k=0.95) and 95% (131/138; k=0.88), respectively. All 99 *ALK*-G tumors by FISH were negative by IHC and RT-PCR.

Conclusions: Our study suggests that FISH should be combined with IHC as a screening strategy for *ALK* fusions in routine practice, with confirmation by RT-PCR as needed. IHC can raise diagnostic accuracy in cases with low % of FISH-positive cells (5-15%). All FISH-positive cases in this low range were confirmed by IHC/RT-PCR. ALK-R tumors are associated with poorly differentiated lung AD (solid, MP). However, signet ring cells are identified in only 15% of ALK-R tumors.

Clinical features of ALK-R and ALK-G Lung AD

	ALK-R (n=48)	ALK-G (n=99)	P value
Age (median)	31-84 (58)	35-87 (63)	
Female	21 (44%)	60 (61%)	0.1029
Male	27 (56%)	35 (39%)	
Non-Smoker	40 (83%)	21 (21%)	<0.0001
Smoker	8 (17%)	78 (79%)	
Stage IA-IIIA	12 (25%)	67 (68%)	<0.0001
Stage IV	36 (75%)	32 (32%)	

1721 Comprehensive Analysis for Clinically Relevant Oncogenic Driver Mutations in 1131 Consecutive Lung Adenocarcinomas.

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Background: Somatic mutations within several signaling molecules of the EGFR pathway have been shown to drive lung adenocarcinoma. With the advent of targeted therapies, the prospective assessment of tumors for a growing number of clinically relevant genetic alterations is becoming necessary. In this study, we aimed to determine the proportion of lung adenocarcinomas with known mutations based on a comprehensive panel interrogating 38 mutations in 8 genes.

Design: Consecutive tumors samples accrued between 1/2009 and 2/2010 at MSKCC were analyzed for recurrent mutations in EGFR, KRAS, NRAS, HER2, BRAF, PIK3CA, AKT1 and MAP2K1 using a combination of methods including standard Sanger sequencing, fragment analysis and mass spectrometry genotyping (Sequenom) assays. Cases negative for EGFR, KRAS and BRAF were subsequently tested for EGFR exon 20 insertions and HER2 exon 20 insertions (by fragment analysis) and EML4-ALK fusions by fluorescent in-situ hybridization.

Results: A total of 1131 specimens were analyzed for all mutations. Of these, 845 were from former/current smokers and 286 from non-smokers; 719 females and 411 males. KRAS mutations were found in 358 tumors (32%), 256 (23%) harbored EGFR mutations, 16 (1.4%) BRAF, 3 (0.2%) AKT, 20 (18%) PIK3CA, 5 (0.4%) NRAS and 20 (1.8%) HER2 mutations. Of all cases tested by FISH for evidence of the EML4-ALK fusion, 40 were positive (8%). The estimated overall rate of 4%. With the exception of PIK3CA mutations and the EGFR T790M secondary mutation, all mutations concurrently tested were mutually exclusive. In all, 718/1131 tumors (63%) harbored a mutation.

Conclusions: To our knowledge, this study represents the largest comprehensive analysis of recurrent oncogenic mutations in lung adenocarcinoma. Prospective testing of lung adenocarcinomas can identify targetable oncogenic mutations in over 60% of samples which may help assign specific kinase inhibitors or aid in the further management of these patients.

1722 Expression and Diagnostic Implication of HPV and p16 in Squamous Cell Carcinoma in Multiple Organ Sites.

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Background: p16 is a cell cycle regulatory protein which maintains tumor supressor activity of pRb gene. Following integration of human papilloma (HPV) viral DNA into host genome, HPV E7 protein degrades pRb resulting in p16 upregulation. HPV has been implicated in squamous cell carcinoma (SCC) in anogenital and upper aerodigestive tracts. Its role in lung SCC is controversial. Studies imply usefulness of p16 in discriminating between cervical SCC with lung metastases and primary lung SCC. We study the presence of HPV16 and the expression p16 in SCC in multiple sites to determine its usefulness as a marker in these sites.

Design: 56 cases of primary SCC were retrieved from our pathology archives. H&E stained slides were reviewed by 2 independent pathologists. Sites were lung: 25 cases, anogenital area: 8 cases, esophagus: 5 cases, skin: 7 cases, head/neck: 11 cases. Cases varied from well to moderately differentiated SCC. Insitu hybiridization (ISH) for HPV16 and p16 immunohistochemical analysis were performed on paraffin sections using an automated system (Ventana 1:200). Scoring for p16 expression was based on proportion of positive cells as follows: negative 0:<10%, weak 1+:10-30%, moderate 2+:50-75%, strong 3+:75-100%. Nuclear or combined nuclear and cytoplasmic stains were considered specific for p16. HPV16 ISH was scored as positive or negative based on nuclear positivity.

Results: Of 25 cases of lung SCC, 40% were positive for p16. Proportion of positive cells was independent of degree of differentiation. All cases from the anogenital area, 40% of skin SCC, 27.3% of head/neck SCC were positive for p16. All cases of esophagus were negative for p16.

By ISH, HPV16 was detected in 4% of lung SCC, 37.5% of anogenital SCC and 27.3% of head/neck SCC. There was no detection of HPV16 in all cases of esophageal and skin SCC.

SCC site, p16 and HPV16 expression

		P16 immunoreactivity score				HPV16 ISH	
SCC site	Number of cases	0	1+	2+	3+	Positive	Negative
Lung	25	15	1	5	4	1	24
Anogenital	8	0	0	0	8	3	5
Skin	7	5	1	0	1	0	7
Esophagus	5	5	0	0	0	0	5
Head/Neck	11	8	0	0	3	3	8

Conclusions: 1) p16 expression and presence of HPV16 DNA are not consistent in lung and anogenital SCC. Other pathways or HPV types may be involved in tumorgenesis in these sites. **2)** Majority of lung SCC are negative for HPV16 DNA; a positive HPV16 case should be considered as metastatic from anogenital or head/neck primary. **3**) HPV16 has no role in the pathogenesis of SCC in the skin and esophagus. **4)** HPV16 ISH can be used as a valuable tool in our daily differential diagnosis.

1723 High-Risk HPV Status in Primary and Metastatic Carcinomas of the Lung.

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Background: Human papillomavirus (HPV) is the major causative agent in squamous cell carcinomas (SqCCs) of the cervix and oropharynx, but its role in lung cancer is unclear. Rates of HPV detection in lung cancer range from 0 – 80%. High detection rates may reflect to some degree the poor specificity of various non-quantitative PCR-based assays. The purpose of this study was to determine the presence of high risk HPV in primary lung carcinomas using a highly sensitive and specific in-situ hybridization (ISH) assay; and to determine the utility of HPV detection as a means of establishing tumor relationships in patients with head and neck SqCC who develop a SqCC in their lung.

Design: High risk HPV in-situ hybridization was performed on 146 primary lung carcinomas from patients without prior head and neck SqCCs, and on 49 lung SqCCs from patients with a prior head and neck SqCC.

Results: Overall, HPV was detected in 8 of 195 (4.1%) cases. HPV was not detected in any of the lung SqCCs from patients without a history of head and neck SqCC. All HPV-positive cases were from patients with a prior oropharyngeal SqCC (Table). For the paired oropharyngeal and lung SqCCs, concordant HPV status was confirmed in 94% of cases. The time interval from treatment of the HPV-positive oropharyngeal carcinomas to detection of the lung carcinoma ranged from 1 to 97 months (mean 41 months). Two HPV-positive cancers were detected in the lung 8 years after treatment of the oropharyngeal primary.

Conclusions: HPV does not appear to play any significant role in the development of primary lung cancer. For patients with oropharyngeal SqCC who develop SqCCs in their lungs, HPV analysis may be helpful in clarifying tumor relationships. These relationships may not be obvious on clinical grounds as HPV-related oropharyngeal SqCCs may metastasize long after treatment of the primary tumor.

Tumor type	**P16 IHC (%)**	**HPV ISH* (%)**
Small cell carcinoma	3/3 (100)	0/3 (0)
Non-small cell carcinoma, NOS	1/12 (8)	0/12 (0)
Adenocarcinoma	15/74 (20)	0/74 (0)
Large cell/pleomorphic carcinoma	4/11 (25)	0/11 (0)
Squamous cell carcinoma		
No prior squamous cell carcinoma	6/46 (13)	0/46 (0)
Prior head and neck squamous cell carcinoma	14/49 (29)	8/49 (16)
- oropharynx	12/24 (50)	8/24 (33)
- oral cavity	1/6 (17)	0/6 (0)
- larynx	1/20 (5)	0/20 (0)
- hypopharynx	0/1 (0)	0/1 (0)

1724 Pleuropulmonary Infection by Paragonimus westermani in the United States: A Clinicopathologic Analysis of Four Cases with Identification of a Unique Risk Factor.

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Background: Infections caused by parasites in the genus *Paragonimus* are a rare cause of pleuropulmonary disease in the United States (US), typically acquired by eating raw crayfish carrying the species *P. kellicotti*. The species *P. westermani* has only rarely been reported in the US. Due to its nonspecific presentation, the diagnosis relies on a history of ingestion and the pathologic review of biopsy material. We report four cases of pleuropulmonary disease caused by US-acquired *P. westermani*.

Design: We identified all cases of *P. westermani* pulmonary infection in the consultation files of the authors. We recorded the clinical history, risk factors and serologic diagnostic data, and reviewed histologic slides.

Results: Four cases (3 men and 1 woman, aged 20-66 years) were identified. Patients presented with pulmonary complaints and chest imaging abnormalities: cavitary infiltrates (2), lung mass (1), pleural effusion (1) and pneumothorax (1). Surgical biopsies showed marked eosinophilic infiltrates including eosinophilic pneumonia with organizing pneumonia in all cases. Granulomatous inflammation with geographic necrosis (3), vasculitis (3), and pleuritis (3) were also encountered. *Paragonimus* organisms/eggs were identified in 2 cases. Serologic studies were positive for *P. westermani* in 3 cases (2 ELISA and 1 immunoblot) and helped confirm the diagnosis in 2 cases lacking organisms or eggs. Three patients ate live crabs at sushi bars (including crabs in martinis, a previously unreported mechanism for infection). In one patient the source of infection was undetermined, although as a manager of a Japanese restaurant it is postulated that he ingested infected imported crabs.

Conclusions: Paragonimiasis should be considered in the differential diagnosis of patients with eosinophilic pleuropulmonary disease in the US. Although eosinophilic pneumonia was a consistent finding, the biopsy findings may be non-specific as the organisms and/or eggs are not always visualized. Unusual features include pleuritis, foci of geographic necrosis and granulomatous vasculitis. A history of ingestion and targeted serologies are key to the diagnosis.

1725 Pulmonary Hypertension in Cirrhosis, Study of 22 Autopsy Cases.

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Background: Portopulmonary hypertension (POPH) is a form of pulmonary arterial hypertension associated with portal hypertension with or without underlying liver disease. Previous autopsy studies have reported a prevalence of pulmonary hypertension in a population with cirrhosis or portal hypertension to vary between 0.25% and 0.73%. However, prospective hemodynamic studies indicate the prevalence of POPH to be 2-4 % in patients with severe liver disease and 5-10 % in patients being evaluated for liver transplantation. The discrepancy in these prevalence rates is unclear. The aim of this study was to assess the prevalence of POPH in a group of subjects with cirrhosis undergoing a post mortem examination.

Design: The electronic autopsy data base at the University of Texas Medical Branch was examined from 1996-2009 for subjects with post mortem findings of cirrhosis. We selected those subjects with the following gross anatomical findings right ventricular hypertrophy, right ventricular dilation, and pulmonary artery atheromas or microscopic histology indicative of pulmonary vascular disease. Subsequently, those subjects with cirrhosis and pulmonary hypertension were identified and the microscopic histology of liver and lung examined by two pulmonary pathologists.

Results: We identified 256 subjects with cirrhosis. From this cohort, 36 subjects with cirrhosis and right ventricular pathology or pulmonary vascular disease were identified. However, 14 patients were excluded from further analysis due to missing pathology (n=8), absent clinical history (3) or a clinical history of left ventricular systolic dysfunction (n=3) resulting in a final cohort of 22 subjects. The mean age of the final cohort was 50 ± 8.3 years with 17 males and 5 females. Cirrhosis was recognized 5.9 ± 5.5 yrs before death. Eight subjects were known to have right ventricular hypertrophy or pulmonary hypertension before death for a mean time of 0.8 ± 0.8 yrs. Mean cardiac weight was 518 ± 237 grams and mean liver weight was 1366 ± 372 grams. Cirrhosis was identified in all hepatic histology sections. The pulmonary vascular histology revealed intimal hyperplasia (91 %), precapillary vessel dilation (59 %), plexiform lesions (77 %) and vascular necrosis (14 %).

Conclusions: In the current autopsy study, we observed the prevalence of POPH 8.9 % to be greater than prior autopsy series and similar to prospective hemodynamic studies.

1726 Performance of Cytologic Specimens in *EGFR* and *KRAS* Molecular Testing with a Focus on Minimal Cellularity Requirements.

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Background: Testing for *EGFR* and *KRAS* mutations is used to guide molecular-targeted therapy for lung adenocarcinoma. We evaluated the performance of cytologic specimens in detecting *EGFR*/*KRAS* mutations in clinical practice with a focus on minimal cellularity requirements.

Design: Cytologic specimens submitted for *EGFR*/*KRAS* molecular testing at MSKCC during a 1-year period (n=128) were reviewed. Most specimens were paraffin-embedded cell blocks, with a mean of 14 sections (5um-thick) submitted for testing after a pathologist's triage as "adequately cellular". Included were FNAs (n=96), pleural

fluids (n=29), and bronchial washings/brushings (n=3). We analyzed the test failure rate, DNA yield, concordance with mutations in other specimens, and clinicopathologic correlates. Manual cell counts were performed to determine the minimal cellularity sufficient to detect the mutations.
Results: Of 128 cytologic specimens, 125 (97.6%) were sufficient for complete *EGFR*/*KRAS* testing, 1 (0.8%) was sufficient for partial analysis (*EGFR* only), and 2 (1.6%) were not analyzable due to PCR failure. Of analyzable samples, 31 (25%) had *EGFR* mutations and 25 (20%) had *KRAS* mutations. The mean DNA yield was 1.13ug (range 0.02-18.14ug). Ten samples with the lowest DNA amounts (range 0.08-0.47ug) which yielded *EGFR*/*KRAS* mutations had a median tumor cell count of 773 (range 112-1687) per representative section, with tumor cells representing on average 59% of the cellularity (range 14-90%). Twenty-one (16%) of 128 patients had an additional surgical or cytologic specimen that had undergone molecular testing. All mutations identified in multiple samples were concordant, except for specimens from patients with multiple primary tumors (n=4). *EGFR* mutations identified in cytologic specimens were strongly associated with never-smoker status (p<0.001) and were more common in females (p=0.28), whereas *KRAS* mutations were associated with smoking (p<0.001).
Conclusions: Various types of cytologic specimens are suitable for *EGFR*/*KRAS* testing. Cell blocks triaged by pathologists as "adequately cellular" yield sufficient DNA for molecular testing, with only rare exceptions. Mutations can be identified in cell blocks containing only several hundred tumor cells. Concordance with mutations from other specimens and clinicopathologic correlates similar to those previously established for surgical specimens further support the validity of mutation analysis in cytologic specimens.

1727 Association of NF2 Loss and MTOR Pathway Activation in Pleural Mesothelioma: Comprehensive Genetic Analysis of NF2 and Immunohistochemistry in 13 Cell Lines and 53 Tumors.

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Background: NF2 deletion has been described in >50% of pleural malignant mesotheliomas (MM) and NF2 inactivation has recently been causally linked to activation of the mTOR pathway in both MM and schwannomas, prompting renewed interest in defining NF2 status in MM and other cancers to guide targeted therapy with mTOR inhibitors.
Design: NF2 status was determined in 13 MM cell lines and 53 MM tumors using array CGH and sequencing. Immunohistochemistry (IHC) for NF2 (Sigma), mTOR, Phospho-mTOR (Ser2448), 4EBP1, Phosho-4EBP1 and Phosho-S6 (all from Cell Signaling) were performed on 47 tumors to look for a correlation between NF2 status and mTOR pathway activation.
Results: By array CGH, 4/13 (31%) MM cell lines showed NF2 loss and 6/13 (46%) MM cell lines showed absence of NF2 protein by western blot. Inactivating mutations were present in 5/13 cell lines (38%) (2 frameshift indels, 3 nonsense mutations). Two of 5 mutations co-occured with single copy NF2 loss (absence of NF2 protein by western in 1/2); the other 3 mutations were associated with normal NF2 copy number by aCGH but absence of NF2 protein by WB. By array CGH, 66% (35/53) of the tumors showed loss of NF2 and this was correlated with decrease of NF2 mRNA by expression microarray (p=0.002) and by IHC (p=0.007; for scoring, the % positive tumor cells was multiplied by the intensity (0 = no staining; 1 = faint staining; 2=moderate staining; 3= strong staining), giving a score between 0 and 300. Scores of triplicate TMA cores were averaged; a group comparison by ANOVA test was used). NF2 mutations were found in 11 patients (20%) (3 nonsense mutations, 1 missense mutation, 5 frameshift indels and 2 splice site mutations), of which 7 also showed single copy NF2 loss. By IHC, NF2 genomic loss was associated with increased Phospho-mTOR expression (p=0.002; similar scoring as NF2). The IHC combination of absent NF2 staining (score<100) and strong Phospho-mTOR (score > 200) was seen in 42% (13/31) of NF2-altered MM and in 12.5% of NF2-unaltered MM (2/16). We did not find significant correlations between NF2 loss and total-mTOR (p=0.08), phosho-4EBP1 (p=0.26), 4EBP1 (p=0.05) or phosho-S6 expression (p=0.22).
Conclusions: Our results confirm a high prevalence of NF2 inactivation in MM. Furthermore, we show an increase of phospho-mTOR expression in tumors lacking NF2 expression confirming the link between NF2 and the mTOR pathway. Staining for NF2 and Phospho-mTOR could be useful in selecting MM patients for trials targeting mTOR pathway activation due to NF2 loss.

1728 microRNAs as a Tool for the Subtyping of Non-Small Cell Lung Carcinoma (NSCLC).

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Background: The classification of NSCLC has become very important in the last few years, because of the specific treatment and clinical side effects implied. There is a large number of miRNAs that can be of use in making the diagnosis of different types of carcinoma. The aim of this study is to establish the utility of different microRNAs in accurately subclassifying malignant lung tumors.
Design: We have studied 146 cases of lung tumors, 72 histologically diagnosed as squamous cell carcinoma and 74 as adenocarcinoma. In this series we studied 79 frozen tissue and 67 formalin-fixed, paraffin-embedded tissue samples. All these cases were analysed by Real Time-PCR for: miR-21, miR-205 and miR-218. Data were analyzed with SPSS 15.0 by using Discriminant analysis and T-Test.
Results: According to our results, 83% of the cases that were originally diagnosed as adenocarcinoma or squamous cell carcinoma had a characteristic expression profile when considering miR-205, miR-21 and miR-218. The level of expression of miR-205 was higher in adenocarcinoma than in squamous cell carcinoma, being this difference statistically significant (p<0.001). The comparison between both tumors did not show any significance when assessing the level of expression of miR-21 and miR-218. In both series of frozen and paraffin-embedded tissue samples, the results were similar and therefore significant.
Conclusions: We conclude that miR205 may become an important aid to ascertain the pathological diagnosis of the main subtypes of NSCLC. These results should encourage further studies to confirm this data, as well as suggest the possible application to serological studies for early diagnosis in patients with lung cancer. Supported by: FIS 08/0135

1729 MASH1: A Specific Immunohistochemical Marker for High Grade Neuroendocrine Tumors of the Lung.

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Background: Mammalian/human achaete-scute homolog 1(M/hASH1) is a member of the basic helix-loop-helix family of transcription factors. MASH1 has been shown to play an obligatory role in the development of neuroendocrine cells. No detailed comparative study has been conducted to explore the immunohistochemical utility of MASH1 in distinguishing different types of lung cancers. We investigated the expression of MASH1 in lung cancers including squamous cell carcinoma (SCC), adenocarcinoma (ADC), typical carcinoid (TC), atypical carcinoid (AC), large cell neuroendocrine carcinoma (LCNEC) and small cell lung carcinoma (SCLC) by immunohistochemistry.
Design: Eighty-eight surgically resected NETL including 38 TCs, 14 ACs, 11 LCNECs and 24 SCLCs as well as tissue microarrays of 183 cases of ADC and 101 cases of SCC of the lung were immunohistochemically studied using a monoclonal antibody against MASH1 (BD Biosciences, clone 24B7.2D11, 1:500). Nuclear staining was considered positive. Staining intensity was graded from 1 to 3 and percentage of tumor cells in each grade was estimated. The product of each intensity scale and the percentage of positive tumor cells for each stain in each case was added to calculate cumulative scores. A case was considered positive if 5% or more tumor cells had positive nuclear staining. Pathologic diagnosis was confirmed with immunohistochemical and mucicarmine stains. A p value of <0.05, as determined by Kruskall-Wallis one way analysis of variance on ranks and Dunn's method using SigmaStat 3.0 (Aspire Software International).
Results: Immunohistochemical studies showed all cases of ADC and SCC were discreetly negative for MASH1. Twenty of 38 TCs (63.2%), 9 of 14 (64.3%) ACs, 8 of 11 (72.7%) LCNECs, and 19 of 24 (79.2%) SCLC were positive for MASH1. TC, AC, and LCNEC had weaker intensity and lower percentage of tumor cells positive for MASH1 than SCLC. The majority of positive TC, AC, and LCNEC demonstrated weak to moderate intensity while SCLC often demonstrated moderate to strong positivity. There were statistically significant differences in median scores between SCLC (170) and low grade NETL, TC (5) and AC (5), but no such difference was detected between LCNEC (40) and SCLC (170).
Conclusions: MASH1 is a specific marker to distinguish NETL from SCC and ADC. Additionally, high grade NETL, especially SCLC have stronger positivity for MASH1 than their low grade counterparts. These findings support that MASH1 is a useful diagnostic marker for segregating SCLC from other NETL and differentiating NETL from non-NETL

1730 KRAS Amplification Is Restricted to KRAS Mutated Lung Adenocarcinomas.

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Background: *KRAS* mutations are well studied in lung carcinoma, but their relationship with *KRAS* amplification was rarely reported. Recent studies suggested that combination of *KRAS* mutation with amplification may indicate worse prognosis. The aim of our study was to determine the frequency of *KRAS* amplification in lung adenocarcinomas (ADC) in respect to oncogenic mutations, and their clinicopathologic characteristics. The allelic imbalance between *KRAS* mutated allele (MA) and wild type allele (WTA) was also assessed.
Design: 122 surgically resected, formalin fixed, paraffin embedded lung ADC (73 *KRAS* mutated, 20 *EGFR* mutated, 29 wild type) were randomly selected for *KRAS* FISH analysis. Dual color FISH was performed using a Spectrum Green- labeled chromosome 12 centromeric probe (Abbott Molecular, Des Plaines, IL) and a Spectrum-Orange labeled, locus specific *KRAS* (RP11-295I5, CHORI, Oakland, CA) probe. *KRAS* /CEP12 ratio ≥ 2 was considered positive for amplification. To characterize the incidence and significance of allelic imbalance between *KRAS* mutated allele (MA) and wild type allele (WTA), sequencing electropherograms (SE) were semi-quantitatively scored and categorized in two groups: MA>WTA or MA≤WTA.
Results: *KRAS* amplification was identified in 5 out of 122 ADC (4%), all of which harbored *KRAS* mutation (5/73; 7%). The 3 cases with *KRAS* mutation and amplification presented at higher stage and experienced worse survival. Four cases showed predominant solid morphology. *KRAS* mutated cases with MA>WTA demonstrated *KRAS* amplification, validating SE as a quantitative method of evaluating the "dosage" of *KRAS* MA. None of *KRAS* mutated cases negative for *KRAS* amplification showed MA>WTA.
Conclusions: *KRAS* amplification seems to occur in KRAS mutated ADC only and may have poor prognostic implications. A semi-quantitative evaluation of *KRAS* allelic imbalance may help to identify cases with KRAS amplification and should be considered to be a part of routine reports of *KRAS* sequencing. Further studies of prognostic and therapeutic impact of the *KRAS* MA level and *KRAS* amplification are warranted.

1731 High Frequency of Coexpression of Maspin with p63 and p53 in Squamous Cell Carcinoma but Not in Adenocarcinoma of the Lung.

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Background: Maspin, a member of the serpin family of protease inhibitors, has been shown to inhibit tumor growth and suppress metastasis in several malignancies, including lung cancer. Previous studies have reported that p63 and p53 control maspin expression by transactivating its promoter. The aim of this study was to determine if maspin expression is correlated with p63 and p53 in squamous carcinoma and adenocarcinoma of the lung.

Design: Tissue microarrays of lung adenocarcinoma (n=161) and squamous carcinoma (n=86) were generated. Immunohistochemical studies were performed using antibodies against maspin, p63, and p53. Both nuclear and cytoplasmic staining for maspin and nuclear staining for p63 and p53 were considered positive and the percentage of positively stained cells was recorded. Concordance was considered if both maspin and p63 or p53 were expressed in the same tumor. The immunostaining intensity was graded as weak, moderate, or strong. The case with >5% of stained tumor cells was considered positive. A p value of <0.05, as determined by Fisher's exact test, was considered statistically significant.

Results: Immunohistochemical studies showed that maspin, p63 and p53 were expressed in squamous carcinomas of the lung with a majority of cases exhibiting strong positivity and the frequencies of their staining were 83/86 (96.5%) for maspin, 79/86 (91.9%) for p63 and 77/86 (89.5%) for p53. Adenocarinomas of the lung showed more variable intensity and much lower positivity for these markers than squamous carcinomas with 82/161 (50.9%) for maspin, 16/161 (9.9%) for p63 and 99/161 (61.5%) for p53. These differences were statistically significant with p<0.01 for maspin, p<0.01 for p63 and p<0.01 for p53. The frequency of concordance between maspin and p63 was 78/86 (90.7%) and 8/161 (5.0%) in squamous carcinoma and adenocarcinoma of the lung, respectively; the frequency of concordance between maspin and p53 was 75/86 (87.2%) and 62/161 (38.5%) in squamous carcinoma and adenocarcinoma of the lung, respectively. The concordance rates between maspin and p63 or p53 in squamous carcinoma and adenocarcinoma were significantly different (p<0.01).

Conclusions: Maspin, p63 and p53 are highly expressed in squamous carcinoma of the lung and expression concordance between maspin and p63 or p53 is much higher in squamous carcinoma, but not in adenocarcinoma of the lung. These findings indicate that both p63 and p53 may control maspin expression in squamous carcinoma, but not in adenocarcinoma of the lung.

1732 Nonsmall Cell Lung Carcinoma (NSCLC), Not Otherwise Specified (NOS) Diagnosed by Fine Needle Aspiration Biopsy (FNAB): Reviewed with Attempt To Reclassify with a Specific Diagnosis, Based on Classic Cytomorphological Features (CCMF).

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Background: Treatment options for lung cancer have made specific cell typing of NSCLC significantly more relevant than in the past. A large proportion of NSCLC present with a clinically advanced stage rendering them inoperable & they are often diagnosed by cytology without tissue sampling. A fraction of NSCLC are interpreted as NOS. The aim of this study is to attempt to reclassify NSCLC, NOS into a specific diagnosis using CCMF, allowing more targeted treatment regimens.

Design: 41 FNABs with an initial diagnosis (IDX) of NSCLC, NOS were independently reviewed by 2 pathologists using previously agreed upon CCMFs, including 8 criteria for adenocarcinoma (ACA) & 6 criteria for squamous carcinoma (SCA), in an attempt to reclassify them more specifically. The 41 cases of NSCLC, NOS were mixed with 33 FNABs that had an IDX of ACA (17) or SCC (16) as control cases. All specimens included direct smears with Diff-Quik and Papanicolaou stains; cell blocks were not used .A third independent pathologist organized cases & tallied results.

Results: 74 cases in total were reviewed. Of the 41 NSCLC, NOS, independent, specific cell type agreement by 2 pathologists occurred in 80% of cases, in the remaining 20% a diagnosis was made by consensus conference. Of the 41 cases of NSCLC, NOS a specific diagnosis was made in 33 or 80% of cases. 27 (66%) were classified as ACA, 5(12%) as SCC, and 1(2%) as adenosquamous carcinoma. 20% remained as NSCLC, NOS. Of the 33 controls the pathologists independently agreed in 94% of cases. The major criteria for reclassifying NSCLC, NOS to ACA were delicate cytoplasm, peripherally located nuclei and rudimentary acinar formation; for SCA, polygonal shapes and centrally placed nuclei were the predominant features. A histologic diagnosis was available in 19 of the 74 total cases. The review cytologic diagnosis (RCD) agreed with the tissue diagnosis in 78% of cases. In 3 of the disagreements, the RCD was ACA, whereas the original tissue diagnosis favored SCA; the reverse occurred in one patient.

Conclusions: Many lung cancer patients are treated soley on a FNAB diagnosis, often without the benefit of cell block material for immunohistochemical studies. Most NSCLC, NOS diagnosed by FNAB can be classified specifically by well recognized CCMF alone, thus allowing more specific therapeutic interventions & improved patient care.

1733 Inconsistent Findings of PET-CT Imaging in Identifying Malignancy in Cytology or Biopsy Proven Small Cell Carcinoma: A 3 Year Experience in 54 Cases.

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Background: It has become accepted that combined Positron Emission Tomography-Computerized Tomography (PET-CT) has a role in the diagnosis and prognostic staging in Non-small cell lung cancers (NSCCs) especially with standardized uptake values (SUVs) > 8 when compared to normal tissues (liver). Small cell carcinomas (SCCs) are reported to have SUVs of 8 or higher but its value in diagnosis or prognosis has been debated. We have offered PET-CT in our facility since mid-2008 and use it in lung cancer staging. We investigated its use in diagnosis and staging of small cell carcinomas of all sites.

Design: We searched our files for all cases of SCC in the lung and at distant sites diagnosed since 2008 and reviewed the clinical, radiographic, cytologic and histologic findings. PET-CT was performed using 18F-FDG PET fused with a low dose non-contrast CT scans. SUVs were reported for primary and metastatic lesions seen. The values were recorded and compared to pathologic data.

Results: There were 93 patients with small cell carcinoma and 54 patients had 1 or more PET-CT scans. SUVs of the primary tumor were as follows: 3 or less (17/31%/avg. 1.1), >3 but <6 (6/11%/avg. 4.5, >6 (29/54%/avg. 13.2) & no value (2/4%). Patients with a value >8 were 24/54 or 44%. Many metastatic lesions (cecum, rectum, lymph node, etc) had SUV's of 0.

Conclusions: PET-CT is useful in the diagnosis, staging and prognosis in NSCCs but is not as useful in small cell carcinomas. A low SUV in a lung mass does not guarantee a benign diagnosis, as seen in this study. Its value in staging may be useful with locations with high SUVs but many metastatic lesions in our study were negative. This may give a falsely low staging in SCC of lung. This may be very important in decisions of resections of limited stage SCC of the lung. Mediastinal staging with tissue diagnosis may still be of value in such patients. PET-CT is a fairly recent modality of staging and only with time and more data in pathologic correlations to reported SUV values will its use be truly determined.

1734 miR-205 and miR-21 Quantification in Adenocarcinomas and Squamous Cell Carcinoma of the Lung.

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Background: Classification of non-small cell lung cancers is of paramount clinical relevance, as novel chemotherapeutic agents show different efficacy in adenocarcinomas (ADC) as compared to squamous cell carcinomas (SQCC). The measurement of miR-205 and miR-21 may be an additional tool for the distinction between ADC and SQCC. Aim of our study is to compare morphological and immunohistochemical classification with miR-205 and miR-21 relative quantification in surgically resected and well characterized lung tumors.

Design: A series of 50 consecutive lung carcinomas (25 ADC, 24 SQCC, 1 adenosquamous) have been histologically confirmed and immunostained for TTF1, p63, p40, desmocollin 3, napsin A, CK5, CK7. Quantification of microRNA expression was carried out using TaqMan MicroRNA Assay kits according to manufacturer's protocol (Applied Biosystems, USA). Real-time RT-PCR assay is based on a stem-loop RT primer design. U6snRNA is a widely used endogenous reference RNA in microRNA quantification experiment. Normalized Ct of miR-205 and miR-21 was calculated according to Lebanony et al. (*J Clin Oncol*. 2009;27:2030-2037.).

Results: miR-21 relative levels are similar in SQCC and ADC, while miR-205 relative levels are lower in ADC (p<0.0001). The miR-205 sample score value is higher in ADC compared to SQCC; accordingly 22 tumors can be classified as ADC and 28 as SQCC, although 8 cases (2 SQCC and 6 ADC) are in the range of "near cut-off values". 4 cases classified as SQCC according to the sample score method corresponded to cases classified as ADC on the basis of morpho-immunohistochemical evaluation.

Conclusions: miR-205/miR-21 relative quantification seems a promising diagnostic tool. However the molecular approach is still not completely satisfactory as it may misclassify a non negligible percentage of cases, but it could be used as an adjunctive diagnostic criterion in selected cases.

1735 Lung Adenocarcinoma in Young Adults: *EGFR/KRAS/ALK* Mutation Status and Clinicopathological Features in 64 Patients under Age 40.

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Background: Lung cancer in patients <40 years old is rare and adenocarcinoma is the most common histological type. Poor outcomes and the availability of targeted therapies prompt the need for an accurate clinicopathological and molecular characterization of adenocarcinomas occurring in this age group. Here, we evaluated 64 consecutive lung adenocarcinoma cases from patients under age 40.

Design: From 09/2004 to 03/2010, 64 lung adenocarcinomas arising in patients 15-39 years old were tested for *EGFR* mutations (exon 19 del and L858R mutation in exon 21) using sensitive mutation-specific PCR assays, *KRAS* mutations (codon 12 and 13 in exon 2) by PCR-sequencing, and *EML4-ALK* rearrangement by FISH. Of 64 cases, 63 and 47 cases had sufficient material for *EGFR/KRAS* and *ALK* testing, respectively. H&E histology and/or cytology slides were reviewed and 56 tumors were histologically categorized as (1) well-, (2) moderately- & moderately to poorly-, and (3) poorly differentiated. Differentiation could not be determined in 8 cases due to scant material. Clinical data on 2964 lung adenocarcinoma patients over age 40 were used for comparison. Associations were tested using the Fisher exact test.

Results: Among these 64 patients, there were 34 (53%) women and 30 (47%) men including 39 (61%) never smokers. Nine of 63 (14%) had *EGFR* mutations (8 exon 19 del and 1 L858R), and 10/63 (16%) had *KRAS* mutations (5 G12D, 2 G12V, 1 G12C, 1 G12A, and 1 G13D). *EML4-ALK* translocation was detected in 7 of 47 (15%) cases. Poorly differentiated tumors were more frequent in men than in women (16/25; 64%

vs. 9/31; 29%, p=0.01). Twelve of 34 (35%) women and none of 30 men presented at stage I (p=0.0002). In comparison to older male patients, men under 40 were more likely to present at stage IIIB/IV (25/30; 83% vs. 633/1099; 58%, p=0.004), and were less likely to present at stage I (0/30; 0% vs. 299/1099; 27%, p=0.0002).
Conclusions: In this subset of patients with lung adenocarcinoma, the high proportion of never smokers suggests an etiology unrelated to tobacco, also supported by the very low prevalence of the smoking-associated *KRAS* G12C mutation and the relative frequency of the *KRAS* G12D mutation typically seen in never smokers. A relatively high percentage of *EML4-ALK* rearrangement in these patients may warrant *EML4-ALK* detection as a first-line/high-priority molecular test in this age group. A relatively higher frequency of late stage at presentation and a higher likelihood of poorly differentiated adenocarcinomas in men <40 may imply more aggressive tumor biology.

1736 Correlation of Twist Expression in Primary Non Small Cell Lung Carcinomas with Histologic Subtypes, Risk of Metastases, and Overall Patient Survival.

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Background: Literature suggests metastasis involves the activity of Twist, a highly conserved basic helix-loop-helix transcription factor that down-regulates E-cadherin expression, allowing epithelial-to-mesenchymal transition (EMT). It is believed EMT allows carcinoma cells to disseminate to distant organs. Twist over-expression has been shown to be an independent marker in predicting metastatic risk and overall prognoses in various cancer types (i.e. melanoma, breast carcinoma, ovarian, and prostate cancer). To our knowledge, Twist expression in NSCL carcinomas has not been well studied. The goal of our study is to assess Twist expression in adenocarcinoma primary lung tumors of varying degrees of differentiation and different histological subtypes, and correlate this with metastasis risk and patient prognosis.
Design: Monoclonal Twist antibody was used to stain sections of primary tumor of resected lung cancer cases in which permission was granted for research purposes.. Twist staining was graded (0-3+) based on an average of positive nuclear stained cells per six 40X fields (0: no staining, 1+: 1-75, 2+ 76-150, 3+ >150). Counts were performed blindly as to TNM status, histologic subtype, and patient survival time.
Results: ANOVA analysis proved significant difference between histologic subtypes ($p<0.05$) and degrees of differentiation in adenocarcinomas ($p<0.05$). Appropriately, bronchoalveolar carcinoma subtype showed absent Twist staining. Significant difference of Twist expression was observed between low and high T-stage cases ($p<0.03$). Twist over-expression was observed in tumors with positive node metastasis compared to tumors without ($p = 0.07$). Kaplan Meier survival curve showed strong correlation with high Twist expression and low overall survival, and low expression with good prognosis.
Conclusions: In agreement with recent studies stating prognoses varies with histologic subtypes, our data shows significant difference in Twist expression amongst corresponding subtypes. Significant difference in Twist expression was noted in poorly differentiated adenocarcinomas, as compared to moderately and well-differentiated forms. Twist was found to be over-expressed in tumors with nodal involvement compared to those without. In conclusion, our findings suggest that high Twist expression is an independent marker in NSCLC prognosis, and can be useful in risk stratification and tailoring therapeutic modalities.

1737 Biopsy Site Changes in Lung Carcinomas Following Core Needle Biopsy: A Potential Pitfall in the Assessment of Stromal Invasion.

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Background: Although biopsy site changes are known to cause diagnostic difficulties in thyroid and breast lesions, especially when assessing invasion, such changes have not been noted in the lung. Assessment of invasion is important in lung cancers to separate bronchioloalveolar carcinoma (BAC) from ordinary adenocarcinoma. The aim of this study is to determine whether biopsy related changes occur in the lung and whether they may impact this differential diagnosis.
Design: Lobectomy specimens were examined from patients who had previous core needle biopsies showing adenocarcinoma suggestive of BAC. The presence of biopsy site changes was noted, and the following features were recorded: histologic appearance, size of biopsy site changes, proximity to tumor and presence of entrapped epithelium. The duration since biopsy was also noted.
Results: There were 22 resected adenocarcinomas (14 minimally invasive adenocarcinomas and 8 BACs) with a prior CT-guided core biopsy. Biopsy site changes were identified in 6 of 22 (27%), including 4 minimally invasive adenocarcinomas and 2 BACs. The interval between core biopsy and resection in these 6 cases ranged from 12-35 days (mean, 21). The biopsy site in all 6 cases consisted of an elongated scar composed of collagen and proliferating fibroblasts. Scattered lymphocytes and plasma cells were present in 5 and hemosiderin-laden macrophages in 4. The biopsy scars ranged from 0.8-7.5 mm in length and 0.4-1.7 mm in width. The biopsy site was in close proximity to the tumor in 3 cases, and was partially present within the tumor in the other 3. Benign entrapped lung epithelium was present within the scar in all 6. The 3 cases in which the biopsy site scar was partially within the tumor all contained entrapped malignant epithelium (2 minimally invasive adenocarcinomas and 1 BAC).
Conclusions: Biopsy site related changes can be identified in a significant proportion of lung tumors following core needle biopsy. They need to be distinguished from tumor related stromal reactions that are considered an indication of invasion and are important in the differentiation of BAC and well differentiated adenocarcinoma.

1738 The Diagnostic Efficacy of Combining Bronchoscopic Tissue Biopsy and Endobronchial Ultrasound-Guided Fine Needle Aspiration for the Diagnosis of Malignant Tumors in the Lung.

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Background: Bronchoscopic tissue forceps biopsy (BBX) is a standard procedure for diagnosis of malignancy in the lung. Endobronchial ultrasound-guided fine needle aspiration (EBUS-FNA) has also proved to be a sensitive alternative to tissue biopsy for the diagnosis and staging of lung tumors. We report our institutional experience of diagnostic yield when combining BBX and EBUS-FNA in the bronchoscopic evaluation of patients who present with lung lesion(s).
Design: The pathology files at our institution were searched for all BBXs performed between 1/09 and 6/10 for the diagnosis of malignancy and to select patients that had undergone combined EBUS-FNA and BBX procedures. The data points included site of the biopsy, cytologic and histopathologic diagnoses and available clinical follow-up.
Results: A total of 288 BBXs were reviewed; 118 patients had BBX combined with EBUS-FNA and 110(93%) patients received a definitive pathologic diagnosis. Malignancy was diagnosed in 96(81%) patients; BBX and EBUS-FNA of the lung lesion only were performed in 22 patients, BBX and EBUS-FNA of the lymph node(s) only in 79 patients with BBX and a combination of EBUS-FNA of the lung lesion and lymph node(s) in 17 patients. Of the 21 patients with malignant diagnoses who underwent BBX and EBUS-FNA of the lung lesion only, 13(62%) had tumor in both, 3(14%) had tumor only in BBX and 5 (24%) had tumor only in EBUS-FNA. Of the 64 patients with malignant diagnoses that underwent BBX and lymph node staging by EBUS-FNA, 29(45%) had tumor in both, 19(30%) had tumor only in BBX and 16(25%) had tumor only in EBUS-FNA(s). Of the 11 patients who underwent BBX and EBUS-FNA of the lung lesion and lymph node(s), 5(45%) had tumor present in all specimens, 2(18%) had tumor in BBX and EBUS- FNA of the lung lesion only, 3(27%) had tumor only in BBX and 1(9%) had tumor in only EBUS-FNA of the lung lesion. Immunostains were performed for 74(77%) patients and molecular testing for 12(13%) patients.
Conclusions: In our experience, diagnostic yield is increased when bronchoscopic diagnostic technologies (BBX and EBUS-FNA) are combined. In a significant number of patients where BBX was negative, EBUS-FNA provided diagnostic material, thus increasing diagnostic yield by 18%. In a subset of these patients the EBUS-FNA also helped in the staging of a primary tumor. By combining these procedures, more tissue was obtained for immunohistochemistry and molecular testing, which facilitated personalized clinical management in a minimally invasive manner.

1739 Detection of NUT Midline Carcinoma in Retrospective Analysis of Thymic and Mediastinal Neoplasms.

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Background: NUT midline carcinomas (NMC) comprise a group of highly aggressive tumors that arise primarily in the head, neck and mediastinum, predominantly in younger individuals. Their defining characteristic is overexpression of the nuclear protein in testis (NUT) due to a chromosomal translocation. Although the earliest cases were described as thymic in origin, an extensive survey for NMC in a series of thymic and unclassified mediastinal carcinomas has not been reported.
Design: We queried our pathology database, including consult files, from 2001-2010 using search terms "thymic", "mediastinal", and "carcinoma"; thymomas or tumors for which another primary site was identified were excluded. Diagnosis of NMC was based on strong, diffuse nuclear staining with a clinically validated NUT-specific monoclonal antibody.
Results: Among 119 cases of thymic and unclassified mediastinal carcinomas identified, 38 cases had material currently available for testing. These consisted of 11 unclassified or undifferentiated malignant epithelioid neoplasms, 7 thymic carcinomas, 7 poorly-differentiated carcinomas, 4 squamous cell carcinomas, 4 favor non-small cell carcinomas, 2 poorly-differentiated neuroendocrine carcinomas, 1 favor small cell carcinoma, 1 sarcomatoid carcinoma, and 1 favor malignant germ cell tumor. Patients screened were on average 48.9 years old (ranging from 8 to 89 years) and included 18 females and 20 males. One NMC was identified in a 61 year old female with a mediastinal mass, previously diagnosed as unclassifiable epithelioid malignant neoplasm. This tumor exhibited a nested arrangement of uniform, moderately-sized, epithelioid cells that contained abundant granular eosinophilic cytoplasm, vesicular chromatin, and prominent nucleoli. The tumor was negative for keratins, p63, neuroendocrine markers, and EMA.
Conclusions: NUT translocations were detected in 2-3% of patients with mediastinal neoplasms in this series. The findings highlight the fact that NMC can arise in patients of advanced age. Although rare, this entity should be considered in the differential diagnosis of any poorly-differentiated mediastinal tumor.

1740 Increase of EZH2 Expression in Bronchiolar Epithelial Cells in Smoking-Related Diseases of the Lung.

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Background: Epigenetic modifications of histones play essential roles in tumorogenesis. Enhancer of zeste homolog 2 (EZH2), a key component of polycomb repressive complex 2, mediates histone H3 methylation at lysine 27. EZH2 has been demonstrated via immunohistochemical staining to be expressed in multiple human malignancies, including squamous cell carcinoma of the lung. However, its role in early tumorogenesis remains unclear. Previously we demonstrated scattered sparse EZH2 expression in basal bronchiolar cells. The aim of this study was to determine if there is differential EZH2 expression in the bronchiolar epithelial cells of patients with a smoking history and smoking-related lung diseases, specifically respiratory bronchiolitis (RB) and

desquamative interstitial pneumonia (DIP) as compared to those of patients with no history of smoking.
Design: Twenty-five surgically resected lung specimens were immunohistochemically studied using a monoclonal antibody against EZH2 (Leica), including 9 cases of RB, 7 cases of DIP, and 9 cases from non-smokers. Clinical history was examined to confirm that all patients with RB and DIP were current or past smokers. For each case two bronchioles with similar luminal circumferences were examined. For each bronchiole, 100 contiguous non-neoplastic, non-metaplastic bronchiolar epithelial cells were examined and the percentage of EZH2 positive cells was recorded. The percentage of EZH2 positive cells was averaged for each case. Nuclear staining was considered positive for EZH2. A p value of <0.05, as determined by Fisher's exact test, was considered statistically significant.
Results: Immunohistochemical studies showed that the majority of the EZH2 positive cells were localized in the basal layer of bronchiolar epithelium. The values (Mean±SD) of EZH2 positive bronchiolar epithelial cells were 5.11±1.16% in non-smoking patients, 21.71±7.27% in patients with DIP, and 16.72±3.50% in patients with RB. There was no significant difference between the percentage of EZH2 positive bronchiolar epithelial cells in the RB and DIP groups (p=0.51). However, there was a statistically significant difference in EZH2 positivity in bronchiolar epithelial cells in patients with RB and DIP as compared to examined cases from non-smokers without these pathologies (p=0.01).
Conclusions: Our study shows that smoking-related respiratory diseases (RB and DIP) are associated with an increase of EZH2 expression in the bronchiolar epithelial cells, which indicates smoking induced EZH2 expression may be involved in the early stage of carcinogenesis.

1741 EZH2 Expression Is Correlated with Histologic Grade of Lung Adenocarcinoma.

JJ Findeis-Hosey, LA McMahon, Q Yang, F Li, H Xu. University of Rochester, NY.
Background: Enhancer of zeste homolog 2 (EZH2) is the catalytic subunit of polycomb repressive complex 2 with histone methyltransferase activity. EZH2 has been demonstrated via immunohistochemical staining to be expressed in multiple human malignancies, including prostate carcinoma, small and large cell neuroendocrine carcinoma of the lung, and squamous cell carcinoma of the lung. The aim of this study was to examine EZH2 expression in atypical adenomatous hyperplasia (AAH), bronchioloalveolar carcinoma (BAC), and primary lung adenocarcinoma.
Design: A total of 183 cases of surgically resected lung specimens were immunohistochemically studied using a monoclonal antibody against EZH2 (Leica), including 6 cases of AAH, 7 cases of BAC, and 170 cases of primary lung adenocarcinoma. The lung adenocarcinomas were further segregated by histologic grade, and included 20 cases of well-differentiated (WD), 88 cases of moderately differentiated (MD), and 62 cases of poorly differentiated (PD) adenocarcinoma. All 170 lung adenocarcinoma cases and 2 BAC cases were examined as part of a tissue microarray, while the remaining 6 cases of AAH and 5 cases of BAC were examined as representative resection sections. The percentage of positively stained tumor cells was recorded and the staining intensity was graded as negative, weak, moderate, or strong. Nuclear staining in greater than 5% of tumor cells was considered positive for EZH2. A p value of <0.05, as determined by two-tailed Fisher's exact probability test and Chi-square test, was considered statistically significant.
Results: Immunohistochemical studies showed EZH2 staining in only 5 (25%) of 20 cases of WD adenocarcinoma, as compared with 53 (85.5%) of 62 cases of PD adenocarcinoma and 69 (78.4%) of 88 cases of MD adenocarcinoma. There was EZH2 staining in 1 (16.67%) of 6 cases of AAH, with 40% of atypical cells demonstrating moderate EZH2 positivity. There was EZH2 staining in 1 (14.29%) of 7 cases of BAC, with 10% of neoplastic cells demonstrating strong EZH2 positivity. There is a statistically significant difference in EZH2 staining among the 3 histologic grading subtypes (p<0.0001).
Conclusions: This study demonstrates that there is differential EZH2 immunohistochemical expression in preinvasive lesions (AAH and BAC) and invasive lung adenocarcinoma. Specifically, there is low frequency of EZH2 expression in AAH and BAC, and high frequency of EZH2 expression in invasive adenocarcinoma, with the majority of cases of MD and PD adenocarcinoma being positive for EZH2. These findings indicate that EZH2 expression may be associated with an aggressive biological behavior.

1742 EZH2 Expression in Invasive Squamous Cell Carcinoma of the Lung and Its Precursor Lesions.

JJ Findeis-Hosey, LA McMahon, Q Yang, F Li, H Xu. University of Rochester, NY.
Background: Enhancer of zeste homolog 2 (EZH2) is the catalytic subunit of polycomb repressive complex 2 with histone methyltransferase activity. EZH2 has been reported to be expressed in multiple malignant neoplasms, but with only limited studies in squamous cell carcinoma (SCC) of the lung and no published reports of EZH2 expression in squamous precursor lesions. The aim of this study was to examine EZH2 expression in invasive SCC of the lung and its precursor lesions, including mild, moderate, and severe dysplasia and carcinoma in situ (CIS).
Design: Tissue microarrays constructed from 99 invasive SCC cases were immunohistochemically studies using a monoclonal antibody against EZH2 (Leica). A separate cohort of 17 resected lung SCC cases with squamous precursor lesions was immunohistochemically studied for EZH2 expression. 2004 WHO definitions of squamous precursor lesions were utilized. Nuclear staining for EZH2 was considered positive. The percentage of positively stained cells was recorded and the staining intensity was graded as negative, weak, moderate or strong. A p value of <0.05, determined by Fisher's exact test, was considered statistically significant.
Results: Ninety (90.9%) of 99 cases of invasive SCC were immunohistochemically positive for EZH2. Normal respiratory epithelium generally included scattered weakly to moderately EZH2 positive cells in the basal layer, with only 2 (11.1%) of 17 cases demonstrating moderate to strong staining in a majority of bronchiolar epithelial cells. In contrast, nearly all areas of squamous metaplasia, dysplasia and CIS were moderately to strongly positive for EZH2 in the majority of cells, with 4 (80%) of 5 cases of squamous metaplasia, 10 (90.9%) of 11 cases of mild dysplasia, 12 (100%) of 12 cases of moderate dysplasia, and all 5 (100%) of 5 cases of severe dysplasia demonstrating moderate to strong EZH2 positivity. Interestingly, only 9 (69.2%) of 13 cases of CIS and 13 (76.5%) of 17 cases of invasive SCC were moderately to strongly EZH2 positive. The frequency of EZH2 expression was significantly different (p<0.0001) between bronchiolar epithelial cells and squamous metaplasia, dysplasia, CIS or invasive SCC.
Conclusions: Our studies showed that EZH2 expression is present in a high proportion of SCC of the lung, as well as its squamous precursor lesions, including squamous metaplasia, squamous dysplasia and CIS, as compared with only limited expression in normal bronchiolar epithelium. These findings indicate that EZH2 expression may be involved in the early stages of carcinogenesis.

1743 Pulmonary Arterial Remodeling in End-Stage Liver Disease.

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Background: Pulmonary arterial remodeling is a well-recognized phenomenon associated with end-stage liver disease (ESLD). Two major pulmonary manifestations of ESLD exist: the hepatopulmonary syndrome and portopulmonary hypertension (POPH). Though both diseases are reported to be of low prevalence, screening for POPH is routine, as POPH is a relative contraindication to orthotopic liver transplantation. Little is known about the pathogenesis of POPH, and there are relatively few detailed quantitative studies of the pulmonary vasculature in ESLD. We reviewed autopsy specimens from 38 patients with ESLD, 11 with known POPH, in order to quantify pulmonary vascular morphology compared to normal controls.
Design: Using trichrome/elastic stained histologic sections, we performed computer-assisted morphometry of pulmonary arteries in 38 patients with ESLD and 12 patients with no known pulmonary or hepatic pathology. Eleven of the patients with ESLD had known POPH. A mean of 75 arteries per patient (range = 22-123) were analyzed, with diameters ranging from 50-1000 micrometers. Using calibrated software, tracings were made of external and internal elastic laminae, and endothelial surfaces, so as to calculate the areas occupied by the intima, media, and lumen of each vessel.

	Cases (males)	Mean Age	No. Arteries
POPH(-) ESLD	27 (18)	52	1907
POPH(+) ESLD	11 (4)	54	827
Control	12 (10)	38	748

Results: Pulmonary arteries in all patients with ESLD exhibited significant intimal thickening compared to controls (p < 0.01), greatest in patients with POPH (p = 0.02). Significant medial atrophy was present in ESLD patients without POPH (p = 0.01). Media area in POPH was not statistically different from controls (p = 0.45). Plexiform or thrombotic lesions were not present in any cases.

	Media Area		Intima Area	
	Mean	Std Dev	Mean	Std Dev
ESLD (no POPH)	7968	5069	9500	3680
POPH	10827	6550	11531	8241
Control	12850	6070	5118	2324

Area in square-microns
Conclusions: In this study, pulmonary arterial remodeling occurred in all patients with end-stage liver disease. These patients all exhibited pulmonary arteries with marked intimal thickening, usually accompanied by significant medial atrophy. However, in cases of POPH, the medial area is not significantly different from controls, suggesting the arterial media is either regenerated or spared. Whether the preservation of medial thickness is the cause or effect of pulmonary hypertension is unknown. Nevertheless, our findings suggest that the prevalence of pulmonary vascular pathology in ESLD is greatly underestimated.

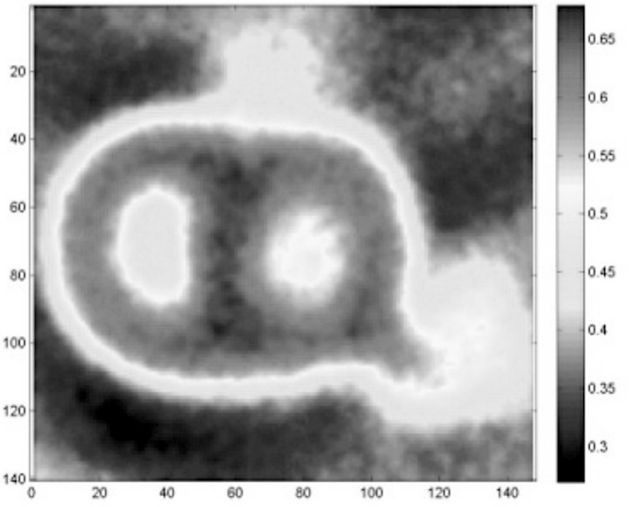
1744 Prognostic Factors for Stage I Non Small Cell Lung Cancer: A Single Institution Experience.

F Franzi, A Imperatori, N Rotolo, L Dominioni, C Capella, F Sessa. University of Insubria, Varese, Italy; Multimedica IRCCS, Milan, Italy.
Background: The 5-years survival rate of patients with stage I non-small cell lung cancer (NSCLC) ranges greatly from 50% to 90%. The aim of this single Centre study was to evaluate the expression and the prognostic significance of some biological markers in resected stage I NSCLC patients.
Design: The expression of p53, Ki-67, ERCC-1 and TTF-1 was immunohistochemically analyzed in 157 resected stage I NSCLC, according to the 7th ed. TNM classification, (79 adenocarcinomas (AC); 61 squamous cell carcinomas (SQC); 11 bronchioloalveolar carcinoma (BAC);4 large cell carcinomas; 2 adenosquamous carcinomas); the median follow-up was 60 months. TTF-1 immunoreactivity was documented by using the commercially available clones, 8G7G3/1 and SPT24. Univariate and multivariate analysis was performed for clinico-pathological and immunohistochemical parameters.
Results: The 5-year overall survival was 49,6%. The overexpression of p53 was significantly more frequent in males and in SQC patients and was associated with high tumor grade and high Ki-67 proliferative activity. A significant correlation was also shown between the high expression of Ki-67 and high tumor grade. ERCC-1 was detected in 50% of NSCLC patients with a significantly higher frequency in SQC; moreover, ERCC-1 expression was correlated with high Ki-67 proliferative activity. TTF-1 expression was significantly greater in AC and in BAC. The SPT24 antibody

detected a significantly higher number of AC than the 8G7G3/1 clone (p=0.0009). TTF-1 expression was significantly inversely correlated with Ki-67 proliferative activity, p53 overexpression and with ERCC-1 expression. TTF1 positive immunoreactivity correlated with higher tumour differentiation and with better prognosis. The factors significantly correlated to better survival at the univariate analysis were: stage IA, low p53 protein expression and low Ki-67; at multivariate analysis, only stage IA and low p53 protein expression were independent favorable prognostic factors.
Conclusions: This study confirms that in early stage NSCLC pathological stage IA and a low p53 expression are independent favorable prognostic factors. We also demostrated a relevant prognostic role for Ki-67. TTF1 expression might have a slight prognostic implication based on its correlation with better tumour differentiation, low Ki-67 proliferative activity and tendency for better survival. In addition we showed that the SPT24 antibody is more sensitive than the 8G7G3/1 clone for labeling lung adenocarcinomas.

1745 A Novel Computer Image Analysis Algorithm Improves Inter-Rater Reliability in the Evaluation of Respiratory Mucosal Ciliary Ultrastructure.

K Funkhouser, M Neithammer, J Carson, B Baker, S Minnix, M Leigh, M Knowles, W Funkhouser. UNC, Chapel Hill, NC.
Background: Primary ciliary dyskinesia (PCD; MIM 242650) is a genetic disease causing defects in the structure or function of cilia. It is characterized by recurrent infections of the upper and lower respiratory tract due to reduced mucociliary clearance. Presently, diagnosis requires identification of dynein arm loss in transmission electron microscopy (TEM) images. These images exhibit highly variable background signal in the axoneme, which can confound interpretation of dynein arm presence or absence. We developed a novel computer image analysis algorithm in the MATLAB(c) environment to align axonemal microtubular pairs. The purpose of this study was to determine whether inter-rater reliability of PCD diagnosis could be improved through the use of this method.
Design: In a randomized, double-blind experiment, two morphologists evaluated dynein arm loss in 32 cases of possible PCD. Each evaluated the presence of inner and outer dynein arms using three distinct methods. In the first method, each morphologist was given all of the scanned TEM images for the cases. For the second and third methods, 10 in-focus axonemes from each case were processed by our software. In the second method, each morphologist was given 10 averaged images (1 averaged image of the 9 microtubular pairs for each of 10 axonemes) for each case. In the third method, each morphologist was given a single averaged image of all 90 microtubular pairs in the 10 axonemes for each case.
Results: Cohen's kappa (K) was calculated for each of the three methods. For the "gold-standard" TEM technique, we found K=0.68. Using 10 computer-generated axonemal averages per case, we found K=0.81. Using only the single computer-generated average of all microtubular pairs per case, we found K=0.94. An example of a computer-generated average displaying inner dynein arm loss is shown below.



Conclusions: Our novel image alignment algorithm improves inter-rater reliability for evaluation of dynein arm loss in ciliary axonemes. Studies are underway to determine whether the accuracy of TEM-based PCD diagnosis is also improved.

1746 Prognostic Significance of Histological Subtypes, and Calretinin, Mesothelin, p16 and p53 Expression in Pleural Malignant Mesothelioma: A Study of 311 Cases from the MESOPATH IM@EC Center.

F GalateauSalle, N Le Stang, M Paciencia, C Drougard, V Abonnet, Referral Center MESOPATH IM@EC. Chu Caen, France.
Background: Age, epithelioid type, T and N staging are conventional prognostic factors in pleural malignant mesothelioma [PMM] useful for the management of the patients. We aim to analyse whether histological subtyping, together with immunohistochemical markers (calretinin, mesothelin, p16, p53) can predict a better outcome.
Design: We reviewed H&E slides of 311 DPMM patients from the MESOPATH files during 1980-2008. We classified the tumors according to the WHO 2004 classification and we subclassified the epithelioid type into predominantly trabecular, predominantly papillary (either micro or macropapillary) and solid type. We also evaluated the presence of necrosis. Additionally we evaluated calretinin Zymed, mesothelin, p53 and p16 expression. A score was attributed taking account the number of cells (0 to 100%) of stained cells and the intensity of staining and were graded (1;0-24%, 2;25-74%,3;75-100%). Log Rank tests and Cox models were used to analyse the relation between histological and immunohistochemical variables and survival.
Results: There were 223 male (72%) and 88 female (28%) with an average age of 70 years old (range 28-96). Asbestos exposure was observed in 204 patients (66%). The histological type was epithelioid (n= 248, 80%), biphasic (n=27,9%), sarcomatoid (n=28, 9%) and desmoplastic (n= 8, 2%). Patients with sarcomatoid type had the worst median survival with 5 months followed by biphasic 7 months and epithelioid type 14 months. Among the 248 epithelioid type, the solid variant (n= 124, 50%) had the poorer survival with a median of 11months followed by trabecular variant 16 months(n=16, 28%), while papillary subtype (n=24, 50%) had a median survival of 24 months. Calretinin expression was higher expressed in papillary (78%, grade 3) than trabecular (67%), solid variant (56%) and sarcomatoid (32%). On univariate analysis age p=0.02, epithelioid type p<0.0001, calretinin grade 3 p<0.0001, mesothelin (p<0.0001), p53 expression(1; 0-24%, p=0.001) and p16 expression (25-100% p=0.0005) were indicators of better survival. In multivariate analysis sarcomatoid type (p=0.03)and necrosis p=0.02 were predictors of short survival.
Conclusions: Our study supports classification by histological type and subtype for epithelioid variant to better stratify survival. Evaluation of calretinin and p 16 expression are indicators of better survival by univariate analysis. In multivariate analysis sarcomatoid type and presence of necrosis favor short survival.

1747 Pulmonary Graft-Versus-Host Disease: A Reterospective Review of a Distinct Clinicopathological Entity.

PA Grieshaber, PP Olivieri, JL Farber. Thomas Jefferson University Hospital, Philadelphia, PA.
Background: Pulmonary graft-versus-host disease (PGVHD) is an increasingly recognized complication of bone marrow transplantation (BMT). The histologic diagnosis of PGVHD has focused on obliterative bronchiolitis (OB) and constrictive bronchiolitis (CB) in the context of chronic GVHD. PGVHD begins as a lymphocytic bronchiolitis (LB) and alveolitis (LA). Recognition of these early features of PGVHD clarifies its natural history and allows a broader basis for its recognition. Using LB and LA as criteria in addition to OB and CB, the present study reviewed 19 patients with PGVHD.
Design: Nineteen cases of PGVHD were retrieved from the electronic database of the pathology department. The criteria for the diagnosis included 1) engraftment of the bone marrow transplant, 2) no evidence of an infectious etiology (bacterial, viral, or fungal), 3) no evidence of recurrence of the original disease or history of recent chemotherapy, and 4) a compatible histology (LB and LA, or BO with or without organizing pneumonia, or CB). The electronic medical records of the 19 patients were reviewed to obtain the clinical data.
Results: The 19 patients (13 males, 6 females) ranged in age from 21 to 67 years (mean 46). Fifteen were white, 3 were African-American, and 1 Hispanic. Malignant diseases necessitated BMT in 16 patients (8 leukemia, 4 lymphoma, 3 multiple myelome, and 1 Hodgkin lymphoma). Two patients had myelofibrosis and 1 aplastic anemia. All transplants were allogenic. Conditioning regimins included chemotherapy alone (14) or chemotherapy plus total body radiation (5). The mean time from BMT to diagnosis of PGVHD was 15 months (range 3-46). The clinical indication for lung biopsy was rarely a suspicion of PGVHD. All except one of the patients had a prior diagnosis of GVHD in one or more organs (skin 5, GI 3, skin and GI 10). All but 2 of the patients were currently on immunosuppressive therapy for GVHD at the time of lung biopsy. Eleven patients expired, 4 are alive, and for 4 have been lost to follow up for the last 2 years.
Conclusions: PGVHD as defined here presents as a distinct entity. LB and LA evolve into OB, CB, organizing pneumonia, and a nonspecific, chronic interstitial pneumonitis. Clinically, PGVHD is a late complication of allogenic BMT, almost invariably in association with previously diagnosed GVHD in another organ (skin or GI tract) and in the context of ongoing immunosuppression. The fact that the diagnosis of PGVHD is followed by a significant mortality rate emphasizes the importance of the recognition of the early phases of this disease (LB and LA) as emphasized here.

1748 Expression and Clinical Significance of Prohibitin in Primary Lung Squamous Cell Carcinoma.

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Background: Lung cancer is the leading cause of cancer death for both man and women in the world. Studying the carcinogenesis of lung squamous cell carcinoma with proteomic approaches may identify carcinogenesis-associated proteins. The purpose of the study is to discover potential biomarkers for diagnosis, treatment and prevention of the lung squamous cell carcinoma by using proteome-based approaches.
Design: Protein samples from 10 paired lung squamous cell carcinoma (SqCC) tissues and noncancerous tissues were compared by fluorescent 2-D differential gel electrophoresis (2-D DIGE) .Validation of a candidate biomarker was carried out with Western blotting and immunohistochemistry analysis of 71 cases of lung SqCC. The immunostaining in tissue sections was divided into high and low expressions based on the intensity (1+ to 3+) and proportion (<40%, >40%) of positive cells. The correlation between PHB expression and clinicopathological indicators of lung SqCC was determined by using x^2 test. Kaplan-Meier methods were used to compare survival rates. Cox proportional hazards models were used to identify independently significant variables.

Results: Thirty-six proteins whose expression altered significantly in the tumor tissue were identified by mass spectrometry. Among them, a 30-kDa protein prohibitin (PHB) was up-regulated in lung SqCC, which was confirmed by immunoblotting. In immunohistochemistry, high expression of cytoplasmic PHB was seen in 41 out of 71 SqCC patients, and was associated with UICC classification of tumor grade (p=0.011), tumor size (p=0.03), lymph node metastasis (P=0.01) and pathological stages (p=0.002). During the median 56 months of follow-up, patients who had the high expression of PHB had low over-all(P=0.008) and disease-free survival rates (P=0.034) by Univariate analysis. Multivariate analysis indicated that PHB was an independent prognostic factor for SqCC (P=0.04).
Conclusions: The expression of PHB may play an important role in the development and progression of human squamous cell carcinoma. PHB is likely to be a clinical prognostic biomarker and may be a potential therapeutic target for lung cancer.

1749 EGFR Mutations in Exons 18, 19 and 21 Are Closely Associated with TTF-1 Positive Lung Adenocarcinoma.

M Guo, Q Si, Y Gong, N Kalhor, S Chen, C Moran. The Univerisity of Texas M.D. Anderson Cancer Center, Houton.
Background: Molecular testing to detect EGFR and KRAS mutations are frequently utilized for targeted therapy in patients with non-small cell lung carcinoma, especially adenocarcinoma. Thyroid transcription factor 1 (TTF-1) positivity has been used as supportive evidence of primary lung adenocarcinoma or metastatic adenocarcinoma of lung origin. However, published data on the associations of EGFR or KRAS mutations with the TTF-1 status in lung adenocarcinoma is scant. In this study, we compared the EGFR and KRAS mutations in lung adenocarcinomas with positive or negative TTF-1 immunostain results.
Design: From our pathology data between January 2009 and June 2010, we identified 629 cases of lung adenocarcinoma with available EGFR and KRAS mutation results. The mean age of the patients was 64 years (range, 35-89). The results of EGFR or KRAS mutations detected by DNA sequencing were compared with TTF-1 status measured by immunostains in lung adenocarcinoma.
Results: Of the 629 adenocarcinomas, EGFR mutations were detected in 146 (23.2%), and KRAS mutations were observed in 147 (23.4%). Of the 344 adenocarcinomas that had TTF-1 results, 292 (85%) tumors were positive for TTF-1. The distribution of EGFR or KRAS mutations in adenocarcinomas with positive or negative TTF-1 results is listed in the Table 1. The frequencies of EGFR mutations were significantly higher in TTF-1-positive adenocarcinomas than those of TTF-1-negative adenocarcinomas (P = 0.016), specifically in exons 19 (44.7%, 34/76 vs. 0/76), 21 (40.1%, 31/76, vs. 1/4) and 18 (9.2%, 7/76 vs. 0/76). Significantly higher frequency of EGFR exon 20 mutation was detected in TTF-1-negative carcinomas (75%, 3/4) compared to that in the TTF-1- positive carcinomas (5%, 4/76) (P = 0.002). KRAS mutations were not significantly different between TTF-1-positive and TTF-1-negative adenocarcinomas (P = 0.314).

Table 1. Distribution of EGFR and KRAS mutations in lung adenocarcinoma with positive or negative TTF-1 immunostain results

TTF-1	Case No.	EGFR (%)	P-value	KRAS (%)	P-value
Positive	292	76 (26.0)	0.016	65 (22.3)	0.314
Negative	52	4 (7.7)		16 (30.8)	

Conclusions: TTF-1 positive status in adenocarcinoma of lung is significantly associated with EGFR mutations in exons 18,19 and 21. EGFR exon 20 mutation is significantly associated with TTF1-negative lung adenocarcinoma. KRAS mutations are not associated with the TTF-1 status in lung adenocarcinoma.

1750 Histopathological Features of Acute Hypersensitivity Pneumonitis.

LP Hariri, EJ Mark, ML Onozato, Y Yagi, B Shea, S Digumarthy, A Fraire, O Matsubara, M Mino-Kenudsen. Massachusetts General Hospital, Boston; University of Massachusetts Memorial Medical Center, Worcester; National Defense Medical College, Tokorozawa, Japan.
Background: Hypersensitivity pneumonitis (HP) is an interstitial disease which develops in response to antigen exposure, characterized by a bronchiolocentric chronic inflammatory response. Cases can be stratified by antigen load and exposure duration into acute, subacute, and chronic HP. While the pathologic features of subacute HP are established, those of chronic HP have only recently been described. No definitive pathological features of acute HP exist, because patients with acute presentation or rapid symptom resolution are infrequently biopsied.
Design: We evaluated 21 cases of clinically confirmed HP: 4 acute HP (acute, high-dose exposures without a background of subacute or chronic HP), 3 acute exacerbations in a background of subacute/chronic HP, 11 subacute HP, and 3 chronic HP. We semiquantitatively evaluated the bronchiolocentricity of chronic inflammation, loose histiocytic aggregates, giant cells, bronchiolitis obliterans, and organizing and/or fixed fibrosis. Interstitial inflammatory infiltrates were also evaluated (lymphocytes, plasma cells, eosinophils, and neutrophils). Fibrin content was assessed with phosphotungstic acid hematoxylin. Statistical comparisons were conducted using the Student's t-test.
Results: In acute HP and acute HP exacerbation, the extent of fibrin deposition was significantly greater (> 10% surface area with fibrin deposition/total area of disease) compared with subacute and chronic HP (p = 0.0016). Interstitial neutrophils were increased in the former (> 5 per HPF) when compared with the latter (p=0.0127). Fixed fibrosis was absent in all cases of acute HP, but was present in 1 of 3 cases of HP with acute exacerbation. One of the cases of acute HP had intraalveolar fibrin so marked it resembled acute fibrinous and organizing pneumonia. There was no statistically significant difference in the other characteristic features for HP between the 4 groups.

	Acute	Acute exacerbation	Subacute	Chronic	p-value
No. patients	4	3	11	3	
Sex	2F/1M	3F/0M	5F/6M	2F/1M	
Age	44.7 ± 11.5	44.7 ± 29.1	49.7 ± 19.8	55.7 ± 13.0	
Bronchiolocentric (y/n)	3/4	3/3	10/11	3/3	
Loose histiocytic aggregates (0–4)	2.75 ± 0.95	1.67 ± 0.6	2.3 ± 0.8	1.3 ± 0.6	
Giant cells (0–4)	0.25 ± 0.5	1.3 ± 1.5	1.8 ± 1.1	1.67 ± 0.6	
Bronchiolitis obliterans (y/n)	3/4	2/3	9/11	3/3	
Organizing collagen (0–4)	2.25 ± 1.5	2.0 ± 1.7	2.0 ± 0.6	2.0 ± 0.0	
Fixed collagen (0–4)	0.0	0.67 ± 1.1	0.6 ± 0.6	2.0 ± 1.0	0.0012
Interstitial lymphocytes (0–4)	1.5 ± 0.6	2.0 ± 1.7	1.6 ± 0.9	1.3 ± 1.5	
Interstitial plasma cells (0–4)	0.5 ± 0.6	2.0 ± 1	1.3 ± 1.0	1 ± 0.0	
Interstitial eosinophils (no./hpf)	4.25 ± 0.95	4.3 ± 0.6	3.0 ± 3.0	1.3 ± 1.15	
Interstitial PMN (no./hpf)	25 ± 16.8	13.3 ± 7.6	1.7 ± 3.2	0.0	0.0127
PTAH (% of diseased tissue)	42.5 ± 28.7	7.5 ± 3.5	1.9 ± 3.2	0.67 ± 0.6	0.0016

Conclusions: Fibrin greater than 10% in the area of inflamed lung and more than 5 neutrophils/HPF characterized acute HP or acute exacerbation of an underlying subacute or chronic HP. The finding of fibrin in these biopsies should raise suspicion for an acute antigen exposure.

1751 High-Resolution Volumetric Imaging of Lung Pathology: Improving Biopsy Sampling and Evaluation.

LP Hariri, M Mino-Kenudsen, EJ Mark, MB Applegate, GJ Tearney, BE Bouma, MJ Suter. Massachusetts General Hospital, Boston.
Background: Histologic diagnosis of lung biopsies can be limited by small size or incomplete sampling. Larger tissue volumes can improve diagnostic yield, but may be difficult to obtain. Optical frequency domain imaging (OFDI) is a high resolution imaging modality providing 3-dimensional views of tissue microstructure to depths approaching 2mm. Large area volumetric OFDI is a possible adjunct to traditional biopsy to provide tissue images larger than biopsies and direct biopsy sampling. We performed OFDI through two approaches (bronchoscopic airway centered imaging and pleural based parenchymal imaging) and correlated to histopathologic findings.
Design: Airway and/or parenchymal images were obtained with a specialized bronchoscopic 2.4 French catheter and a bench-top external imaging system for 13 surgical and autopsy specimens. Corresponding tissue samples were processed for histologic comparison.
Results: OFDI was performed in 7 lung carcinomas (2 squamous cell, 1 adenosquamous, 1 adenocarcinoma, 1 undifferentiated carcinoma, and 2 mucinous bronchioloalveolar carcinomas [BAC]), 2 tracheal mucoepidermoid carcinomas, 1 carcinoid tumor, 1 cartilaginous hamartoma, and 2 interstitial fibrosis cases. Adjacent normal tissue was assessed when present. OFDI of normal airway allowed high resolution visualization of epithelium, lamina propria, submucosal glands, cartilage, blood vessels and alveolar attachments. Carcinomas exhibited architectural disarray, loss of normal airway and alveolar structures, and rapid light attenuation. Squamous cell carcinomas showed nested architecture on OFDI, while malignant glandular formations could be appreciated in adenocarcinomas and mucoepidermoid carcinomas. Mucinous BACs showed alveolar wall thickening with intra-alveolar mucin.

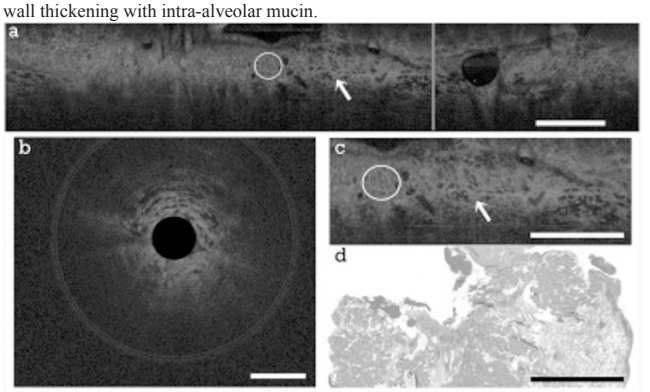

Figure 1. Pleural based imaging of mucinous bronchioloalveolar carcinoma. (a) Longitudinal section, scale bar: 5 mm. (b) Cross-section, taken at location of line in panel (a), scalebar: 1mm. (c-d) Higher magnification of longitudinal section and corresponding histopathology, scalebar: 5 mm. Thick arrow: Alveolar wall thickening, Circle: Intra-alveolar mucin.

Conclusions: This study is the first demonstration of volumetric high-resolution microscopy with correlation to tissue-based diagnostics for evaluating lung tumors. While tissue biopsy remains essential as the gold standard, OFDI may provide a tool for *in vivo* identification of malignant features and improved tissue sampling with targeted guidance.

1752 Acquired *BRAF* Gene Mutations in Pleural Malignant Mesothelioma: A Potential Therapeutic Target.

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Background: Pleural malignant mesothelioma (PMM) remains an aggressive tumor with a short median survival and a poor response to conventional treatment modalities. Current treatments, including chemotherapy, radiotherapy, and surgical resection, are aimed mainly at palliation. Research into alternative therapies using molecular targeted therapy, gene therapy, and immunotherapy has not been conclusive at this time. Profiling of the genetic alterations responsible for tumorigenesis is needed to advance our understanding of the disease and guide future research efforts. Somatic missense mutations in the *BRAF* gene have been identified in a variety of human cancers but have not been reported in PMM.

Design: From the consultation files of one of the authors (KS), paraffin blocks from 40 cases of immunohistochemically confirmed PMM including various histologic subtypes with adequate tumor were pulled to assess for mutations in the *BRAF* gene. Briefly, DNA was extracted from paraffin blocks using QIAamp DNA FFPE Tissue Kit (Qiagen). A 107 base pair in length region of the BRAF gene, including codon 600, was amplified by Real-Time PCR on the LightCycler (Roche) and subjected to high resolution melting curve analysis using the HR-1 High Resolution Melting Instrument (Idaho Technologies). Known *BRAF* wild type and *BRAF* ^{V600E} mutant DNA samples were included as negative and positive controls respectively. All samples with an abnormal melting curve that was not aligned with the wild type melting curve, and was suggestive of a mutation, were further examined by direct DNA sequencing in both forward and reverse directions using BigDye Terminator Cycle Sequencing kit (Applied Biosystems) on an ABI PRISM 3100 Genetic Analyser.
Results: Of 40 PMM, 2 (5%) harbored a *BRAF* mutation. One sarcomatoid subtype demonstrated *BRAF* ^{V600E}, the most common *BRAF* mutation seen in malignant melanoma, papillary thyroid carcinoma, colorectal cancers, and other human malignancies. The second case with mixed epithelial and sarcomatoid histologic subtype revealed a novel *BRAF* ^{D587G} mutation.
Conclusions: *BRAF* is mutated in PMM at a low frequency. Given the dismal prognosis of PMM, the small subset of patients who demonstrate activating *BRAF* mutations in their tumors may benefit from the administration of new BRAF kinase inhibitors (such as PLX4032) that have shown promising results in clinical trials enrolling patients with *BRAF* ^{V600E} mutated melanoma.

1753 The Predictive Value of *EGFR/KRAS/BRAF* Genotyping and TP53 Expression in a Series of 54 Patients with Lung Adenocarcinoma Treated with Tyrosine Kinase Inhibitors.

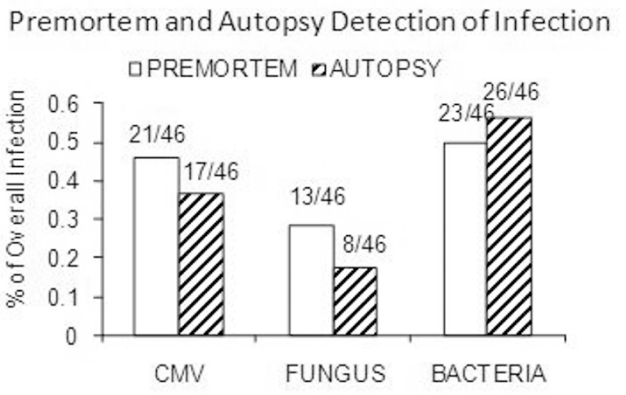
S Houlle, A Perel, F Blanchard, A Lamy, P Courville, L Thiberville, J-C Sabourin. Rouen University Hospital, France.
Background: Adenocarcinomas (ADK) represent 30 to 40% of lung carcinomas. Tyrosine kinase inhibitors (TKI) are used to treat patients with advanced carcinomas harboring activating mutations of the EGF receptor (*EGFR*). Recent studies have demonstrated that TKI efficacy depends on the mutational status of specific molecular markers such as *EGFR* and *TP53* while other activating mutations in *KRAS* or *BRAF* lead to resistance. The aim of our study is to retrospectively analyze lung ADK tumor samples to correlate these 4 molecular markers with clinical and histological data.
Design: 72 patients with lung adenocarcinoma (17 surgical specimens and 55 biopsies) were analyzed; 25 metastasis and 7 recurrences were also studied. Among the 72 patients, 54 patients were treated with TKI. The molecular analysis of *EGFR* was performed by sequencing exons 18, 19, 20 and 21, *KRAS* and *BRAF* genotyping by SNaPshot® multiplex on codons 12, 13 and 61 for *KRAS* and exon 15 for *BRAF*. *TP53* gene expression was quantified by immunohistochemistry on 28 patients with TKI treatment.
Results: 6.9% of *EGFR* mutations and 48.6% of *KRAS* mutations (no mutation in the codon 61 was reported) were identified while no *BRAF* mutation was found. Interestingly we observed only one case with both *EGFR* and *KRAS* mutations. *EGFR* mutations are frequently diagnosed in women and also in none mucinous ADK whereas *KRAS* mutations are predominantly found in mucinous ADK. 64.3% of tumors are TP53 positive. The study of the overall survival of patients with *EGFR/KRAS* mutations and TP53+ is under analysis. Mutational discordances between primary tumors and metastasis or primary tumors and recurrences represent 8% for *EGFR* and 24% for *KRAS*. Different mutations between primary tumor and metastasis were sometimes encountered emphasing the heterogeneity of these tumors.
Conclusions: The identification of molecular alterations in *EGFR/KRAS* genes and TP53 expression seems to be important for the outcome of patients treated with TKI. Today, the use of these inhibitors merely requires detection of activating mutations in *EGFR*. The detection of *KRAS* mutations (more frequent, technically easier to perform and mostly exclusive to *EGFR*) might also be necessary for TKI administration. Our results suggest that *BRAF* is not implicated in the carcinogenesis of these tumors. Mutational discordances between primary tumor site and metastasis encourage the analysis of the metastasis site if accessible.

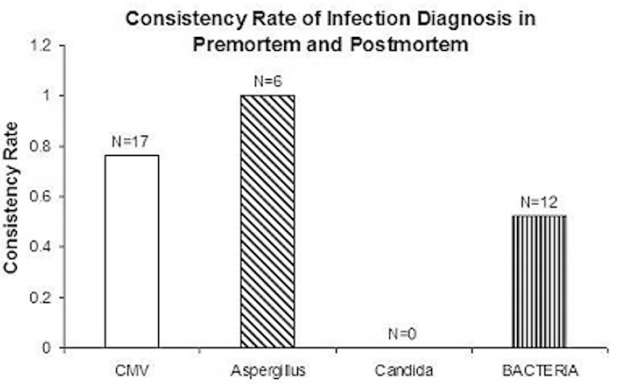
1754 Determining Percent EGFR Activation in Tumor Cells in Intact FFPE Tissue Sections.

C Hoyt, K Lane, G Innocenti, R Wetzel. Cambridge Research and Instrumentation, Inc., Woburn, MA; Cell Signaling Technology, Danvers, MA.
Background: Many areas in oncology research depend on revealing signaling pathway activity in FFPE tissue sections. However, techniques such as immunohistochemistry, micro-array detection, and analysis of sample lysates provide averages from volumes of tissue, including many cells not of interest. These methods blur out key proteomic information that resides at the cellular level, relating to the signaling states of individual cells. The purpose of this study is to demonstrate effective, practical and reliable cytometric analysis of signaling pathway proteins in intact tissue sections, using EGFR expression and activation as examples. In lung cancer, EGFR expression and/or activation have been linked to therapeutic response as well as angiogenesis and metastasis.
Design: The approach integrates multiplexed immuno-fluorescence labeling technology that is robust and specific, automated multispectral high-throughput image acquisition to capture and distinguish multiple labels (Vectra™), and image analysis algorithms to differentiate relevant tissue regions (e.g., malignant and normal epithelia, stroma, necrosis, etc.) and segment cellular compartments. We designed a pilot study using human tumor xenografts to determine whether per-cell co-expression subtypes can be reliably determined. We describe methodology and present results using amplified and unamplified non-small cell lung carcinoma cell lines (H1975/L858R, H3255/L858R, H1650/E746_A750del, and HCC827/E746_A750del). A multi-label immunofluorescence kit from Cell Signaling Technology was used, labeling total EGFR, pEGFR and pERK, plus a DAPI counterstain.
Results: Analysis was performed on sections from multiple xenografts of from each cell line. Vectra's pattern-recognition-guided high power field selection was used to acquire ten 20x multispectral images of tumor regions. Machine-learning-based automated image analysis was performed to locate cancer cells, segment subcellular compartments, and extract IF signals on a per-cell basis from each field. Percentage of EGFR+ cells that were actively signaling (pEGFR+) was calculated for each xenograft. Percent expression levels concur with molecular phenotype.
Conclusions: Together, the multispectral platform and image analysis software, with multiplex panels of activation state-specific antibodies, reveal molecular subtypes, which may lead to new targeted strategies for oncology research and potentially clinical care, to diagnose progression of disease, guide therapy selection, and monitor response.

1755 Comparison of Infectious Pathogen Detection in Pre and Postmortem Specimens from Lung Transplant Patients.

Z Hu, Y Li, J Gagermeier, CG Alex, R Love, MM Picken. Loyola University Medical Center, Maywood, IL.
Background: Infection/sepsis remains a major cause of death post lung tx. Various methods, including transbronchial (tx), play pivotal roles in evaluating infection post lung tx. At autopsy, lung tissue is often cultured to detect infection. However, the consistency and value of these premortem and postmortem methods are still unclear.
Design: 76 lung tx patients, autopsied in the past 2 decades, were retrospectively compared with regard to premortem and autopsy findings on the detection of various infectious pathogens.
Results: Of 76 lung tx patients, 46 pts died of infection/sepsis post tx, caused by bacteria/virus/fungus. CMV was the main viral pathogen. 21 pts were CMV (+) by premortem PCR/culture/BAL/transbronchial bx. 17 were detected at autopsy, with a consistency of 76%. Aspergillus/Candida were the main fungal pathogens. 6 cases of Aspergillus infection were identified premortem by lung bx/culture, which was 100% consistent with autopsy findings. However, none of the 7 cases with Candida infection diagnosed premortem was identified at autopsy. Pseudomonas was the main bacterial pathogen, both premortem and at autopsy. 23 patients were detected with bacterial infection/sepsis by premortem blood/sputum/urine culture, while 26 were found at autopsy by lung tissue culture. Among these patients, 12 had a (+) premortem bacterial culture, consistent with autopsy (52%). However, 11 cases with a (+) premortem bacterial culture were not identified at autopsy, and 24 cases with a (+) bacterial culture at autopsy were not detected premortem.





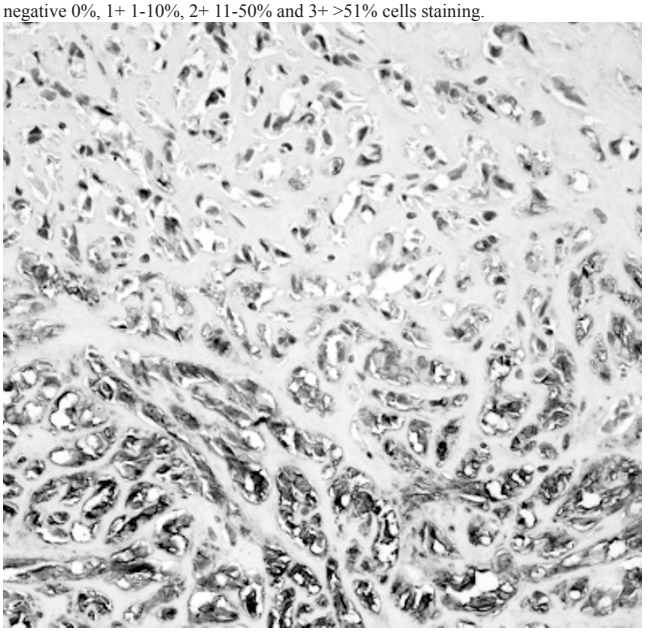
Conclusions: Premortem and autopsy findings showed different consistencies in detecting various pathogens. Aspergillus and CMV were most consistently detected. In contrast, bacteria showed a low consistency rate, which may be due to therapeutic intervention, contamination, or the inaccuracy of the techniques used.

1756 How Useful Is GLUT-1 in Differentiating Mesothelial Hyperplasia and Fibrosing Pleuritis from Epithelioid and Sarcomatoid Mesotheliomas?

AN Husain, AR Gibbs, K Hiroshima, Y Chi, R Boumendjel, N Le Stang, F Galateau-Salle. University of Chicago, IL; Llandough Hospital, Penarth, United Kingdom; Tokyo Women's Medical University Yachiyo Medical Center, Yachiyo, Japan; MESOPATH IM@EC, Caen, France.

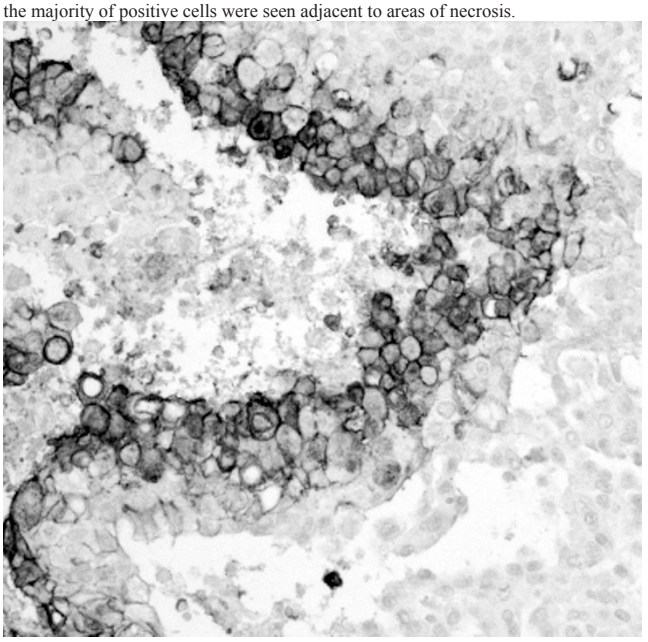
Background: Mesothelial hyperplasia (MH) and fibrosing pleuritis (FP) can be difficult to distinguish from epithelioid (MM-E) and sarcomatoid (MM-S) malignant pleural mesotheliomas. GLUT-1 is one of the isoforms of glucose transporters which is not expressed on most benign epithelia and stromal fibroblasts but is expressed in many malignancies. The few studies published show variable results regarding its sensitivity and specificity.

Design: In this retrospective study, well-characterized diagnostic cases (MH=31, FP=29, MM-E=41, MM-S=29) were collected and stained for GLUT-1 (polyclonal, DAKO). All slides were scored visually by 2 pathologists; membranous staining was scored as negative 0%, 1+ 1-10%, 2+ 11-50% and 3+ >51% cells staining.



Any membranous staining was considered positive for the overall calculation. Sensitivity was calculated as % positivity among patients with disease (MM) and specificity as % of negativity among non-diseasesd (reactive).

Results: All benign cases (n=60) were negative for GLUT-1 while 42 of 70 (60%) MM [21 of 41 (50%) MM-E and 21 of 29 (72%) MM-S] had 1+ to 3+ staining. Of the MM-E, 10 had 1+, and 11 had 2+ staining; of the MM-S 3 had 1+, 15 had 2+ and 3 had 3+ staining. The overall sensitivity was 60% and specificity was 100%. In many cases the majority of positive cells were seen adjacent to areas of necrosis.



Conclusions: Positive staining with GLUT-1 is helpful since it is positive in half of epithelioid and three-quarter of sarcomatoid MM. Although all reactive mesothelial lesions were negative, a negative stain does not exclude the diagnosis of MM. As with all IHC stains, GLUT-1 has to be used as part of a panel, and the results interpreted in the context of clinical, radiological and histological findings.

1757 Immunoreactivity of Napsin A in Non-Lymphoid Tumors of Anterior Mediastinum, a Comparative Study with TTF-1.

PG Hwang, LA Litzky, PJ Zhang. Hospital of the University of Pennsylvania, Philadelphia.

Background: Of the non-lymphoid tumors, primary pulmonary carcinoma, thymoma and germ cell tumor are among the tumors most commonly encountered in anterior mediastinum. Although these can be usually differentiated by their morphologic characteristics, diagnosis can be difficult in certain circumstance such as limited material. In such case, TTF-1 reactivity would strongly favor a lung primary vs. others. Napsin A is a new immunohistochemical marker that is known to be positive in >80% of pulmonary adenocarcinoma. However, napsin A has not been extensively studied in other commonly encountered mediastinal tumors such as thymoma and germ cell tumor. In attempt to better understand its utility in the differential diagnosis of tumors of anterior mediastinum, we evaluated napsin A in thymoma and germ cell tumor and compared it that of TTF-1.

Design: A total of 37 cases of thymoma of various types and 22 cases of germ cell tumor were retrieved from the Surgical Pathology of the Hospital of the University of Pennsylvania and Presbyterian Medical Center. Napsin A (KCG1.1, 1:100, Abcam) and TTF-1 (8G7G3/1, 1:100, Dako) immunostains were performed on representative section of each case with standard immunohistochemical method.

Results: Of 37 thymomas, two (5.4%) showed focal faint positive napsin A reactivity and one (2.7%) showed focal weak nuclear TTF-1 reactivity. Napsin A and TTF-1 were each focally positive in two (9.1%) of 22 cases of germ cell tumor. In these unusual positive cases, the tumors were mixed germ cell tumor and immunoreactivity of either napsin A or TTF-1 was detected in well-differentiated glandular structures of the teratomatous component in the tumor.

Conclusions: Our finding of rare TTF-1 reactivity in thymoma and germ cell tumor are consistent with that of the prior studies. Similar to TTF-1, we also found that napsin A is also focally positive in rare thymoma and germ cell tumor. In germ cell tumor, focal expression of both markers is typically limited to better differentiated glandular element in the teratomatous area. Positive staining of either of these two markers would make the diagnosis of thymoma or germ cell tumor very unlikely and can be used to confirm a lung primary in the differential diagnosis of an anterior mediastinal tumor.

1758 Assessing the Pleura and Other Objective and Routine Findings Predicting Outcome with Stage 1 Non-Small Cell Lung Carcinoma.

SK Jeffus, AS Nagji, BD Kozower, DR Jones, EB Stelow. University of Virginia, Charlottesville.

Background: Stage 1 non-small cell lung carcinomas (NSCLC) have an excellent prognosis. Larger tumor size has been well documented to increase the risk for adverse outcome. The meaning of visceral pleural involvement is less clear, especially as a number of studies have shown that this finding is assessed by different means throughout the pathology community and, furthermore, there is significant interobserver variability associated with its assessment. Other findings, including identifying squamous or glandular differentiation can be no less troublesome, and some immunohistochemical stains are now used to assess differentiation at some institutions. Here, we investigate the use of routine gross, histologic and immunohistochemical findings for predicting behavior of stage 1 NSCLC.

Design: 78 cases of completely resected NSCLC were used. Smoking history and patient age and sex were recorded. Tumor size, differentiation (by H&E), and distance from visceral pleural surface were recorded. A tissue microarray was constructed and immunohistochemistry was performed with antibodies to TTF1, p63, mutant EGFR (L858M and E746-A750del), and ALK1. All results were compared to outcome.

Results: Patients included 36 men and 42 women; 7 were non-smokers and the mean age was 68 years. Tumor size was as follows: >5cm (n=3; 4%), >3cm and <5cm (n=19; 24%), >2cm and <3cm (n=22; 28%), and <2 cm (n=34; 44%). 33 tumors (42%) extended to within 1 mm or less of the visceral pleural surface. Histologically, 32 tumors were squamous cell carcinoma (41%), 38 were adenocarcinomas (49%) (7 were non-mucinous bronchioloalveolar carcinomas), 2 were adenosquamous carcinomas (3%), and 6 were undifferentiated carcinomas (8%). 9 tumors had lymphovascular space invasion. 48% of tumors were immunoreactive with antibodies to p63 and 56% with antibodies to TTF1. 3 tumors were immunoreactive with antibodies to mutant EGFR (4%) and none with antibodies to ALK1. There were 14 recurrences and/or metastases (18%). Smoking, non-squamous phenotype (by H&E), undifferentiated phenotype and distance of less than 1 mm from the pleural surface were the greatest predictors of adverse outcome (p=0.16, 0.03, 0.001, 0.07, respectively).

Conclusions: Our results confirm that histologic sub-typing (by H&E) and grading of NSCLC (especially identifying undifferentiated tumors) are important for predicting behavior. Of note, measurement of tumor from pleural surface may be a more reproducible method for predicting behavior than assessment of pleural invasion.

1759 Nuclear Grading System Predicts Recurrence in Stage I Lung Adenocarcinoma Patients.

K Kadota, K Suzuki, VW Rusch, AL Moreira, PS Adusumilli, WD Travis. Memorial Sloan-Kettering Cancer Center, New York, NY; Memorial Sloan-Kettering, Cancer Center, New York, NY.

Background: There is no established histologic grading system for lung adenocarcinoma. Architectural and cytologic grading systems are widely accepted for prostate (Gleason) and breast (nuclear) cancers, respectively. We sought to investigate whether a nuclear grading system can predict recurrence among stage I lung adenocarcinoma.

Design: H&E slides of 506 stage I lung adenocarcinoma patients from a single institution (1995 to 2005) were evaluated for the following nuclear features: nuclear diameter, nuclear atypia, nuclear/cytoplasmic ratio, chromatin pattern, presence of intranuclear

inclusion, prominence of nucleoli, mitotic count per 10 high power fields (HPF), and presence of atypical mitoses. We also correlated the nuclear grade with histologic subtyping according to the new IASLC/ATS/ERS classification of lung adenocarcinoma based on predominant morphology: lepidic, acinar, papillary, micropapillary and solid. Log-rank tests and Cox proportional hazard models were used to analyze the association between histological variables and disease-free survival (DFS).

Results: Larger nuclear diameter (P=0.024), nuclear atypia (P=0.020), higher mitotic count (P<0.001), and atypical mitosis (P<0.001) were associated with shorter DFS. Based on these results, we developed a scoring system based on the sum of two factors: nuclear diameter (1: small, 2: intermediate, 3: large) and mitotic count (1: 0-1/10HPF, 2: 2-4/10HPF, 3: ≥5/10HPF). We further established a 3-tier grading system based on this scoring.

Table 1

Nuclear grade	N	Recurrence (%)
Grade I (score 2-3)	252	88.6
Grade II (score 4-5)	177	77.7
Grade III (score 6)	77	71.6

By Kaplan Meier analysis, nuclear grading was significantly associated with DFS (P<0.001). The prognostic value of our grading system remained significant in a subset of patients with papillary and acinar subtype (5-year DFS: 84.2%), with grade III (n=48) showing the worst 5-year DFS (78.2%), followed by grade II (n=130, 80.4 %), and grade I (n=193, 88.4%) (P=0.024).

Conclusions: Nuclear grading system based on nuclear diameter and mitotic count predicts recurrence in stage I lung adenocarcinoma patients. This simple system based on light microscopic review of H&E slides is immediately translatable to identify high risk patients for further interventional studies.

1760 Cytologic Grading Is an Independent Prognostic Factor in Epithelioid Diffuse Malignant Pleural Mesothelioma.

K Kadota, K Suzuki, C Sima, VW Rusch, PS Adusumilli, WD Travis. Memorial Sloan-Kettering Cancer Center, New York, NY.

Background: Other than the poor prognostic categories of sarcomatoid and biphasic histologic subtypes, there is no established grading system for epithelioid diffuse malignant pleural mesothelioma (DMPM). For epithelioid DMPM, tumor and nodal stage are the only validated prognostic markers. We sought to investigate whether cytologic grading can further stratify survival among epithelioid DMPM patients.

Design: H&E slides of 232 epithelioid DMPM patients (14 stage I, 54 stage II, 123 stage III, 34 stage IV) from a single institution (1989 to 2009) were reviewed. We classified the tumors into 4 histologic patterns: trabecular, tubulopapillary, micropapillary, and solid. We evaluated cytologic features including nuclear atypia, nuclear/cytoplasmic ratio, chromatin pattern, presence of intranuclear inclusion, prominence of nucleoli, mitotic count, and presence of atypical mitosis. Lymphatic and vascular invasion was also evaluated. Log-rank tests and Cox proportional hazard models were used to analyze the association between histological variables and overall survival.

Results: Overall median survival of epithelioid DMPM was 1.36 years. Patients with < 10% tubulopapillary (P<0.001) and ≥ 10% solid pattern (P=0.006) correlated with poor prognosis. On univariate analysis, nuclear atypia (P<0.001), chromatin pattern (P=0.031), prominence of nucleoli (P<0.001), mitotic count (P<0.001), and atypical mitosis (P<0.001) were associated with poor survival. Tumors were graded into I-III using nuclear atypia and mitotic count.

Table 1

Cytologic grade	N	Median survival (years)
Grade I (score 2-3)	107	2.34
Grade II (score 4-5)	91	1.14
Grade III (score 6)	34	0.41

Cytologic grade was associated with lymphatic invasion (P<0.001), vascular invasion (P<0.001), T stage (P=0.012), and clinical stage (P=0.009). On multivariate analysis, cytologic grade was an independent predictor of overall survival (P<0.001). Moreover, among 159 patients who underwent R1 resection, higher cytologic grade was significantly associated with shorter median time to recurrence (P=0.004).

Conclusions: Our proposed cytologic grading system is an independent predictor of survival in epithelioid DMPM and it correlates well with other prognostic factors in these patients.

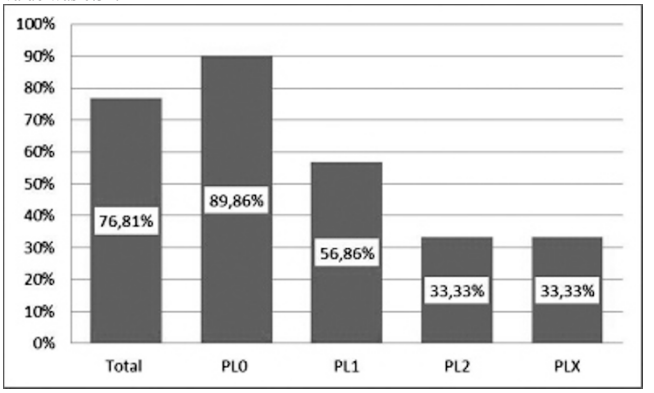
1761 Assessment of Visceral Pleural Invasion (VPI) in Patients with Surgically Resected Non Small Cell Lung Cancer: A Study of Interobserver Agreement.

M Kamionek, A Akalin, K Dresser, A Fraire. One Innovation Drive, Three Biotech, Worcester, MA.

Background: Current standards from the IASLC and the AJCC recommend the use of elastic tissue stain for the assessment of VPI in non small cell carcinoma of lung. The value of this stain has been documented in previous studies including our own (Mod Path 2010, Vol23:405A). However, the interpretation of elastic tissue stains can be difficult. We undertook this study to determine interobserver variability in the assessment of elastic tissue stains. To this end, only tumors located 0.2 cm or less from the pleura were studied.

Design: In this study, VPI was defined as tumor invasion beyond the pleural elastic lamina as follows: PL1 tumor present beyond the elastic lamina, PL2 tumor at the pleural surface and PLX for undetermined cases. From an initial pool of 96 cases and after exclusion of cases with tumors greater than 0.2 cm from the pleura, elastic tissue stains of 69 cases initially staged as T1N0MX were submitted for independent evaluation by 3 observers. Reconciliation of discrepancies was achieved by joint review. Mean interobserver percentage agreement and the kappa statistical method were used to determine interobserver variability.

Results: Interobserver unanimity was achieved in 36 (52.2%) of the 69 cases. The percent agreement was 89.86% for PL0 (among 46 cases), 56.86% for PL1 (among 17 cases), and 33.3% for PL2 (only 1 case) and PLX (among 5 cases) categories. Total percentage agreement was 76.81%. (see bar graph below). The overall kappa value was 0.32.



Conclusions: Our study suggests that interobserver agreement of the integrity of the elastic lamina on basis of elastic stain is excellent (89.86%) in PL0 cases, but only fair at best in the remaining (PL1, PL2) categories. Our findings further suggest that while valuable, elastic tissue stains are probably best assessed by more than one observer.

1762 Mycobacterial PCR Is an Adjunct to Pulmonary Granuloma Diagnosis in Surgical Pathology.

S Kantola, Y-W Tang, JE Johnson. Vanderbilt University Medical Center, Nashville, TN.

Background: Necrotizing granulomatous inflammation in the lung is routinely encountered in Surgical Pathology practice. Acid-fast stains are commonly negative in cases of mycobacterial infection. Rapid, accurate identification of infection with tuberculosis (TB) or non-TB mycobacteria is important for optimal, timely therapeutic decision-making and for protection of nosocomial and public health.

Design: Using SNOMED data, all surgical pathology cases of necrotizing granulomatous inflammation of lung or bronchus from 2004-8 were identified. The slides were reviewed and the H and E interpretation confirmed. "Controls" (n=24) were those in which an etiology other than mycobacterial infection was firmly established, typically with the use of histochemical stains. "Cases" (n=21) were those in which either all stains were negative (n=17), or Ziehl-Neelsen (ZN) or fluorescent AFB stains were positive or suggestive (n=4). Using the H and E slide to direct sampling, the area of the granuloma in the paraffin block was sharply sampled using a scalpel. The DNA was extracted and amplified for a mycobacterial 16S-23S ITS region by using a broad-range primer set. One genus-specific (MYC) and 35 species-specific probes were used for detection, using a Luminex 200 instrument (Austin, TX). This procedure detects and speciates 17 medically important Mycobacterium species.

Results: Mycobacterial DNA was detected in 9 of 21 "cases" and 1 of 24 "controls." Among the 9 PCR-positive "cases," species detected by PCR included M. avium (1), M. intracellulare (1), M.intracellulare/xenopi/smegmatis (1), M. kansasii (2), M. fortuitum (2) M. smegmatis (1), and M. tuberculosis (1). The single PCR-positive "control" sample was GMS-positive for yeast forms consistent with Histoplasma sp.; M. kansasii DNA was detected. PCR-negative"cases" (n=12) included 7 cases in which all stains and cultures were negative; 3 in which either the ZN or fAFB stain was positive; and 1 which was stain-negative but grew M. tuberculosis from a different specimen (BAL).

Conclusions: Mycobacteria can be detected and speciated from archived paraffin-embedded lung sections using PCR technology. Stain- and culture-negative granulomas yield a range of species by PCR, some of which are pathogenic. Up to 1/3 of cases remain negative by all modalities (stains, cultures, PCR). Uncommonly, non-mycobacterial granulomas (e.g. Histoplama sp.) may be associated with PCR positivity for mycobacteria (1/24 in our series; M. kansasii).

1763 Clinicopathological Characteristics of Subcentimeter Adenocarcinomas of the Lung.

F Kato, M Hamasaki, A Iwasaki, H Iwasaki, K Nabeshima. Fukuoka University Hospital and School of Medicine, Japan.

Background: The number of pT1a (≤2 cm in diameter) lung adenocarcinomas, especially of subcentimeter ones, is increasing because of recent development in radiographic techniques. In the current classification, pT1a tumors with a pure lepidic growth pattern (bronchioloalveolar carcinoma, BAC) have excellent prognosis, whereas invasive pT1a carcinomas can lead to recurrence or metastasis in spite of their small sizes. Thus, an accurate pathological diagnosis is critical. The aim of this study was to examine clinicopathological characteristics of subcentimeter adenocarcinomas, which helps in diagnosis of early invasive carcinomas.

Design: We retrospectively reviewed 595 adenocarcinomas resected in 2003-2009 at Fukuoka University Hospital, and analyzed 67 carcinomas that were ≤10 mm in diameter with reference to early invasive features. Invasion was diagnosed when it was associated with reactive fibroblastic proliferation, destruction of elastic fiber frameworks (detected using EvG stain), and discontinuity of subepithelial myofibroblastic layers (using α-SMA immunostaining). We regarded localized fibrous area more than 1 mm in diameter as minute scar (scar).

Results: Of the 67 cases, 38 (56.7%) were invasive and 29 (43.3%) were noninvasive (BAC). BAC and minimally invasive carcinomas (BAC>90%) comprised 48% of all tumors. Invasive carcinomas were significantly more frequent in male than female, and included 32 cases with BAC, and 6 cases without BAC (whole invasive). Moreover, a half of invasive carcinomas had no scar, in which cases we often had difficulty in detection of invasion without EvG stain and α-SMA immunostaining. However, high grade nuclear atypia was always associated with invasive carcinomas and helped in the diagnosis. Compared with adenocarcinomas of 11-20 mm in diameter, those of ≤10 mm included significantly more BAC and less whole invasive carcinomas. Invasive carcinomas with scar or micropapillary pattern were also significantly less.
Conclusions: Being familiar with histopathological characteristics of subcentimeter adenocarcinomas helps in accurate diagnosis of early invasive carcinomas. Moreover, EvG stain and α-SMA immunostaining are useful adjuncts to detect stromal invasion without scar.

1764 Validation of Napsin A/TTF-1 Double Stain in Adenocarcinoma of the Lung Versus Malignant Mesothelioma.

A Khoor, C Cortese, PT Cagle. Mayo Clinic, Jacksonville, FL; The Methodist Hospital, Houston, TX.
Background: Double staining can be performed using two antibodies and a single detection system, if the target antigens are located in different cellular compartments. This can result in cost savings. TTF-1 is a well established pulmonary adenocarcinoma marker; recent studies have indicated that Napsin A may also have a role in separating adenocarcinoma of the lung from malignant mesothelioma. TTF-1 is a nuclear stain, whereas Napsin A shows granular cytoplasmic reactivity.
Design: The objective of this study was twofold: (1) to confirm a role for Napsin A in separating adenocarcinoma of the lung from malignant mesothelioma and (2) to validate the use of Napsin A and TTF-1 as a double stain. Tissue microarrays composed of 114 cases of pulmonary adenocarcinoma and 46 cases of epithelioid malignant mesothelioma were available for the study. Immunohistochemistry was performed using a mouse monoclonal Napsin A antibody (Leica, Newcastle, UK), a mouse monoclonal TTF-1 antibody (Dako, Carpinteria, CA), and the NovoLink Polymer Detection System (Leica) with DAB as the chromogen. The primary antibodies were applied both individually and as a cocktail (double stain).
Results: The 114 pulmonary adenocarcinoma cases stained as follows:

	Single staining method	Double staining method
Napsin A +	92 (80%)	92 (80%)
TTF-1 +	95 (83%)	95 (83%)
Napsin A + and/or TTF-1 +	98 (86%)	98 (86%)

All 46 malignant mesothelioma cases were negative for both Napsin A and TTF-1 with either staining method.
Conclusions: These data indicate that (1) immunohistochemistry for Napsin A is a useful aid in separating adenocarcinoma of the lung from malignant mesothelioma and that (2) Napsin A and TTF-1 can be applied as a double stain without any loss of sensitivity or specificity.

1765 Histological Spectrum of Pulmonary Manifestations in Kidney Transplant Recipients.

S Kirby, A Satoskar, S Brodsky, A Pope-Harman, D Nunley, C Hitchcock, R Pelletier, T Nadasdy, K Shilo. The Ohio State University, Columbus.
Background: After the introduction of novel effective immunosuppressive therapies, kidney transplantation became the treatment of choice for end stage renal disease (ESRD). While these new therapies lead to increased graft survival, they can also cause unwanted complications. Only small series or case reports describe pulmonary pathology in renal allograft recipients. Therefore, we undertook a systematic review of all lung biopsy specimens of kidney transplant recipients and report a spectrum of histological findings.
Design: Laboratory information system database search (from January 2002-2010) revealed 28 renal allograft recipients who required a lung biopsy for respiratory symptoms. They included 19 males and 9 females, 25 to 77 years old (mean age 53 years) and comprised approximately 1% of over 2,100 kidney transplant recipients. There were 41 biopsies including 7 (17%) open lung biopsies. Retrospective analysis of electronic clinical records and correlation with histological findings were performed.
Results: The time from transplantation to lung biopsy ranged from 4 to 345 months (mean 81 months). The majority of patients (15/28 or 54%) received a deceased donor kidney. The main cause of ESRD was diabetic nephropathy (10/28; 36%). Immunosuppression regimen of 16 (57%) patients included Rapamycin. Based on chest CT findings, 9 (32%) patients had diffuse process (ground glass opacities and/or reticulations including 2 with five-lobe consolidation). Localized lesions, including nodules or mass-like consolidation, were seen in 19 (68%) patients. Among 9 patients with diffuse lung disease, biopsy revealed diffuse alveolar hemorrhage as predominant finding in 5 cases, organizing pneumonia in 1 and diffuse alveolar damage in 1 case. Three of 9 with diffuse lung disease had clinical concern for Rapamycin toxicity and improved after drug discontinuation. In 19 patients with localized lesions, biopsy showed neoplasia in 8, necrotic nodules/granulomas in 5, and organizing pneumonia in 1 case. Lung biopsies in 8 (29%) patients had only minimal/nonspecific findings.
Conclusions: Pulmonary changes requiring tissue diagnosis are uncommon in kidney transplant recipients. Among localized lesions, the majority are infectious nodules and neoplasms. Histological spectrum among diffuse lung diseases includes diffuse pulmonary hemorrhage and acute lung injury. Together with infectious etiology, Rapamycin toxicity becomes another important differential diagnostic consideration in these patients.

1766 Chemokine Receptor CXCR4 Expression Correlates with Tumor Grade in Pulmonary Neuroendocrine Carcinomas.

S Kirby, N Mohd, J Jen, W Travis, T Franks, C Hitchcock, W Zhao, R Ganju, K Shilo. The Ohio State University Medical Center, Columbus; Mayo Clinic, Rochester, MN; Memorial Sloan Kettering Cancer Center, New York, NY; AFIP, Washington, DC.
Background: Chemokine receptor-4 (CXCR4) expression has been implicated in promoting metastasis in a variety of cancers including non-small cell lung carcinoma (NSCLC). However, there are conflicting results regarding the significance of its expression patterns (nuclear vs. cytoplasmic) and their relationship to outcome. Little is known about its expression in small cell lung carcinoma (SCLC), the lung carcinoma with the greatest metastatic potential. The aim of this study was to investigate CXCR4 expression in a wide range of pulmonary neuroendocrine carcinomas (NEC), including SCLC, and its possible association with clinical-pathological findings.
Design: Clinical-pathological features of 156 patients with NEC (73 men and 83 women, mean age of 59±15 years; range 19–83; median 62 years) were analyzed with regard to CXCR4 expression. Tissue microarray based samples were studied for CXCR4 (1:50, rabbit polyclonal, ab2074, Abcam, Cambridge, MA) expression by immunohistochemistry. The results were evaluated in comparison to normal lung parenchyma and recorded as negative, cytoplasmic, and nuclear. Correlation of the CXCR4 expression with clinical-pathological variables was performed utilizing statistical package JMP 8.0 (SAS Inc., Cary, NC).
Results: Cytoplasmic CXCR4 expression was detected in 53.8% (86/158) of pulmonary NEC: 26.3% of typical carcinoid (TC), 13.5% of atypical carcinoid (AC), 4.5% of large cell neuroendocrine carcinoma (LCNEC), and 10.9% of small cell lung carcinoma (SCLC). Nuclear CXCR4 expression was detected in 45.0% (72/158) of pulmonary NEC: 0.6% of TC, 4.5% of AC, 9.6% of LCNEC, and 28.9% of SCLC. Nuclear CXCR4 expression correlated with tumor grade ($p<.05$) but not with other clinical-pathological findings.
Conclusions: CXCR4 is observed in a significant percentage of pulmonary NEC. Nuclear CXCR4 expression correlates with grade of pulmonary NEC. The association between CXCR4 and SCLC suggests that aberrant nuclear CXCR4 expression may play role in metastasis as reported in NSCLC.

1767 Expression of Melanocyte Differentiation Markers in Pulmonary Lymphangioleiomyomatosis Offers Potential for Immunotheraputic Targeting.

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Background: Lymphangioleiomyomatosis (LAM), a hyperproliferative disorder of smooth muscle cells, involves formation of tumor nodules in the lungs in premenopausal women, following mutations in both alleles of *TSC-1* or *TSC-2*. Tumor growth is slow, with a life expectancy of approximately 10 years at diagnosis. Although Rapamycin alleviates symptoms of LAM, it does not provide lasting disease remission and LAM is ultimately lethal unless salvage lung transplantation is performed. The detection of smooth muscle cells co-expressing HMB45 is diagnostic. However, a subset of LAM cells also express gp100 and a melanoma-associated antigen recognized by T cells (MART-1); melanosomal markers are also observed by electron microscopy (EM). Immunotherapy has been applied to treat patients with melanoma. This study aims to explore the feasibility of targeting tumors in LAM by melanoma immunotherapy.
Design: The presence and abundance of melanosomal markers gp100, MART-1, TRP-1, TRP-2, tyrosinase as well as GD3, a ganglioside widely expressed in human malignant melanoma cell lines and tumors, and immune infiltration were studied in cryosections of LAM and normal lung. Cultured LAM cells displaying early melanosomes by EM were subjected to Cytotoxic T Lymphocytes (CTL) and included in complememnt-dependent cytotoxicity (CDC) and antibody-dependent cell-mediated cytotoxicity (ADCC) assays using antibodies to GD3.
Results: Expression of gp100 was abundant and only superseded by TRP-1. In serial sections, expression of separate melanosomal markers was observed in different cells. Among CD4+ and CD8+ T cells, dendritic cells and macrophages, only macrophage infiltration was markedly increased in LAM. Remarkably, melanoma-derived, HLA-matched gp100 reactive CTL and autologous T cells from LAM lung reacted with cultured LAM cells. Also, antibodies to GD3 mediated CDC and ADCC responses.
Conclusions: Separate melanosomal markers are expressed in different LAM cells, with TRP-1 and gp100 being most abundant, and gp100-reactive CTL cells react with cultured LAM cells. Macrophage infiltration is markedly increased in LAM. These findings suggest that occult expression of immunogenic target molecules does elicit marked T cell responses and that targeting multiple antigens may be the strategy of choice for eradicating LAM tumors. Since LAM tumors are slow growing, use of immunotherapeutic strategies may be more successful than in melanomas which are rapidly progressing.

1768 Correlation of a Proposed Scoring System for Lung Adencoarcinoma with Molecular Alterations.

VE Klepeis, EJ Mark, D Dias-Santagata, AJ Iafrate, M Mino-Kenudson. Massachusetts General Hospital, Boston.
Background: A myriad of proposed revisions to the classification of lung adenocarcinomas, which often show heterogenous morphology, have attempted to tighten the correlation between histology and prognosis, and supplementation of histology by molecular markers may prove necessary. Recently, a three-tiered histologic pattern-based scoring system was developed for stage I lung adenocarcinomas which stratified patients into low, intermediate, and high-risk categories for disease recurrence (Sica G, et al. AJSP 2010). The aim of the current study was to correlate score with molecular alterations.

Design: One-hundred and thirty two lung adenocarcinomas including 80 stage I tumors surgically resected and submitted for molecular testing were classified based on the three tier histopathologic scoring system. Briefly, comprehensive histologic subtyping was performed in a semiquntitative manner with the percentage of the 5 possible histologic subtypes quantified in 10% increments. A BAC pattern was classified as Grade I, acinar and papillary patterns as Grade II and solid, micropapillary and the other variants as grade III. A score was then obtained for each tumor by adding together the 2 most predominant grades. For mutational analysis of each scored tumor, 110 described mutations of 13 genes were studied using mutiplex PCR. The association between score and tumor genotype was evaluated.

Results: While EGFR (37/132) or K-ras mutations (42/132) were detected in 60% of the tumors studied, 36% showed no detectable mutations. The association of EGFR mutants with a low-risk category (score 2 or 3) was statistically significant compared to the other molecular alterations (p = 0.044 for the entire cohort, p=0.0087 for the stage I).

Table 1: Molecular alterations and Scoring in 132 Lung Adenocarcinomas

	2-3	4	5-6	Total
EGFR	18 (49%)	6 (16%)	13 (35%)	37
KRAS	12 (29%)	9 (21%)	21 (50%)	42
TP53	0	0	3 (100%)	3
PIK3CA	1 (50%)	1 (50%)	0	2
BRAF	0	0	1 (100%)	1
None	15 (32%)	16 (34%)	16 (34%)	47
Total	46	32	54	132

Table 2: : Molecular alterations and Scoring in Stage I Lung Adenocarcinomas

	2-3	4	5-6	Total
EGFR	13 (68%)	2 (11%)	4 (21%)	19
KRAS	10 (36%)	8 (25%)	11 (39%)	29
TP53	0	0	2 (100%)	2
PIK3CA	0	1 (100%)	0	1
BRAF	0	0	1 (100%)	1
None	10 (36%)	13 (46%)	5 (18%)	28
Total	33	24	23	80

Conclusions: The scoring that has been developed to discriminate patients with different risk of disease-recurrence in stage I lung adenocaricnomas also correlates with molecular alterations.

1769 Association of c-Met Activation with Micropapillary Pattern and Small Cluster Invasion in pT1 Lung Adenocarcinoma.

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Background: Lung adenocarcinomas with micropapillary pattern (MPP) are associated with frequent nodal metastasis. However, little is known about the mechanisms that underlie MPP-associated nodal metastasis. We have previously reported that carcinomas with MPP tend to undergo small cluster invasion (SCI) in scars and invade lymphatics in pT1 lung adenocarcinomas [Modern Pathology (2007) 20: 514, Virchows Arch (2009) 454: 61]. SCI was defined as markedly resolved acinar-papillary tumor structures with single or small clusters of carcinoma cells invading stroma within fibrotic foci. In this study, we hypothesized that c-Met activation may be involved in the MPP-SCI sequence, since the c-Met tyrosine-kinase receptor and its ligand hepatocyte growth factor play an important role in tumor cell motility and invasion. We tested this hypothesis by analyzing the c-Met activation status immunohistochemically.

Design: We analyzed 146 cases of pT1 lung adenocarcinomas with reference to the presence of MPP, small cluster invasion (SCI), and lymphatic involvement. Expressions of c-Met protein and phosphorylated c-Met were so far examined immunohistochemically using monoclonal antibodies (c-28, Santa Cruz; No. 248 (Tyr 1235), IBL) in 53 of the 146 cases.

Results: SCI was significantly more frequent in the MPP-positive group (P<0.0001) and was significantly associated with lymphatic involvement (P<0.0001) and nodal metastasis (P=0.0073). c-Met protein was detected in all cases examined as membranous and cytoplasmic staining. Phospho-c-Met was positive in 51/53 cases (96%), and was expressed at high levels in 36/53 cases (68%). High levels of phospho-c-Met expression was significantly associated with MPP (P=0.0054) and SCI (P=0.0009). Patients of the high phospho-c-Met expression group tended to show shorter overall survival compared with those of the low expression group.

Conclusions: C-met activation may play roles in the sequencial process of MPP-SCI-lymph node metastasis.

1770 Expression of SOX2 and p63 Do Not Have Independent Prognostic Significance in Stage I Lung Adenocarcinoma.

AE Kovach, EJ Mark, M Mino-Kenudson. Massachusetts General Hospital, Boston.

Background: A myriad of proposed revisions to the classification of lung adenocarcinoma have attempted to tighten the correlation between histology and prognosis, and supplementation of histology by immunohistochemical and molecular markers may prove necessary. The SOX2 oncogenic transcription factor is expressed in lung squamous cell carcinomas and a subset of lung adenocarcinomas; its overexpression has recently been reported to be a prognostic marker for stage I adenocarcinomas. p63 also may be expressed in lung adenocarcinomas in a patchy manner; the significance of this is unclear. We sought to evaluate SOX2 and p63 expression in a cohort of stage I lung adenocarcinomas in the context of histologic subtype and clinical outcome.

Design: Lung adenocarcinomas resected at MGH between 2000 and 2004 were reviewed. Tissue microarrays were constructed from multiple sections in each of 104 cases. SOX2 and p63 expression were assessed by immunohistochemistry and compared to clinicopathologic features and disease free survival (DFS). For each antibody, nuclear expression in ≥ 5% of tumor cells was considered positive.

Results: The cohort consisted of 59 women and 45 men, with an average age of 68 yrs (SD 9 yrs). The majority of cases (82, 78%) were of mixed histologic subtype, with acinar subtype being the most common predominant pattern (42, 40%). SOX2 expression was seen in 83 (80%) cases and p63 expression in 19 (18%). Of the p63 positive cases, 15 (80%) were also positive for SOX2. There was no significant association between the expression of SOX2 or p63 and predominant histologic subtype; however, p63 tended to be overexpressed in BAC components. Expression of both proteins was also not associated with age, gender, tumor stage (Ia vs. Ib), or tumor grade. Survival analysis showed that patients with stage I adenocarcinoma with SOX2 expression had a slightly shorter DFS than those without (5 year DFS rate: 68% vs. 84%), but the difference did not reach statistical significance (log-rank p=0.2788). There was no difference in outcomes between tumors with p63 overexpression and those without.

Conclusions: SOX2 and p63 do not have independent prognostic significance in stage I lung adenocarcinomas in this study. The prevalence of SOX2 expression in stage I lung adenocarcinomas in our cohort was much higher than that previously reported. Differences in populations, different lots of antibodies used, and/or thresholds of observers for positive expression must be considered when evaluating outcome results as studied here.

1771 Evaluation of Cyto-Histologic Agreement for Defining Histologic Subtype of Lung Carcinoma.

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Background: The differential therapeutic efficacy and toxicity of targeted therapies has made differentiation of non-small lung cancer (NSCLC) into squamous cell carcinoma (SCC) and adenocarcinoma (AC) subtypes mandatory. The use of immunohistochemistry (IHC) in core needle biopsies has been recently reported to improve accuracy. This study aimed to review lung fine needle aspirates (FNAs) to assess the overall impact of IHC in subtyping NSCLC and the agreement between the final cytological and surgical diagnoses.

Design: An electronic search retrieved all cases with lung FNAs and subsequent surgical resection during the period between 2001 to 2009. All metastatic tumors, non epithelial neoplasms, inflammatory, negative or suspicious for malignancy, and unsatisfactory results were excluded from the analysis. Only NSCLC cases with cytological and corresponding surgical specimen were selected. Overall agreement between cytological and surgical diagnoses was assessed for cases with specific cytology subtypes. NSCLC cases without subtyping were stratified by year and the cell block (CB) slides were reviewed to identify the reasons why subtyping was not stated. The number of cases in which IHC was performed, the antibodies used and the purpose of the assay were also tabulated.

Results: A total of 605 cytology cases were retrieved from the database. 433 (71.6%) cases had a final cytological diagnosis of either AC or SCC. In 172 cases, no subtypes were defined and were reported as NSCLC, NOS (not otherwise specified) (28.4%). The number of cases in this category has decreased since 2001, reaching the lowest rate in 2009 (17.4%). Among NSCLC cases cytology subtyped into AC and SCC, an agreement with surgical diagnosis was found in 377 (94.0%) cases. When stratifying the reasons for no subtyping, 40 cases had no CBs, 9 CB had no tumor cells, and in 28 cases IHC was performed but was not contributory. IHC was performed in 157 cases, of which 56 were done specifically for subtyping NSCLC. The median number of antibodies used was 3. TTF-1 was the most frequently (91.1%) used antibody, followed by CK7 and HMWK (44.6%).

Conclusions: Specific subtyping can be achieved in a high proportion of lung FNAs with high level of agreement with surgical diagnosis. The percentage of NSCLC with no further subtype has been decreasing in recent years. The use of IHC increased the accuracy of the cytological diagnosis, and TTF-1, CK7 and HWMK were the most used antibodies.

1772 HuR Involvement in PTHrP Expression in Human Lung Adenocarcinoma.

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Background: PTHrP posses growth factor-like properties and is involved in the growth and invasion of multiple cancers. We have previously shown that, in lung adenocarcinoma, both PTHrP and cytoplasmic HuR expressions are correlated with a worse clinical outcome. Recently, it has been reported that, in renal cell carcinoma, HuR is involved in PTHrP upregulation, so we studied 54 lung adenocarcinomas, to ascertain whether HuR is involved in the regulation of PTHrP expression.

Design: Immunohistochemical analysis was performed on neoplastic tissue from 54 lung adenocarcinomas, with anti-HuR and anti-PTHrP antibodies. In order to further investigate the role of HuR, in vitro studies were performed on HCC44 human lung adenocarcinoma cell line, by siRNA transfection.

Results: Double immunohistochemical staining revealed that HuR and PTHrP are co-expressed in tumor cells. Moreover, by quantitative immunohistochemistry, we found that the expression of HuR (measured as nuclear/cytoplasmic ratio: N/C) and that of PTHrP are inversely correlated. With a median follow-up of 36 months (range 3-194), metastases occurred in 19 out of 54 cases and 12 out of 54 patients had died of cancer. Cox's regression analysis, using PTHrP as continous covariate, showed that PTHrP levels were directly associated with the risk of death and metastases. We further evaluated whether the adjustement for HuR N/C status of the tumors added information to the relationship between PTHrP levels and the metastases-free and overall survival rates. Plots of the Cox's model estimates of the overall and metastasis-free survival as a function of PTHrP levels, for patients with HuR N/C high or low tumors, showed that, at 5-year follow-up, the estimated proportions of metastasis-free surviving patients, at the

mean value of PTHrP, were 90.1% and 70.2% for those with tumors displaying high and low HuR N/C, respectively. Relative to the overall survival, the estimated proportions of surviving patients were 92.5% and 55.0% for those with tumors displaying high and low HuR N/C, respectively. In HCC44 human adenocarcinoma cell line, knockdown of HuR expression by siRNA tranfection reduced cell growth and this effect was partially prevented by addition of exogenous PTHrP; immunocytochemical and western blot analyses revealed that knockdown of HuR reduced the levels of PTHrP.
Conclusions: These observations suggest that in human lung adenocarcinoma the expression of PTHrP is regulated by the RNA-binding protein HuR, adding further interest on HuR, also as a molecular target for tumor therapy.

1773 Sonic Hedgehog (Shh) Pathway Signaling and Clinical Outcome in Non Small Cell Lung Cancers (NSCLC).

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Background: Desert hedgehog (Dhh), patched-1 (PTCH1), smoothened (Smo) and glioma oncogene 1 (Gli-1) proteins are Shh pathway members and that have been implicated in human organogenesis and neoplasia, but have not been widely studied in clinical NSCLC specimens.
Design: FFPE sections from 110 NSCLC, including 31 squamous cell carcinomas (SCC), 41 adenocarcinomas (AC), and 38 bronchoalveolar carcinomas (BAC), were immunostained by automated methods (Ventana) with goat polyclonal Dhh, Smo, and Gli-1 and rabbit polyclonal patched1 antibodies (Santa Cruz Biotech). The staining pattern was semiquantitatively assessed based on staining intensity and distribution and the results were correlated with morphologic and prognostic variables.
Results: Immunoreactivity for Dhh, patched1 and Gli-1 was predominately cytoplasmic, with both nuclear and cytoplasmic noted for Smo. Dhh overexpression was noted in 47% tumors and correlated with tumor type [77% SCC vs 44% AC vs 26% BAC, p<0.0001] and high grade [p=0.036]. Strong diffuse patched overexpression was noted in 46% tumors, correlating overall with tumor type [60% BAC vs 49% AC vs 26% SCC, p=0.019] and early stage [p=0.031]; while within the SCC subgroup only, correlating with high grade [p=0.025], female gender [p=0.031], and disease recurrence [p=0.05]; and within the ACs, correlating with early stage [p=0.047] and lengthened survival [p=0.032]. Strong diffuse Smo overexpression was noted in 58% tumors overall [58% SCC vs 45% BAC vs 39% AC]; with cytoplasmic localization in 30% tumors correlating with lengthened survival [p=0.002] overall and within AC [p=0.013]; and nuclear localization in 20% tumors correlating with tumor type [36% SCC vs 16% BAC vs 11% AC, p=0.027], small tumor size (<= 3.0 cm) [p=0.038], and lack of disease recurrence [p=0.016]. Strong diffuse Gli-1 was overexpressed in 41% tumors overall [50% SCC vs 48% AC vs 25% BAC, p=0.063] and correlated with non-recurrent disease [p=0.028] overall and within AC [p=0.05]. On multivariate analysis advanced stage [p<0.0001], lack of strong diffuse cytoplasmic Smo localization [p=0.005], and loss of nuclear Smo localization [p=0.048] independently predict shortened survival.
Conclusions: Shh pathway proteins are differentially expressed in NSCLC and are associated with NSCLC histologic subtype, tumor stage and variably with clinical outcome. Of the Shh proteins studied, Smo expression loss was the most noteworthy associated with advanced primary tumor stage and independently predicting shortened patient survival.

1774 Prognostic Significance of Aurora A Kinase (AURKA) Expression in Non Small Cell Lung Cancer (NSCLC).

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Background: AURKA is a serine/threonine kinase that stimulates mitotic progression through the regulation mitotic spindles and centrosomes. AURKA inhibitors are in early clinical trials for the treatment of solid tumors.
Design: Formalin-fixed, paraffin embedded sections from 111 NSCLC, including 30 squamous cell carcinomas (SCC), 44 adenocarcinomas (AC), and 37 either pure bronchioloalveolar carcinomas (BAC) or AC with BAC features were immunostained by automated methods (Ventana Medical Systems, Inc, Tucson, AZ) with a monoclonal antibody to Aurora A (Abcam, Cambridge, MA). Cytoplasmic and nuclear immunoreactivity was semiquantitatively assessed in the tumor for all cases. Scoring was based on staining intensity (weak, moderate, intense) and percentage of positive cells (focal <= 10%, regional 11-50%, diffuse >50%). Results were correlated with clinicopathologic variables.
Results: Both cytoplasmic and nuclear AURKA immunoreactivity was observed. Overexpression was noted in 60/111 (54%) tumors overall and correlated with tumor type [30% SCC vs. 11% AC vs. 32% BAC, p=0.05], small tumor size (<= 3.0 cm) [p=0.04], male gender [p=0.02], and shortened survival [p=0.049]. Within the SCC subgroup, nuclear AURKA overexpression correlated with small tumor size (<= 3.0 cm) [p=0.005], male gender [p=0.05] and shortened survival [p=0.03]; while cytoplasmic overexpression showed a trend toward association with early stage [p=0.10] and node negative [p=0.09] disease. Within the BAC subgroup, cytoplasmic Aurora overexpression correlated with advanced stage [p=0.02], while showing a trend toward correlation with node positive [p=0.10] disease. On multivariate analysis, only disease stage independently predicts shortened survival.
Conclusions: AURKA is expressed in the majority of NSCLC and is significantly associated with histologic subtype and patient survival. Continued clinical trials of AURKA inhibitors for the treatment of NSCLC appear warranted.

1775 Transbronchial Lung Biopsy Is Not an Optimal Method for Evaluating Chronic Rejection in Lung Transplant Patients.

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Background: Lung transplantation is a rescue therapy for patients with end-stage lung disease. Chronic rejection post transplant (tx)
remains a frequent cause of death. Hitherto, the diagnosis of rejection has been made by transbronchial or wedge biopsy (bx). However, the sensitivity of lung bx in the diagnosis of rejection is still unclear.
Design: We reviewed biopsy and autopsy records of the past 19 years from a single lung tx center, and compared the findings from the patient's premortem bx to that of the autopsy (= the gold standard). We also evaluated the diagnostic potency of chronic rejection vs. bx satisfaction rate using the presence of at least 5 fragments of alveolated lung tissue as the criteria.
Results: Bx and autopsy findings from 71 lung tx patients [25F/46M] were reviewed. Most chronic rejection occurred > 6 months post lung tx. The latest bx of 75% of patients was performed within one month prior to the autopsy, with a median of 14 days (ranging from 1 day to 7 months). There were 6 patients diagnosed with chronic rejection from premortem transbronchial bx, whereas 19 patients were diagnosed at autopsy. Hence, the sensitivity for diagnosis of chronic rejection by transbronchial bx was 36.8%, and the specificity was 100%. Wedge bx was performed on 5 patients, none of whom were diagnosed with chronic rejection and this was completely consistent with their autopsy findings. 15 patients were diagnosed with acute rejection by the latest bx, but only 4 of them at autopsy.

Comparison of Diagnosis of Rejection at Bx and Autopsy

	Transbronchial Bx	Autopsy
Acute Rejection	15	4
Chronic Rejection	6	19

All patients with a premortem diagnosis of chronic rejection had a satisfactory transbronchial bx (6/6, 100%) while those that were not diagnosed premortem had a bx satisfaction rate of 55% (7/13).

Diagnosis of Chronic Rejection by Transbronchial Bx vs. Bx Satisfaction Rate

Bx Diagnosis	# of Patients (n=19)	Bx Satisfaction rate (>=5pcs of alveolated lung tissue)
Chronic rejection	6	100%
Other	13	55%

Conclusions: Diagnosis of chronic rejection by txbx is less sensitive than by autopsy. The diagnostic rate of acute rejection was 4 times higher in transbronchial bx than by autopsy, probably due to the immunosuppressant therapy instigated upon diagnosis. Wedge bx is a more reliable tool for the exclusion of chronic rejection than transbronchial bx. A satisfactory transbronchial bx is required for the accurate diagnosis of chronic rejection.

1776 Pleuropulmonary Synovial Sarcoma: Analysis of 15 Cases with FISH and TLE-1.

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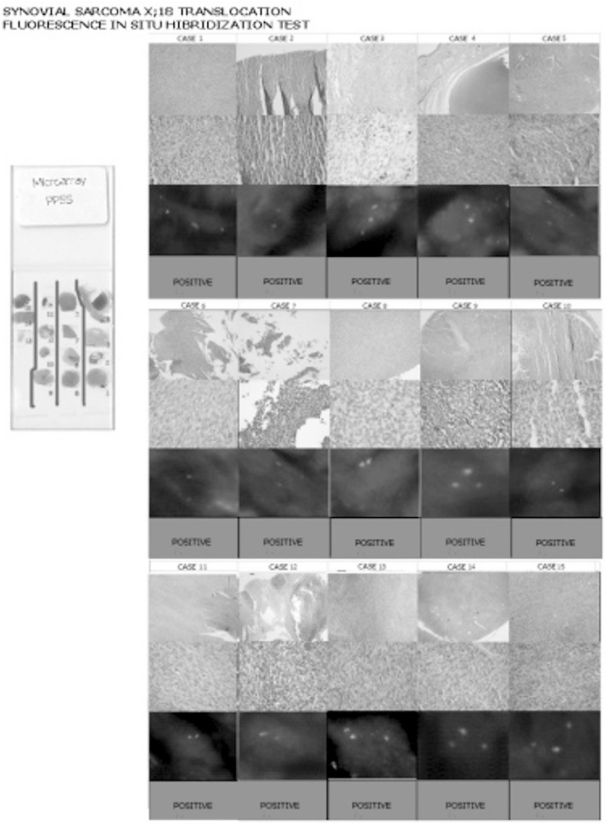
Background: Pleuropulmonary synovial sarcoma (PPSS) is a recently described rare entity. histological, immunohistochemical and molecular characteristics are similar to synovial sarcoma of soft tissue (STSS) including t(X:18)(p11.2;q11.2) with *SYT/SSX* fusion. Immunohistochemical expression of bcl-2 and vimentin, while epithelial markers are focal positivity. In recent studies, the novel antibody (TLE-1) was positive in STSS. There are 120 published cases of PPSS, with t(X;18) in one series of 36 cases, however there are no studies exploring the expression of TLE-1 in PPSS.
Design: The main objective was to explore the immunohistochemical profile and the sensitivity of TLE-1 in a group of PPSS studied by FISH in a tissue microarray (TMA). The secondary objective was to know the usefulness and cost-benefit of a FISH test looking for a specific t(X; 18) in TMA.



From a total of 20 cases of PPSS, 15 demonstrated Split apart signal characteristic of t(X;18) by FISH. Five cases with negative or non evaluable signal were elmiminated. Immunohistochemistry was performed in the same TMA, the panel of antibodies included: TLE-1, vimentin, CD99, CD56, bcl-2, Cytokeratin (AE1-AE3), EMA, CD34, CK7, CK19, calponin and S-100 protein.

Results: For the analyses were 14 cases of monophasic SS and 1 case of biphasic. TLE-1 resulted positive in 11 cases (73.3%), bcl-2 and vimentin were positive in all (100%), calponin and CD56 in 4 (26.6%), CK AE1-AE3, CK19, CK7 and EMA were focal in 2 cases, CD99 in 2 cases, CD34 in one case, and S100 negative in all. The only biphasic SS was positive for cytokeratins and EMA in the epithelial, and negative in the spindle cell component.

Conclusions: The predominant subtype of PPSS was monophasic. TLE1 is expressed in 73% of PPSS (PPV 73%), a value inferior to that reported in STSS, thus, TLE1 may be of value in the differential diagnosis of PPSS, but should be used in correct histological context, complemented by a panel of antibodies. The most constant immunohistochemical profile in molecularly proved PPSS is:Vimentin and bcl-2 positive (100%), and TLE-1 in three fourths of cases. It is possible to perform FISH in TMA, a cost-efficient technique that yields excellent results.

1777 Root Cause Analysis of Problems in Frozen Section Diagnosis of Bronchioloalveolar Carcinoma and Early Invasive Adenocarcinoma of the Lung.

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Background: Pathologists at our hospital are asked to distinguish between bronchioloalveolar carcinoma (BAC), early invasive adenocarcinoma (BAC with <5mm invasion;EIA), and mixed adenocarcinoma (MAC) of the lung at intraoperative frozen section (FS). BAC patients generally undergo wedge/segment resection; most EIA and MAC patients undergo lobectomy. A root cause analysis (RCA) approach was used to review our FS experience.

Design: FS from 224 consecutive single primary resected pulmonary tumors (27 BAC, 46 EIA, and 151 MAC) (median size <2cm) were reviewed and compared with the final diagnoses. Discrepancies were classified as errors or deferrals and as benign vs. malignant, EIA vs. BAC, and BAC or EIA vs. MAC. Reasons for the FS errors and deferrals were tabulated.

Results: FS diagnoses were correct in 183 (8l.7%) of the cases. The 41 (18.3%) discrepancies included 27 (12%) errors and 14 (6.3%) deferrals. These cases had been interpreted by experienced pulmonary pathologists and 15 of the 41 had been reviewed by ≥2 pathologists at the time of FS. One piece of tissue had been frozen in 25 (61%) and ≥2 were frozen in 16 (39%) cases with ≥2 levels examined intraoperatively in 37 (90.2%) cases. There were no false positive diagnoses of malignancy. FS errors involved 21 EIA (13 read as MAC, 5 as benign, 3 as BAC) and 6 BAC (2 read as benign, 2 as EIA, 2 as MAC). Deferrals included 12 benign vs. malignant and 2 EIA vs. BAC. Errors and deferrals were retrospectively attributed to FS misinterpretation in 23 cases as a result of scar with inflammation (9), inflammation with reactive atypia (4), obscuring inflammation (4), scar with inflammation and difficulty estimating extent of invasion (3), minimal cytological atypia (1), scar with inflammation and extensive reactive atypia (1), difficulty estimating extent of invasion (1) and to technical problems in 15 cases resulting from tissue sampling in 11 cases (9 with tumor or invasion only in blocks not frozen and 2 with tumor or invasion seen only at deeper levels of the FS block) and poor fixation in 4 cases. FS slides were not available for review in 3 cases.

Conclusions: -RCA suggests that 19 (46.3%) of the 41 errors and deferrals in FS diagnosis of BAC, EIA, and MAC can be attributed to potentially reducible problems that involve sampling, fixation, and estimating extent of invasion. -Confounding scar and/or severe inlammation remain as more difficult diagnostic problems in 8.5% of the 224 cases.

1778 Higher Metabolic Activity in Fibroblastic Foci of UIP Than in Buds of Fibrosis in Intra-Alveolar Buds in COP/BOOP.

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Background: Fibroblastic foci in profusion contribute to irreversible fibrosis in UIP. Polypoid granulation tissue plugging small airways (intra-alveolar bud) is one of the pathologic characteristics of COP/ BOOP. UIP and COP/BOOP are clinically distinct. The ubiquitin gene is expressed in eukaryotic cells and plays a role in the regulation of cell cycle, apoptosis and signal transmission. We compared the expression of ubiquitin, polyubiquitin, ubiquitinized proteins, various growth factors and an apoptotic marker in UIP and COP/BOOP.

Design: Immunostaining for ubiquitin (P4D1 mouse mAb, Cell Signaling Technology), transforming growth factor (TGF) beta-1, connective tissue growth factor (CTGF), proliferating cell nuclear antigen(PCNA)and Ki-67 was carried out in paraffin-embedded sections of lung from 10 thoracoscopic biopsies with COP/BOOP and 10 biopsies with UIP using a standard indirect avidin-biotin horseradish peroxidase method with various antigen retrievals. Apoptotic DNA strand break by nick end-labeling technique (TUNEL) was also examined.

Results: Ubiquitin and ubiquitinized proteins were expressed in the cytoplasm and nucleus of bronchial and bronchiolar epithelial cells but not in pneumocytes in normal controls. It was expressed in regenerative type 2 pneumocytes and alveolar macrophages both in UIP and COP/BOOP. Ubiquitin and ubiquitinized proteins were expressed strongly in myofibroblasts in fibroblastic foci but faintly in intra-alveolar buds. Growth factors were expressed strongly in fibroblastic foci, regenerating type 2 pneumocytes and bronchiolar epithelial cells in UIP but faintly in intra-alveolar buds and seldom in pneumocytes adjacent to the lesions in COP/BOOP. PCNA-positive pneumocytes, bronchiolar epithelial cells and fibroblasts were frequent in UIP but not in COP/BOOP. TUNEL signals were seldom seen either in fibroblastic foci or in intra-alveolar buds.

Conclusions: Expression of ubiquitin, ubiquitinized proteins and growth factors in the fibroblastic foci in UIP suggest up-regulation of cell cycles and the ubiquitin proteasome system. Thus, there is higher metabolic activity in the fibroblastic foci in UIP when compared to the intra-alveolar buds in COP/BOOP. The difference may contribute to the different natural history of the two diseases.

1779 Pattern of Spread to N2 Lymph Nodes Predicts Survival in Patients with Biphasic Pleural Malignant Mesothelioma.

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Background: In patients with diffuse malignant mesothelioma (MM), metastases to extrapleural N2 lymph nodes are a poor prognostic characteristic. Studies from our group have shown that metastases to N2 lymph nodes from biphasic MM have either both epithelioid and sarcomatoid histologies or epithelioid histology alone, but the clinical significance of this observation is unknown. In this study we investigated the clinical significance of the component metastatic to N2 lymph nodes from patients with biphasic MM.

Design: We identified 715 consecutive patients with MM (462 epithelioid, 231 biphasic, and 22 sarcomatoid type) treated by extrapleural pneumonectomy at Brigham and Women's Hospital between 1988 and 2009. Of the 231 biphasic MMs, we found 74 with metastases to mediastinal N2 lymph nodes. We evaluated the N2 lymph node metastases of 65 of these patients with biphasic MM and available pathology material and correlated the findings with overall survival.

Results: All 65 patients (11 female/54 male; mean age 60.1; range 31-88) had a diagnosis of biphasic MM metastatic to N2 lymph nodes. Thirty-eight patients (58%) with biphasic MM had both epithelioid and sarcomatoid components in the N2 lymph nodes and twenty-seven patients (42%) showed spread only of the epithelioid component to the N2 lymph nodes. The mean follow-up period after surgery was 10.7 months. The median survival of patients with mixed histology in the N2 lymph nodes was 8.8 months versus 11.5 months for those with an epithelioid component alone (p=0.040).

Conclusions: Our data indicate that the presence of a mixed component in the N2 lymph nodes predicts a worse overall survival in patients with biphasic MM. The results of our study emphasize the importance of histologic classification of not only the surgical specimen but also the lymph node metastases and highlight the biologic complexity of disease progression in biphasic MM.

1780 Patterns of Metastases to N2 Lymph Nodes from Biphasic Pleural Malignant Mesothelioma.

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Background: Pathologic classification of diffuse malignant mesothelioma (DMM) into epithelioid, sarcomatoid, and biphasic types, according to the current WHO criteria, is an important predictor of survival. Studies have shown that patients with extrapleural

lymph node metastases have a poor prognosis following surgery. However, there are no studies of biphasic DMM examining which component is more likely to spread to N2 lymph nodes. The goal of this study was to characterize the histology of metastases to N2 lymph nodes from patients with biphasic DMM.
Design: We identified 715 consecutive patients with MM (462 epithelioid, 231 biphasic, and 22 sarcomatoid types) treated by extrapleural pneumonectomy (EPP) at Brigham and Women's Hospital between 1988 and 2009. Of the 231 biphasic MMs, we found 74 patients who also had sampling of mediastinal lymph nodes with a diagnosis of DMM metastatic to those lymph nodes. We evaluated 65 of these patients with biphasic DMM, N2 lymph node metastases, and available pathology material for the presence of epithelioid, sarcomatoid or both histologies in the positive N2 lymph nodes.
Results: All 65 patients (11 F/54 M; mean age 60.1; range 31-88) had a diagnosis of biphasic DMM metastatic to N2 lymph nodes. Thirty eight patients (58%) with biphasic DMM had both epithelioid and sarcomatoid components in the N2 lymph nodes and 27 (42%) showed spread only of the epithelioid component to the N2 lymph nodes. There were no biphasic DMMs that had N2 spread of the sarcomatoid component alone. A mixed histology in the lymph nodes is highly specific of a biphasic DMM (specificity=100%), whereas an epithelioid histology in N2 lymph nodes indicates that the primary tumor may be either an epithelioid or a biphasic DMM (sensitivity=70.4%).
Conclusions: Our data suggest that a diagnosis of mixed DMM in patients with N2 lymph node metastases is highly predictive of biphasic DMM in the primary tumor ($p<0.0001$, Fisher exact test). However, the finding of an epithelioid component alone in the mediastinal lymph nodes does not preclude from a diagnosis of biphasic DMM in the EPP specimen. The results of our study emphasize the importance of histologic classification and highlight the biologic complexity of tumor morphogenesis and progression in biphasic DMM.

1781 Correlation of Pre-Transplantation Clinical Diagnoses with Post-Transplantation Pathologic Diagnoses in Lung Transplantation Patients: A Single Institution Study of 748 Transplants.

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Background: Lung transplantation is the main therapy option for many endstage pulmonary diseases. However, accurately diagnosing the specific lung pathology prior to transplantation may be challenging. Evaluation of the explanted lung provides an accurate measure of the pre-clinical diagnosis.
Design: We reviewed clinical and pathologic records for lung transplantations performed at the Cleveland Clinic between the years of 1995 and 2009. For each transplant, the preoperative clinical diagnosis was compared with explanted lung pathologic findings diagnosed by pulmonary pathologists, (CF and AA). Discrepancies between pre-operative and post-operative diagnoses were tabulated. A discrepancy was counted as a major discrepancy if the explanted lung demonstrated a pathology inconsistent with the pre-operative diagnosis.
Results: Of the 748 lung transplantations performed between the years of 1995-2009, 744 pathology reports were available for review. The patients ranged from 2 to 75 years of age (mean 50.7 years). The most common lung diseases were chronic obstructive pulmonary disease (32.6%), idiopathic pulmonary fibrosis (29%), cystic fibrosis (14.6%), alpha-1 antitrypsin deficiency (6.3%), and sarcoidosis (3.6%).
Major discrepancies between the pre-transplantation clinical diagnosis and the post-transplantation pathologic diagnosis were found in 0.8% (6) patients. Three of these cases demonstrated UIP on the explant evaluation while the pre-operative diagnosis was NSIP.
Unexpected neoplasms were found in 1.9% (14) of explanted lungs. Adenocarcinoma was found in 8 explanted lungs and squamous cell carcinoma in 5 explanted lungs. One patient was found to have both adenocarcinoma and squamous cell carcinoma. The remaining two neoplasms were a BALT lymphoma and a metastatic non-small cell carcinoma of unknown primary found in a lymph node.
Micro-organisms were identified histologically in 9.1% (68) of explanted lungs. *Histoplasma* was identified in 50% (34) of those lungs, *Aspergillus* in 29.4% (20) and *Mycobacteria* in 20.6% (14). *Pneumocystis* and Herpes simplex virus were each found in one lung.
Conclusions: Pre-operative clinical evaluation of lung transplant candidates is very accurate in defining their pulmonary disease. Neoplasms are missed in pre-clinical work-up of lung transplantation in 1.9% of patients. Explanted lungs show micro-organisms by tissue stains in 20.6% of the cases.

1782 The Diagnostic Utility of *p16* FISH and GLUT-1 Immunohistochemistry in Mesothelial Proliferations.

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Background: Distinction between malignant and benign mesothelial proliferations can be challenging, therefore, ancillary studies have been proposed to improve diagnostic accuracy. Two of the most promising ancillary approaches include immunohistochemical (IHC) assessment of GLUT-1 and FISH assay for the *p16* deletion. In this study, the diagnostic utility of *p16* FISH and GLUT-1 IHC in the diagnosis of mesothelial proliferations was compared in a large series of surgical and cytopathology specimens.
Design: Surgical pathology and cytopathology cases with a diagnosis of benign/reactive mesothelial cells, atypical mesothelial proliferation, and malignant pleural and peritoneal mesotheliomas were randomly selected from the archives of the University of Pittsburgh Medical Center. The study protocol was reviewed and approved by the institutional review board of the University of Pittsburgh Medical Center. The diagnosis of mesothelioma was rendered based on the morphology, the results of ancillary studies, and the imaging studies.
Results: A total of 154 cases with the diagnosis of benign/reactive (70 cases; 45%), atypical mesothelial proliferation (16 cases;11%), or malignant mesothelioma (68 cases; 44%), including pleural (27 cases; 40%) and peritoneal (41 cases; 60%) mesotheliomas, were included. Of the benign/reactive cases, none had a deletion of *p16* and 5 (8%) cases were positive for GLUT-1. Of the mesotheliomas, 40 (59%) had a deletion of *p16* (sensitivity 59%; specificity 100%) and 27 (39%) were positive for GLUT-1 (sensitivity 40%, specificity 93%). FISH for *p16* was more sensitive in pleural than in peritoneal mesotheliomas (sensitivity 70% vs. 51%). GLUT-1 IHC showed a significantly lower sensitivity for the detection of malignancy in both pleural (sensitivity 56%, specificity 92%) and peritoneal specimens (sensitivity 29%, specificity 100%) (p value 0.004).
Conclusions: Our results demonstrate that FISH testing for the *p16* gene deletion is a more sensitive and specific test than IHC testing for GLUT-1, and can be a more reliable ancillary tool to help support the diagnosis of mesothelioma, particularly in small tissue samples.

1783 Causes of Granulomatous Inflammation in Lung Biopsies and Resections from Diverse Geographic Settings: An International, Multi-Institution Retrospective Study of 500 Cases.

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Background: Despite the central role of histology in the diagnosis of pulmonary granulomas, there is little data regarding the full spectrum of causes of granulomatous lung disease in pathologic specimens. The aims of this study were to identify the causes of pulmonary granulomas and their incidence in pathologic specimens, to determine whether these causes vary by geographic location, and to ascertain the proportion of cases of unknown etiology.
Design: Lung biopsies (467) and resections (33) showing granulomatous inflammation (n=500) were reviewed retrospectively by pathologists from 10 institutions (4 from the United States [US] and 1 each from Brazil, Scotland, Austria, Turkey, India and Japan). Fifty consecutive cases from each location were classified by diagnosis, incorporating clinical and microbiologic data where available.
Results: Specific diagnoses were made in 58% (290/500); the most common were sarcoidosis (136/500, 27%) and infections (125/500, 25%). Nearly all infections were mycobacterial (n=72) or fungal (n=51). Mycobacteria were identified in 19% cases (56/300) outside the US vs. 8% (16/200) within the US. In contrast, fungi accounted for 19% cases (38/200) in the US vs. 4% (13/300) in other locations. In 42% of all cases (210/500; 85 necrotizing, 125 non-necrotizing), a definite etiology could not be determined.
Conclusions: This is the largest and most geographically inclusive study of granulomatous lung disease to date. Across several geographic settings, sarcoidosis and infections are the most common causes of granulomatous lung disease diagnosed in pathologic specimens. Infectious causes of granulomatous lung disease show geographic variation, in that fungi are more commonly identified than mycobacteria within the US, whereas the reverse is true in other countries. A definite etiology cannot be demonstrated in more than a third of all cases of granulomatous inflammation diagnosed on lung biopsies and resections.

1784 Comparison of Napsin A and TTF-1 Expression in Metastatic Carcinomas from Various Sites: An Immunohistochemical Study of 143 Cases.

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Background: TTF-1 is currently the best immunohistochemical marker of lung origin in metastatic adenocarcinomas of unknown primary. The aim of this study was to compare the utility of the novel marker napsin A to TTF-1 for the accurate identification of pulmonary origin in metastatic carcinomas.
Design: One hundred forty three metastatic carcinomas, including 54 adenocarcinomas of pulmonary origin and 89 carcinomas of non-pulmonary origin, were stained with antibodies to TTF-1 and napsin A. Non-pulmonary carcinomas included 57 adenocarcinomas and 32 other carcinomas that may enter the differential diagnosis of a metastatic pulmonary adenocarcinoma.

Results: The results are shown in Table 1.

Table 1. Napsin A and TTF-1 in 143 Metastatic Carcinomas

Origin	No. of cases	Napsin A - pos (%)	TTF-1 - pos (%)
LUNG	54	42 (78)	44 (81)
NON-PULMONARY	89	11 (12)	9 (10)
Colon	12	0	0
Breast	12	0	0
Kidney (RCC)	11	5 (45)	0
Thyroid*	10	4 (40)	9 (90)
Pancreas	8	0	0
Urothelial tract (UC)	6	0	0
Prostate	6	0	0
Stomach	5	0	0
Liver (HCC)	5	1 (20)	0
Esophagus	5	0	0
Endometrium	5	1 (20)	0
Ovary	4	0	0

RCC=renal cell carcinoma; *7 papillary, 2 medullary, 1 follicular; UC=urothelial carcinoma; HCC=hepatocellular carcinoma

The sensitivity and specificity of napsin A for adenocarcinomas of pulmonary origin were 78% and 88%, respectively. TTF-1 was 81% sensitive and 90% specific. Three metastatic adenocarcinomas of pulmonary origin were TTF-1-positive but napsin A-negative, while 1 was positive for napsin A but negative for TTF-1. In 2 additional adenocarcinomas of pulmonary origin, TTF-1 was weak and present only in rare cells whereas napsin A staining was strong and diffuse. The napsin A-positive endometrial tumor was a FIGO grade 2 endometrioid adenocarcinoma metastatic to a pelvic lymph node; the napsin A-positive tumor of hepatic origin was a well-differentiated hepatocellular carcinoma metastatic to the adrenal.

Conclusions: Napsin A is expressed in a wider variety of metastatic carcinomas than TTF-1. Its expression in endometrial adenocarcinoma and hepatocellular carcinoma has not been previously reported. Although the sensitivity and specificity of napsin A for metastatic pulmonary adenocarcinoma is slightly lower than TTF-1, occasional adenocarcinomas of pulmonary origin are negative or equivocal for TTF-1 but strongly positive for napsin A, suggesting that napsin A may have diagnostic utility in this setting.

1785 The EBUS-TBNA Follow-Up of Cases with Cytological Diagnoses of Atypical/Suspicious Cells in Lung Cancer Patients. A Retrospective Study among 661 Cases.

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Background: Endobronchial ultrasound guided transbronchial needle aspiration (EBUS-TBNA) has been increasingly used by clinicians in the US. Our published data and others have shown that EBUS-TBNA has higher sensitivity and specificity than conventional TBNA in staging of lung cancers. Our data also showed that EBUS-TBNA has a significantly lower non-diagnostic rate (8.7%) compared to TBNA (28.3%, P<0.05) in staging lung cancers. However, 5 to 10% of EBUS-TBNA specimen has been diagnosed as atypical cells and/or suspicious for malignancies. In this study, we have investigated the follow-up EBUS-TBNA cytological diagnostic yield and histological correlation of these cases in clinically suspicious lung cancer patients.

Design: A computer search of the cytopathology archives at a major teaching hospital revealed 661 EBUS-TBNA cases over a 50 month time period. Among them, 340 cases (51%) and 208 cases (31%) were diagnosed as malignant and benign lesions, 82 cases (12%) were diagnosed as atypical and/or suspicious for malignancy. 47 cases had follow-up repeat EBUS-TBNA. All cytological material was correlated with corresponding follow-up cytological/surgical material.

Results: Of 47 repeat EBUS-TBNA (23 LN cases and 24 lung cases), the most frequently sampled locations were station 4R, 7, right upper lobe and right lower lobe. Malignancy was diagnosed in 24 of 47 cases (51.1%). The most frequent malignant diagnosis was lymphoma (29%), followed by adenocarcinoma (21%), squamous cell carcinoma (21%) and non-small cell carcinoma (17%). 23 of 47 cases (48.9%) were diagnosed as benign; 16/23 cases were later found to have cancer diagnosed by surgical follow-up or clinical progression. Among repeat EBUS-TBNA, the sensitivity and specificity were 91.7% and 60.0% in lymph node sampling; and were 91.7% and 16.7% in lung sampling. In addition, repeat EBUS-TBNA was able to provide sufficient tumor tissue for EGFR and KRAS studies in 5 of 5 adenocarcinoma cases.

Conclusions: Our data showed that repeat EBUS-TBNA was able to further confirm the clinical suspicion of malignancy, particularly in locally advanced and/or metastatic lung cancer patients. It was also able to provide tumor tissue for molecular study. In addition to sampling error, the sensitivity and specificity of repeat EBUS-TBNA may also relate to the complexity, size, and location of lesions.

1786 Variant Pulmonary Alveolar Proteinosis: A Syndrome with Distinct Clinical and Pathologic Features.

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Background: Pulmonary alveolar proteinosis (PAP) is a rare condition in which pulmonary macrophages fail to clear surfactant from the lungs, resulting in the alveolar accumulation of lipoproteinaceous debris. The histopathology of PAP is typified by the diffuse filling of alveoli and terminal bronchioles with eosinophilic, periodic acid-Schiff (PAS)-positive acellular material. We describe distinctive morphologic variants of PAP with heterogeneous clinicopathologic features.

Design: The clinicopathologic features of five cases of lung disease with prominent lipoproteinaceous alveolar exudates resembling pulmonary alveolar proteinosis were examined by light microscopic, ultrastructural, and immunohistochemical analysis. Serum antibodies to granulocyte-macrophage colony-stimulating factor (GM-CSF) were assayed in one patient.

Results: These cases of "variant proteinosis" were distinguished by an abundance of degenerating foamy alveolar macrophages, weak PAS staining of intraalveolar lipoproteinaceous material despite accumulation of surfactant apoprotein A, and a paucity of lamellated surfactant bodies by ultrastructural examination. Large, lamellated concretions of surfactant were visible by light microscopy in one case. Cholesterol crystals were frequent. Only one patient showed the computed tomography finding of mosaiform "crazy-paving", and only this patient had the opalescent bronchoalveolar lavage fluid characteristic of alveolar proteinosis. Circulating antibodies to GM-CSF were not detected. Three patients showed either a partial or near complete resolution of disease in response to high-dose corticosteroid therapy, a treatment approach that is generally ineffective in pulmonary alveolar proteinosis. In one patient, therapeutic lung lavage based on a presumptive diagnosis of pulmonary alveolar proteinosis exacerbated respiratory distress and hypoxemia.

Conclusions: Distinguishing "variant proteinosis" from classical PAP can be clinically important. Despite the otherwise typical appearance of granular lipoproteinaceous alveolar material in lung biopsies, the presence of abundant degenerating foamy macrophages, atypical histochemical, ultrastructural, and radiographic features, and the absence of antibodies to GM-CSF suggest a steroid-responsive form of proteinosis that is likely pathogenetically distinct and may not be amenable to whole-lung lavage.

1787 Histologic and Cytomorphologic Features of *ALK*-Rearranged Lung Adenocarcinomas.

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Background: Chromosomal rearrangements leading to constitutive activation of anaplastic large cell lymphoma kinase (ALK) define a category of lung adenocarcinomas that may be amenable to targeted therapy. Our previous study with a limited number of *ALK*-rearranged (ALK+) tumors has shown their association with a solid histology with numerous signet ring cells; however, experience of others appears to be different. Thus, we evaluated the histologic and cytomorphologic characteristics of ALK+ lung adenocarcinomas from a large cohort of patients.

Design: We examined biopsy, resection, and cytology specimens from 119 patients with lung adenocarcinomas that were positive for an *ALK* rearrangement by fluorescence in situ hybridization. Histologic subtypes and distinctive cytomorphologic features were assessed in each case and were compared with 117 *ALK* rearrangement-negative (ALK-) lung adenocarcinomas. In mixed-subtype tumors, the predominant histologic subtype was used in the analysis.

Results: The 119 ALK+ lung adenocarcinoma specimens consisted of 60 resections or excisional biopsies of primary or metastatic tumor, 49 core or transbronchial biopsies, and 10 cell blocks. The most common predominant histologic subtype was solid pattern (58%), followed by micropapillary (17%), acinar (14%), papillary (7%), and bronchioloalveolar (3%). In one specimen, the tumor was predominantly infiltrative with single cells and cords. Tumors with a predominantly solid subtype were more frequent in ALK+ cases compared to ALK- cases (58% vs. 31%, P < 0.0001). In 35% of the ALK+ tumors, signet ring cells comprised 10% or more of the tumor cells, and a solid histology with numerous signet ring cells was seen in 24% (vs. 3% of ALK- tumors, P < 0.0001). Large hepatoid cells with abundant eosinophilic cytoplasm and prominent nucleoli were observed in 26% of the ALK+ cases but were rarely seen in the ALK- tumors.

Conclusions: The evaluation of a large cohort of ALK+ lung adenocarcinomas confirmed a solid subtype with numerous signet ring cells as their characteristic histology and cytomorphology. Identification of this particular pattern as well as cells with hepatoid features may be useful in predicting the subset of lung adenocarcinomas harboring *ALK* rearrangements.

1788 High Expression of Folate Receptor Alpha in Lung Cancer Correlates with Adenocarcinoma Histology and EGFR Mutation.

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Background: Folate receptor alpha (FRa) and receptor folate carrier-1 (RFC1) regulate cellular uptake of folate molecules inside the cell, and are potential biomarkers of tumor response to antifolate chemotherapy. Information on the protein expression of these receptors in non-small cell lung carcinoma (NSCLC) is limited.

Design: Expressions of FRa and RFC1 were examined by IHC in 320 surgically resected NSCLC (202 ADC and 118 SCC) tissue specimens using TMA. The findings were correlated with patient clinicopathologic characteristics. To address FRa expression level with tumor progression, analysis of specimens from advanced tumors and brain metastasis was performed. *FOLR1* mRNA expression was examined using publicly available microarray datasets. FRa expression was correlated with tumors' thymidylate synthase (TS) in NSCLCs, and with *EGFR* and *KRAS* mutations in ADC.

Results: NSCLC frequently over expressed both FRa and RFC1 in samples from early and advanced stages of the disease. In a multivariate analysis, lung ADC were more likely to express FRa in the cytoplasm (odds ratio [OR] = 4.39; *P*<0.0001) and membrane (OR = 5.34; *P*<0.0001) than SCC. Tumors from never-smokers were significantly more likely to express cytoplasmic (OR = 3.35; *P*<0.03) and membrane (OR = 3.60; *P*=0.0005) FRa than those from smokers. FRa and RFC1 expressions did not correlate with NSCLC patients' outcome. In ADC, *EGFR* mutations correlated with higher expression of membrane FRa and *FOLR1* gene expressions. FRa and TS expression inversely and significantly correlated (*P*=0.03).

Conclusions: Membrane transporter FRa and RFC1 proteins are frequently over expressed in NSCLC tissues at all tumor stages. The higher levels of FRa correlate with lung ADC histotype and presence of *EGFR* mutation.
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1789 Utility of Ancillary Studies in the Classification of Lung Adenocarcinoma and Squamous Cell Carcinoma in Cytology Specimens.

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Background: Histologic subtyping of non-small cell lung carcinoma (NSCLC) is becoming important because the efficacy and toxicity of new treatments for NSCLC highly depend on tumor histology. Majority of lung cancers are not surgically treated, and only limited tissue is available for diagnostic and molecular studies. The aim of this study was to assess the diagnostic accuracy of classification of lung adenocarcinoma (ADC) and squamous cell carcinoma (SQC) on cytology and biopsy specimens based on cytomorphology alone or in combination with ancillary studies when compared to resection specimens.
Design: 448 diagnostic preoperative cytology specimens (transthoracic CT-guided FNAs, bronchial brushings, and washings) of a primary lung ADC (263 cases; 59%) and SQC (185 cases; 41%) that underwent surgical resection at the Universitiy of Pittsburgh Medical Center between January 2000 and January 2010 were included in the study. Histologic classification of the tumor on the cytology and resection specimens, the results of histochemical (mucicarmine, PAS with diastase) and immunohistochemical (IHC) studies (cytokeratin (CK) 7, 20, 5/6; TTF-1, surfactant protein A, and p63) reported in the Pathology electronic records were documented. Classification accuracy of cytology specimens was defined as histologic concordance between the cytologic classification and the final surgical resection diagnosis. CK5/6 and p63 were used asIHC markers for SQC and TTF-1 and surfactant protein A were markers for ADC.
Results: The diagnosis of SQC was based more often on cytomorphology alone (75% SQC vs. 41% ADC)(P=0.001). More frequent use of IHC in cytology was noted after introduction of *EGFR* targeted therapies in clinical practice (14% ADC and 8% SQC before 2005 vs. 86% ADC and 89% SQC after 2005). Use of IHC resulted in increased diagnostic accuracy for ADC (56% before 2005 vs. 83% after 2005)(p=0.0001), but not for SQC (77% before 2004 vs. 74% after 2005)(p=0.7290). IHC reduced the number of ADC, but not SQC, classified as NSCLC. ADC showed high expression of CK7 (100%), TTF-1 (86%), surfactant A (81%) and PASD (80%). All of SQC were positive for CK5/6 and p63. Subsets of ADC and SQC were interpreted as positive for p63 and TTF-1 respectively.
Conclusions: Implementation of *EGFR* targeted therapies in lung cancer treatment resulted in increased use of ancillary studies for the classification of NSCLC. IHC appears to be useful in diagnosis of ADC, but not SQC. The panel of p63 and PASD may be help to exclude the squamous differentiation on diagnostically limited specimens.

1790 Proteomic Analysis of the Lung in Rats with Hypobaric Hypoxia-Induced Pulmonary Hypertension.

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Background: The experimental pulmonary hypertension that develops in hypobaric hypoxia is characterized by structural remodeling of the lung.
Design: Using proteomics -- perhaps the most powerful way to uncover unknown remodeling proteins involved in the enhancement of cardiovascular performance -- we analyzed lungs from 150 male Wistar rats housed in a chamber at the equivalent of the 5500m altitude level for up to 21 days.
Results: After 14 days' exposure to hypobaric hypoxia, pulmonary arterial pressure (PAP) was significantly increased. In lung tissue, about 140 matching protein spots were found among 8 groups (divided by hypobaric periods) by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) (pH4.5-pH6.5, 30kDa-100kDa). In hypobaric rats, three spots were increased two fold or more (vs. control rats) using differential in-gel electrophoresis (DIGE). The increased proteins were identified, by matrix-assisted laser desorption ionization time of flight (MALDI-TOF), as one isoform of protein disulfide isomerase associated 3 (PDIA3), and two isoforms of heat shock protein 70 (HSP70). These two proteins were confirmed by Western blotting analyses using 2D-PAGE.
Conclusions: Conceivably, PDIA3 and HSP70 may play roles in modulating the structural remodeling that occurs in the lung due to pulmonary hypertension in hypobaric hypoxia.

1791 Increased Caspase-9 Gene Expression Contrast with Normal Type V Collagen and VEGF Isoforms Gene Expression in Lung Chemical Carcinogenesis.

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Background: Recently, we demonstrated an association between decreases in type V collagen and apoptosis in human and mouse lung chemical carcinogenesis, but the mechanisms involved still remains uncertain (Parra et al 2010; Souza et al 2010). Remodeling of the tumoral microenvironment was evaluated in a model of chemical carcinogenesis in the mouse lung.
Design: Ultrastructural analysis, tridimensional reconstruction and mRNA gene expression were used to evaluate the amount, structure and genic expression of collagen V, vascular endothelial growth factor (VEFG) isoforms (120,164, 168) and caspase-9 in two groups of male Balb/c mice: a) animals that received two intraperitoneal doses of 3 g/kg urethane carcinogen (urethane group = 15); and b) animals submitted to a sham procedure, comparable to the test group (control group = 4). Both groups were sacrificed after 120 days.
Results: All the urethane animals had multiple pulmonary neoplasms. Histologically, the lung tumors present adenoid pattern and ultrastructurally they consisted predominantly of Clara cells and of cells morphologically resembling type II alveolar epithelial cells immersed in a loose stroma composed by reduced collagen fibers. Tridimensional reconstruction showed normal spatial organization of the fibers, normal synthesis in cellular cultures and normal gene expression of type V collagen and VEFG isoforms. An important significant higher Caspase-9 expression was observed in tumoral areas when compared with control group.
Conclusions: The results suggest that chemical lung carcinogenesis doesn't induce type V collagen and VEFG issoforms genes damage but increased the expression of caspase-9 gene. The decrease observed of this collagen in the tumoral parenchyma probably is caused by active destruction of the fibers after their synthesis and in this context the activation of caspase-9 probably is not sufficient to stop the growth neoplastic cells. Other studies are necessary to evaluate if these results are only causal or consequence.
Financial support: FAPESP, CNPq.

1792 Different Cytokines and Growth Factors Have Different Expression in Limited or Diffuse Systemic Sclerosis.

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Background: Systemic sclerosis (SSc) is a multisystem connective tissue disorder characterized by excessive accumulation of extracellular matrix, resulting in progressive fibrosis and dysfunction of a number of organs. In this regards many studies suggest that several cytokines and growth factors have been implicated in the SSc. The aim of this study was examined different factors, cytokines and growth factors, implicated in the fibrotic process in limited and diffuse SSc.
Design: Lung specimens were obtained from 23 patients with limited (n=10) or diffuse (n=13) SSc. Immunohistochemical staining of alpha-smooth muscle actin (α-SMA), anti-interleukin (IL)-4, anti-IL-13, anti-*basic fibroblast growth factor* (bFGF), anti-transforming grow factor beta (TGF-β), anti-telomerase and anti-endothelin-1 (ET-1), and morphometric analysis were used to score in epithelial, endothelial, myofibroblast and smooth muscle cells and correlated with pulmonary functions.
Results: We observed higher epithelial and bronchiolar smooth muscle cells expression of bFGF and higher endothelial IL-4 and IL-13 expression in diffuse SSc when compared with limited SSc ($p<0.05$). A significant negative association existed between myofibroblast ET-1 expression and VC (r=-0.594, p=0.02); endothelial bFGF expression and DCO (r=-0.583, p=0.03); and a positive association between endothelial telomerase expression and FEV1, FVC, and DCO/VA (r=0.435, p=0.04; r=0.585, p=0.02; and r=0.595, p=0.02; respectively).
Conclusions: The evidence suggests that increased from epithelial and bronchiolar smooth muscle cells expression of bFGF and endothelial IL-4 and IL-13 expression in diffuse SSc can be partially mediated by the extension of the disease, but more importantly provide a potential histochemical biomarker for differentiating limited SSc from diffuse SSc. However, further studies are needed to determine whether or not these overexpressions are causative or indicative of SSc progression.
Financial support: FAPESP, CNPq.

1793 Common Markers (Cytokeratins 5/6&7, p63, TTF1, Vimentin) on Small Biopsies of Non-Small Cell Lung Cancer Effectively Parallel Profiling and Eventual Diagnoses of Surgical Specimens.

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Background: More detailed typing of non-small cell lung cancer (NSCLC) upon small biopsy specimens is increasingly required, albeit demanding with morphology alone. Little, however, is known about the likelihood of immunohistochemistry (IHC)-assessed small biopsies to effectively parallel profiling and hence eventual diagnoses of surgical specimens.
Design: Sixty-three preoperative biopsies and corresponding surgical specimens from 30 consecutive squamous cell carcinomas (SCC), 22 adenocarcinomas (AD), two adenosquamous carcinomas (ADSCC), eight pleomorphic carcinomas (PLC) and one yolk sac tumor were jointly evaluated semiquantitatively for cytokeratin 5/6 and 7, p63, TTF1 and vimentin immunoreactivity. Surgical specimens were the gold standard for morphology and IHC.
Results: Unsupervised clustering of surgical specimens and biopsies showed a nonrandom and overlapping distribution of the relevant markers, which closely correlated with each other and the diverse tumor categories, as confirmed by mosaic plot analysis. There were no differences in AUC-ROC curves for each marker between any two samples, with the exception of p63 that paralleled more effectively squamous cell carcinoma on biopsy than surgical specimen. 59/63 (94%) lesions were correctly classified by IHC on biopsy compared to 53/63 (84%) by revised morphology, with predictive positive value of diagnostic accuracy of 97% for SCC, 88% for AD, and 100% for PLC and ADSCC. Yolk sac tumor and three PLC, however, failed any diagnostic recognition.
Conclusions: Diverse cell differentiation lineages of NSCLC may be consistently detected by IHC in small biopsies, making the eventual typing of tumors effective in most cases.

1794 Presence and Absence of Chondroitin Sulfate in Lung Tissues as a Potential Diagnostic Marker of Lung Cancer.

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Background: The relationship between the extracellular matrix(ECM) components and cancer cells have an important role on cancer development and progression.Between the most important molecules present on the ECM are the glycosaminoglycans(GAGs) and studies have reported that they have different behaviours when in the presence of malignant tissues.The aim of this study was to analyse the GAGs concentration in normal and tumoral areas of patients with lung cancer(LC).

Design: Normal and tumoral tissue specimens were obtained from 41 patients with LC(stages I until IIIB).Sulfated GAG chains(heparan, dermatan and chondroitin sulfate – HS, DS and CS)were obtained after incubation with a proteolytic enzyme.GAGs were precipitated with ethanol and the pellet was centrifugated, dried and dissolved in DNAse(5 µl/mg).The different types of sulfated GAGs and their concentration in the lung samples were identified after gel electrophoresis in diaminopropane buffer.

Results: We observed a distinct profile of GAG between normal and tumoral areas.HS showed significantly higher concentration in tumoral than in normal areas(p=0.02)(FIG 1A).This data correlated with the different histologic types.The adenocarcinomas had higher amounts of HS than squamous cell carcinoma specimens(p=0.02)(FIG 1B).One hundred % of tumoral areas presented CS while the normal areas did not(p<0.0001) (FIG 1C).

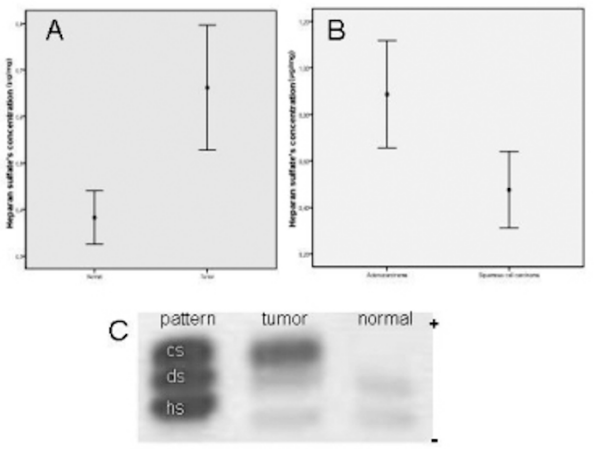

FIG 1: **A** - Difference between HS's concentration (µg/mg of tissue dry weight) in normal and tumoral areas. p=0.02. **B** - Difference between HS's concentration (µg/mg of tissue dry weight) in adenocarcinoma and squamous cell carcinoma samples. p=0.02. **C** - PDA gel electrophoresis assay showing the different GAG types and concentration in a pattern, normal and tumoral tissues.

Conclusions: The presence of CS and higher concentrations of HS in patients with lung cancer suggest a possible role of these GAGs in this pathological condition, but more importantly provide a potential biochemical marker for differentiating normal from lung cancer patients.The correlation between the histologic types and the amounts of HS provide a possible role of this GAG on the development of tumor agressiveness considering that one of its functions is to bind itself to growth factors and regulate their action.However, further studies are needed to determine whether or not these GAGs concentrations are able to be diagnostic/prognostic markers of LC and what is their role regarding the histologic types.

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1795 Intrapulmonary Solitary Fibrous Tumors – A Clinicopathologic Analysis of 25 Cases.

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Background: Solitary fibrous tumor (SFT) was originally described arising in the pleura; later recognized to be ubiquitous in origin. SFT arising within lung has been reported only sporadically, and is therefore not well recognized. Majority of SFT in all locations are benign; a small proportion are aggressive. Although several features have been associated with such behavior, the biology of these tumors remains somewhat unpredictable. For intrapulmonary SFT, the problem is compounded by its relative rarity. This study comprises 25 cases of intrapulmonary SFT. To our knowledge, this is the largest series of this entity, with long term clinical follow up.

Design: Twenty five (25) cases of intrapulmonary SFT form the basis of this study. Clinical, radiologic and pathologic findings were evaluated to include those cases conclusively intraparenchymal in location. Aggressive histologic features including mitoses, atypia and necrosis were particularly assessed. Immunohistochemical stains including CD34, CD99, Cytokeratin AE1/AE3, EMA, SMA, bcl-2, MIB-1, Calponin, and Vimentin were performed on 19 cases. Clinical follow up was available in 19 cases, with long term follow up (> 5 years) available in 5 patients.

Results: 2/25 patients had needle core biopsies; 23/25 had resections. 6/23 resected tumors were > 10 cm in size. 3/6 tumors > 10 cm in size had > 10 mitoses/10 hpf with accompanying necrosis. 5 patients had long term follow up (> 5 years) – 2 alive and well at 13 and 14 years respectively; 2 dead of metastatic disease at 5 and 7 years respectively; 1 patient alive with chest wall metastases at 5 years. A 6th patient was dead of metastatic disease at 4 years. Of 6 patients with tumors > 10 cm, long term follow up was available in two patients – 1 alive and well at 14 years; other patient had chest wall metastases. Of 3 patients dead of metastatic disease; 1 patient had high grade sarcoma at presentation with focal classic SFT areas (CD34 focally positive); and 2 patients had classic SFT at presentation (CD34 positive), with high grade sarcoma at autopsy (retaining CD34 positivity).

Conclusions: Intrapulmonary SFT is a rare entity. This series of 25 cases with significant follow up suggests that there are no reliable predictors of behavior. Regardless of histologic features; close clinical follow up appears to be the most prudent course of action at this time.

1796 Antibody Mediated Rejection (AMR) in Lung Allografts: Pathologic Features in Patients with Clinically Diagnosed Humoral Rejection.

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Background: AMR is a newer concept in lung transplant pathology, and the features are less defined than for renal and cardiac allografts. We undertook this study to detail histologic features in cases with clinically diagnosed humoral lung allograft rejection.

Design: The clinical files of our institution were searched, for examples of "humoral rejection." Selected cases met the NIH working definition of solid organ AMR, with allograft dysfunction, tissue pathology, complement deposition, and circulating donor antibodies. Cases were then reviewed for relevant histologic findings, including hemorrhage, necrosis, vasculitis/capillaritis, interstitial neutrophils, acute and organizing lung injury, and cellular rejection. Immunostains for complement were also reviewed.

Results: Eighteen cases fit the clinical definition for AMR. The time from transplant to the index biopsy for the AMR diagnosis ranged from 1 to 120 weeks (mean 40 weeks). All patients had allograft dysfunction, and 17/18 had antibodies (14/18 with circulating donor-specific antibodies; 3/18 with anti-HLA antibodies only) Fourteen patients had C4d immunodeposition in the lung, 2 cases had equivocal staining, and 2 cases were not tested. Acute interstitial pneumonitis with neutrophils was the most common histologic feature, seen in 13/18 cases; the second most common finding was organizing pneumonia/BOOP (11/18). Vasculitis/capillaritis was seen in 8/18 cases, with extensive hemorrhage in 5/18, and alveolar necrosis in 3/18. Four cases showed concomitant acute cellular rejection, including 2 cases each of A1 and A2. The 14 cases with positive C4d staining ranged from strong diffuse capillary staining, to more focal endothelial staining, sometimes in larger vessels only. Two equivocal cases showed only C4d deposits in the interstitium, or in areas of fibrin exudate. There was high background elastic tissue staining complicating interpretation in most cases.

Conclusions: AMR is an important process in the lung transplant setting, and may occur well outside of the early post-transplant setting. The most common finding is acute interstitial pneumonitis, followed by organizing injury patterns; fewer cases show classic features of vasculitis or related lesions. The significance of C4d immunostains, and the delineation of patterns to be considered positive remains a point for further study. AMR should be in the differential diagnosis when there is lung injury without clear-cut infection or other etiology.

1797 Temporal Evolution of Histologic and Cell-Kinetic Parameters of Recurrent and Metastatic Thymic Neoplasms.

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Background: Thymic neoplasms are generally considered as low grade malignancies, which can recur or metastasize. The temporal evolution of histologic and cell-kinetic features in recurring and metastasizing thymic neoplasms has not been studied.

Design: Medical records from 15 patients (pts) (0.05%) treated for thymic neoplasm, 1970-2006, who developed recurrence (recur) and/or metastasis (met) were reviewed. Two pathologists classified all cases (WHO) with consensus diagnosis. Ki-67 LI was expressed as %pos/100 epithelial cell (EC) nuclei (mean of 1000 EC counted over 3 areas) and mitotic activity (MA) as mitoses/10Hpf (mean of 50Hpf counted).

Results: 10 men & 5 women with a median age of 50.3 yrs underwent complete resection of the original tumor (OT) (n=14), or had a biopsy (n=1). 7 pts had additional therapy. Masaoka stages were I (n=2), II (n=6), III (n=5), IV (n=2). OTs were classified as WHO types A (n=1, 6.7%), B1 (n=2, 13.3%), B2 (n=7, 46.7%), B3 (n=2, 13.3%), thymic carcinoma (n=2, 13.3%), atypical thymic carcinoid (n=1, 6.7%). For comparison, all thymic neoplasms treated during that time were classified as A (10.9%), AB (16.1%), B1 (22.3%), B2 (27.4%), B3 (12.4%), thymic carcinoma (7.3%), others (3.6%). Pts had 1 (n=5), 2 (n=4), or 3 (n=2) recurrences. 13 pts had mets, 8 of which also had recurrence(s). The mean interval between initial therapy and 1st recur/met was 6.0 yrs (range, 0 – 14.2 yrs). 30 resection specimens and 7 biopsies were evaluated for histology, Ki67 LI (n=33), and MA (n=31). In 5 cases, WHO type of the recur/met was different from the OT (Table 1).

Temporal evolution of histopathology

	Original tumor (OT)	1st recur/met	2nd recur/met	3rd recur/met
Case	WHO (yrs after OT)			
2	B2	B2 (2.5)	B3 (5.7)	Thymic carcinoma (13.4)
3	B2	B3 (6.0)		
8	B1	B2 (5.6)		
9	B2	B2 (4.4)	B3 (10.2)	
11	B2	B1 (8.8)		

Median Ki67 level and MA increased > 20% in 4 (of 13) and 6 (of 13) 1st recur/met and 1 (of 5) and 4 (of 4) 2nd recur/met specimens when compared to OT and 1st recur/met, respectively. F/u was available in all pts with a mean f/u time of 13.3 yrs (range, 0.6 – 18.9 yrs). 10 pts died, 4 of disease.
Conclusions: The histologic type in most recur/met thymic neoplasms remains unchanged from the OT and the proportion of type B2 and thymic carcinoma is higher when compared to all thymic tumors. Occasionally, B2 thymoma can recur/metastasize as a B3 or thymic carcinoma. Cell-kinetic parameters increase only in the minority of recur/mets. The relative low rate of progression in tumor type and in cell-kinetic parameters from OT to recur/met may explain the bland behavior of most thymic neoplasms.

1798 Differential Expression of microRNA Markers in Pulmonary Neuroendocrine Tumors: Correlation with Histologic Grade.

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Background: Pulmonary neuroendocrine (NE) tumors include low-grade typical carcinoid (TC), intermediate-grade atypical carcinoid (AC) and high-grade large cell NE carcinoma (LCNEC) and small cell lung carcinoma (SCLC). These tumors have been a difficult challenge for pathologists to diagnose. MicroRNAs (miRNAs) have a critical effect on carcinogenesis through post-transcriptional modification and are considered as potential biomarkers for cancer diagnosis.
Design: The expression of five selected miRNA (miR-21, miR-34, miR-135, miR-155, and let 7a) was evaluated in 60 pulmonary NE tumor tissues (18 TCs, 6 ACs, 21 LCNECs and 15 SCLCs) to explore whether the differential expression of miRNAs can be used as a diagnostic tool in grading pulmonary NE tumors. Total RNA was extracted from formalin-fixed paraffin-embedded tissue using RecoverAll Total Nucleic Acid Isolation kit. Quantitative RT-PCR for each miRNA species was performed using the miRNA TagMan RT-PCR kit. Control amplification for endogenous small RNA U6 was performed in all samples. Mean Ct values were calculated for each case and then normalized against the corresponding U6 Ct values, calculated as ($Ct_{experimental\ miRNA}$-$Ct_{u6\ RNA}$). The fold differences in miRNA levels between groups were also noted ($2^{\Delta Ct}$).
Results: Significant overexpression of miR-21 and miR-155 was found in high-grade NE tumors compared to carcinoid tumors, with mean fold difference ranging from 1.6 to 7.8. Although high-grade NE tumors tended to show lower expression of let-7a than in carcinoid tumors, no significant difference was found (p=0.08). No difference was found between LCNEC and SCLC and between TC and AC for miR-21, miR-155, and let-7a, respectively. In contrast, no significant difference was seen for miR-34 and miR-135 (p=0.12 and 0.64, respectively).
Conclusions: To the best of our knowledge, this is the first study to evaluate miRNA expression pattern in pulmonary NE tumors. We concluded that miRNAs, particularly miR-21 and miR-155, might potentially be adjunct markers for distinguishing high-grade NE tumors from carcinoid tumors. Further miRNA-based approach with a large number of cases would allow us to identify novel miRNA for accurate classification of pulmonary NE tumors.

1799 Immunohistochemical Analysis of ALK Fusion-Positive Lung Cancers: Correlation with FISH, Molecular Findings and Morphology.

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Background: Activating fusions involving the gene encoding anaplastic lymphoma kinase (ALK) have been detected in a subset of lung cancers and define a clinically relevant molecular subset sensitive to ALK tyrosine kinase inhibitors. The majority of ALK fusions involve the upstream partner, EML4. This study correlates immunohistochemical (IHC) staining for ALK with tumor morphology, IHC for other common lung cancer antigens, and FISH and/or RT-PCR in a large cohort of non-small cell lung cancers.
Design: ALK IHC (Cell Signaling, rabbit monoclonal D5F3) was performed on 14 tissue microarrays (TMA) containing 434 adenocarcinomas and 226 squamous cell carcinomas. FISH was performed using an ALK break apart probe (Abbott Molecular, Abbott Park, IL). RT-PCR sequencing used 3 different 5' EML4 primers (that detect all known EML4 variants) and 1 ALK primer that hybridizes to all known ALK fusions. All ALK D5F3 stains were compared with routine IHC for CK7, CK20, TTF-1, CK5/6, p63, CDX-2 mucicarmine, and ALK1 (Dako, monoclonal mouse CD246). The presence of a fusion was verified by either FISH or RT-PCR in all cases.
Results: 8 ALK-positive cases were identified: 4 by TMA, 2 by FISH, 2 by RT-PCR. All cases were adenocarcinoma. 7 tumors were positive by IHC (D5F3 antibody). 5 of these were positive by the Dako antibody with generally weaker positivity and more nonspecific staining in FISH-negative tumors. ALK (D5F3) showed moderate-strong staining in >90% of cells in 5 cases, and weaker staining in >30% of cells in 2 cases. One case with a known EML4-ALK E20;A20 fusion by RT-PCR showed very weak staining in <30% of tumor cells (IHC neg). This sample and the 2 weaker ALK IHC positive cases all showed prominent mucinous features. Other histologic patterns included micropapillary to cribriform or solid architecture, sometimes mixed with mucinous/signet ring cells. 7/8 ALK positive tumors had material for IHC panel correlation and were positive for: CK7(7/7). TTF-1(7/7), p63(4/7), and mucicarmine(4/7), while CK5/6, CK20 and CDX2 were essentially negative.
Conclusions: ALK IHC (D5F3) antibody showed good correlation with FISH and RT-PCR and was easier to evaluate than ALK1 (CD246). Mucinous tumors may show lesser staining with ALK IHC. ALK positive tumor morphology can be suggested by a micropapillary-cribriform-solid architecture especially if mixed with mucinous features. Unlike some mucinous adenocarcinomas of the lung, CDX2 and CK20 were negative in these tumors.

1800 Napsin A Expression in Sclerosing Hemangiomas of the Lung.

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Background: Sclerosing hemangiomas (SH) are lung tumors with two distinct cell types: surface cuboidal cells resembling type II pneumocytes, and round stromal cells. SH is now thought to be of primitive respiratory epithelial origin due to its immunohistochemical (IHC) and electron microscopic features. Napsin A, a human aspartic proteinase expressed primarily in type II pneumocytes and alveolar macrophages, is emerging as a helpful IHC marker in characterizing lung neoplasms. We sought to evaluate napsin A staining in SH to help further characterize this unusual tumor.
Design: H&E stained slides from lung resections for SH performed at our institution between 1997-2010 and cases from the authors' consultation files (n=6) were reviewed. IHC was performed using an avidin-biotin-peroxidase complex method on an automated immunostainer (Ventana-Biotech, Tucson, AZ). IHC stains were evaluated for presence of staining, localization of positivity if present, and strength of staining.
Results: Napsin A staining was available for all cases; TTF-1 for 5 cases; cytokeratin (CK) cocktail (CAM 5.2 and AE1/AE3) for 2, CK7 for 1, and CK AE1/AE3 for 1. TTF-1 demonstrated nuclear positivity in both surface and round cells in all 5 cases. CK was positive in surface cells and negative in round cells in all 5 cases. Round cells had focal napsin A staining in 1 case (17%) and surface cells showed positive staining in all 6 cases. In one, surface cell staining was limited to the tumor edge. In one case, most surface cells showed strong granular cytoplasmic staining. In the remaining 4 cases, there were both areas of strong granular staining in surface cells and areas where surface cells were completely negative; negative staining corresponded to weaker keratin expression in these cases.
Conclusions: Similar to others, we observed TTF-1 positivity in both surface and round cells in all SH and CK staining only in surface cells, suggesting primitive respiratory epithelium as the possible cell of origin of SH. Previous reports of surfactant staining in surface, but not in round cells, suggest surface cells are more differentiated than round cells. Our napsin A findings support this, with positivity in surface cells of all tumors (100%), and round cell staining in only one (17%). This suggests that surface cells are type II pneumocytes, which normally express napsin A in a similar granular cytoplasmic pattern. The presence of focal staining in one tumor suggests that round cells may derive from a common progenitor cell. Additional IHC studies on napsin A staining in primitive lung tissue may be of use in further elucidating the origin of SH.

1801 Differentiation of NUT Midline Carcinoma in Humans and Mice by Epigenomic Reprogramming.

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Background: NUT midline carcinoma (NMC) is a lethal tumor defined by the presence of BRD-NUT fusion proteins that act by arresting differentiation. Here, we explore the mechanisms underlying the ability of BRD4-NUT to prevent squamous differentiation.
Design: In vitro studies were conducted with five NMC cell lines, 797, per403, 690, 143, and 10326, and primary cells derived from the index patient. In vivo studies were conducted with three NMC cell lines, 797, per403, and patient-derived cells. Knockdown of BRD-NUT was used as a positive control.
Results: We find that overexpression of the twin bromodomains of BRD4 or a portion of NUT that binds the histone acetyltransferase (HAT) p300 induces NMC cells to differentiate, suggesting a model in which BRD4-NUT sequesters HATs in areas of acetylated chromatin. Consistent with this idea, knockdown of BRD4-NUT caused global increases in histone acetylation, whereas enforced expression of BRD4-NUT had the opposite effect. Sequestration of HATs appears to occur in nuclear BRD4-NUT foci, which correspond to specific genomic regions that are rich in acetylated histones but transcriptionally inactive. Of therapeutic interest, treatment of NMC cells with histone deacetylase inhibitors (HDACi) dispersed nuclear foci, restored histone acetylation, induced NMC cells to differentiate in vitro, and had anti-tumor effects in NMC xenograft models. Based on these data, a child with NMC was treated with the FDA-approved HDAC inhibitor vorinostat. An objective response was obtained after five weeks of therapy, suggesting that this rational therapeutic approach merits further evaluation in patients with NMC.
Conclusions: In summary, we describe: 1) the rationale for targeted use of HDAC inhibitors in NMC; 2) the utility of a pharmacodynamic drug response (acetylation) as a mechanism of oncoprotein redistribution; and 3) the first example (to our knowledge) of true differentiation therapy since the seminal studies of retinoic acid in the treatment of acute promyelocytic leukemia.

1802 Lymphocyte Distribution in Biopsies of Interstitial Lung Disease Predicts Response to Monoclonal Antibody Therapy.

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Background: Interstitial lung disease (ILD) is a heterogeneous collection of illnesses associated with inflammatory and fibrotic changes within interalveolar spaces. The etiologies of ILD include idiopathic (such as usual interstitial pneumonia, or UIP) or those related to underlying autoimmune diseases where there may be significant inflammation; of this latter set, some cases have been associated with increased B cells in

lung tissue. Generalized immunosuppression is the mainstay of therapy in autoimmune ILD, but these agents have variable efficacy and significant side effects. As such, more targeted agents, such as the monoclonal antibody directed against CD20, rituximab, are under investigation for use in these populations.

Design: H&E slides were examined from surgical lung biopsies obtained from patients with ILD being treated with rituximab (two or four doses of 1000 mg). In each, histologic features, including diagnosis, degree of inflammation, and proportion of B-cells and T-cells present, were correlated with response to therapy as determined by pulmonary function testing and clinical assessment.

Results: A total of seven cases were examined. Histologically, three cases showed follicular bronchiolitis (FB), two cases showed UIP, and two cases showed nonspecific interstitial pneumonia (NSIP). In all cases, we observed an excess of CD20-positive cells (indicated by germinal center formation; quantitatively 6-48 lymphoid follicles per cm^2 and 2-to-10-fold CD20 over CD3). Of these, one has just initiated therapy and follow-up is pending, while the others have all shown clinical stabilization or improvement while taking rituximab (mean follow-up 20 months, range 5-30 months).

Pathology	Clinical	CD20:CD3	Follicles/cm^2	Clinical Response
FB	RA	4:1	12	stable
FB	Sjogren's	at least 2:1	unknown	improved
FB	RA	> 10:1	48	improved
NSIP	Wegener's	3:1	12	stable
NSIP	RA	2:1	6	stable
UIP	UIP	5:1	25	pending
UIP	RA	4:1	39	improved

RA: rheumatoid arthritis

Conclusions: In this limited series of patients with ILD, an increased proportion of CD20+ B cells correlates with response to rituximab therapy. These results suggest that the distribution of lymphocytes within an interstitial lung disease biopsy may identify a subset of patients who may benefit from therapy with targeted biologic modalities, even in the absence of known autoimmune disease.

1803 Oncofetal Protein IMP3, a New Biomarker To Distinguish Malignant Mesothelioma from Reactive Mesothelial Proliferation.

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Background: Distinguishing malignant mesothelioma (MM) from a benign reactive mesothelial proliferation remains a major challenge for surgical pathologists. No reliable diagnostic biomarker has been found to distinguish between malignant and reactive mesothelial cells. In this study, we investigated whether IMP3, an oncofetal protein, can be used as a diagnostic biomarker to distinguish between these two entities.

Design: A total of 109 cases (MM, n=45; reactive mesothelial cells, n=64) obtained from the formalin-fixed, paraffin-embedded block archives of two tertiary medical centers were examined by immunohistochemistry for IMP3 expression. Among the MM, 27 (60.0%) cases were epithelial type, 10 (22.2%) cases were mixed type and 8 (17.8%) were sarcomatous type. All cases were collected between January of 1997 and April 2010, and the diagnoses were confirmed by at least two pathologists.

Results: IMP3 showed strongly cytoplasmic staining in 33 of 45 (73%) MM cases. In contrast, none of 64 cases (0%) of benign reactive mesothelial proliferation exhibited IMP3 expression. Among the IMP3 positive MM, 27 (82%) exhibited diffuse IMP3 expression in more than 50% of malignant cells, 2 (6%) in 25-50% of malignant cells, and 4 (12%) in 5-25% of malignant cells. There was a positive IMP3 expression in 21/27 (78%) of epithelial, 8/10 (80%) of mixed and 4/8 (50%) of sarcomatous type. Importantly, two cases which were previously diagnosed as atypical mesothelial proliferation but were confirmed to be MM later showed IMP3 positivity in more than 50% of malignant cells.

Conclusions: Our findings indicate that IMP3 is a sensitive and specific biomarker for malignant mesothelioma and can separate malignant mesothelioma from a reactive mesothelial proliferation. Expression of IMP3 can increase the level of confidence in establishing the diagnosis of malignant mesothelioma.

1804 Pulmonary Neuroendocrine Carcinomas Show Alteration of Nrf2/Keap1 Pathway.

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Background: Nuclear factor erythroid 2-related factor 2 (Nrf2) is a transcription factor that regulates expression of molecules involved in cellular protection from toxins, oxidative stress and chemicals. Nrf2 expression is repressed by Kelch-like ECH associated protein 1 (Keap1). Phosphorylation of Nrf2 at serine-40 has been shown to mediate a dissociation of Nrf2 from Keap1, therefore playing a critical role in regulating Nrf2/Keap1 pathway. Although, recently Nrf2/Keap1 alterations have been reported in non-small cell lung carcinomas (NSCLC), not much is known about this pathway in neuroendocrine carcinomas (NEC). The aim of this study was to investigate expression of phosphorylated Nrf2 and Keap1 in a wide range of pulmonary NEC and assess their possible association with clinical-pathological findings.

Design: Clinical pathological findings of 178 patients were analyzed with regard to Nrf2 and Keap1 expression. Tissue microarray based samples of 48 typical carcinoids (TC), 31 atypical carcinoids (AC), 27 large cell neuroendocrine carcinomas (LCNEC), and 72 small cell lung carcinomas (SCLC) were studied for phosphorylated Nrf2 using antibody that recognizes phosphoserine residue located at amino-acid 40 (1:500, EP1809Y, Epitomics, Burlingame, CA) and for Keap1 (1:200, poly, ProteinTech, Chicago, IL). The staining was evaluated in comparison to normal lung parenchyma and recorded as negative, cytoplasmic, and nuclear with stratification of intensity as high versus low.

Results: Phospho-Nrf2 was localized to the nucleus and was detected in 76.2% (125/164) of NEC. High levels of phospho-Nrf2 were seen in 27.9% of TC, 37.9% of AC, 76.9% of LCNEC and 73.4% of SCLC. Nrf2 expression strongly correlated with tumor grade ($p<.001$) but not with patient's age, gender, tumor size, stage or outcome. Keap1 expression was seen in cytoplasm and at high levels was observed in 34.6% (56/162) of NEC. High levels of Keap1 were detected in 26.1% of TC, 31.0% of AC, 26.1% of LCNEC and 45.3% of SCLC. There was a trend towards a better outcome in NEC with negative Keap1 ($p=.1$).

Conclusions: Phosphorylated Nrf2 is observed in a significant percentage of pulmonary NEC and shows strong correlation with NEC grade with most frequent expression at high levels in LCNEC and SCLC. Keap1 expression may have prognostic significance in NEC similar to what is known in NSCLC. These studies suggest a possible involvement of the Keap1/Nrf2 pathway in NEC pathogenesis.

1805 PET/CT in Pleural Mesothelioma after Talc Pleurodesis and Induction Chemotherapy: Histologic Findings at Hot and Cold Spots.

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Background: The role of computed tomography-positron emission tomography with [18F]-fluoro-deoxy-D-glucose (FDG-PET/CT) in evaluating the effect of induction chemotherapy in malignant pleural mesothelioma (MPM) is unknown. FDG uptake may vary at different sites due to tissue heterogeneity. We aimed for comparing histologic findings at tumor sites with high versus low FDG uptake.

Design: 20 MPM patients with talc pleurodesis during diagnostic thoracoscopy and induction chemotherapy followed by extrapleural pleuro-pneumonectomy (EPP) were included. All patients received a FDG PET/CT scan prior to surgery. Orthogonal whole sections (n total = 86) were taken at areas of maximum (SUVmax, n = 53) and of no FDG uptake (n = 33) and assessed for several histologic parameters (in mm, percentage or semi-quantitatively 0-3) using dichotomized data and Chi-squared tests.

Results: Overall median SUVmax was 2.20 ($p<0.001$ for hot/cold spots correlated with SUVmax). Hot spots were significantly associated with increased thickness of total pleural rind ($p=0.046$) and fibrosis ($p=0.023$) but not thickness of tumor or giant cell reaction. No relation was found with percent sarcomatoid histotype, tumor amount, tumor density, tumor viability and peritumoral chronic inflammation, apart a trend for higher vessel density ($p=0.088$). Further, hot spots were significantly associated with high proliferation rate Mib-1 of tumor ($p=0.044$) but not of giant cells. No relation was found for maximal Glut-1 expression intensity in either tumor or giant cells.

Conclusions: Interpretation of PET/CT data in MPM is difficult after talc pleurodesis and induction chemotherapy due to complex tissue composition which particularly includes a mixture of tumor and giant cells, both positive for Glut-1. Most consistently, hot spots are localized to pleural rind areas with high fibrotic activity.

1806 Testing of New IASLC/ATS/ERS Criteria for Diagnosis of Lung Adenocarcinoma (AD) in Small Biopsies: Minimize Immunohistochemistry (IHC) To Maximize Tissue for Molecular Studies.

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Background: In advanced lung AD patients, recent advances have led to new therapeutic options that now require pathologists to make the distinction from squamous cell carcinoma (SQ) and non-small cell lung carcinoma (NSCLC) not otherwise specified (NOS). As 70% of lung cancer patients present in advanced stages this is a major paradigm shift for pathology diagnosis in small biopsies. A new IASLC/ATS/ERS Classification of lung adenocarcinoma provides new diagnostic criteria for small biopsies and recommends at most one AD marker or one SQ marker in tumors lacking clear AD or SQ morphology with the goal of preserving as much tissue as possible for molecular studies.

Design: Based on small biopsies from 115 patients suspected to have NSCLC all diagnoses made by one pathologist (WDT) during the past year using the new adenocarcinoma classification were reviewed. The number and type of stains utilized as well as the results of molecular testing for *EGFR* and *KRAS* mutation and EML4-ALK fusion testing by FISH were recorded.

Results: Diagnoses included: 58 AD, 13 NSCLC favor AD, 13 SQ, 8 NSCLC favor SQ, 4 NSCLC NOS (NOS), 2 NSCLC with giant cells (GC), 4 suspicious for AD (SAD), 2 metastatic breast cancer (MBC) and 1 metastatic malignant melanoma (MM). No immunostains were performed in 59 (51%) of cases as histologic classification was clear on H&E and/or the amount of tissue was so scant, all available tissue was needed for molecular testing. Positive TTF-1 in 41 AD confirmed a primary lung tumor. Negative TTF-1 in 17 cases suspected to be AD or NOS raised concern for metastasis or SQ: p63 staining in 4 of these cases confirmed SQ (n=4) while ER/PR or S100/HMB45 confirmed diagnosis of MBC (n=2) or MM (n=1). In 81 AD or Favor AD cases molecular testing was performed successfully in 72 (89%). *EGFR* mutations (7- Exon 19 deletion, 4-Exon 21 L858R mutation) were found in 11 (17%) *KRAS* mutations (8-G12C; 9-G12D; 3-G12V) in 20 (30%), and EML4-ALK fusion in 2 (3%). Both GC tested showed KRAS mutations (2-G12C). No mutations were found in 2 NOS cases.

Conclusions: Our data support that accurate diagnosis of lung AD in small biopsies can be achieved in most cases with minimal or no IHC rather than by using panels of multiple AD and SQ IHC markers. This aids in strategic management of tissue to maximize tissue available for molecular studies. This strategy results in a high yield (89%) of successful molecular testing.

1807 Molecular Changes Including the Status of Mismatch Repair Proteins in Malignant Mesothelioma.

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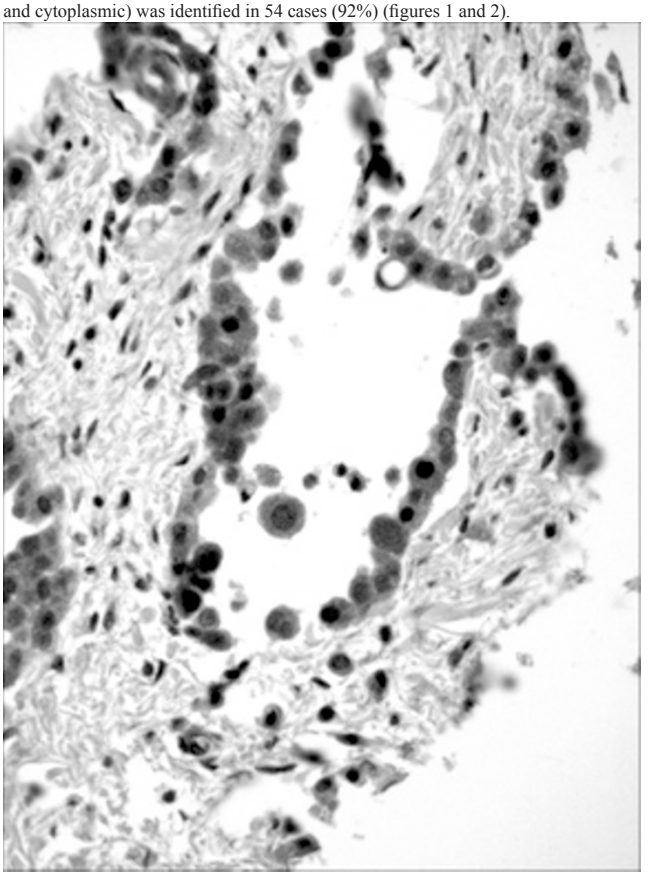
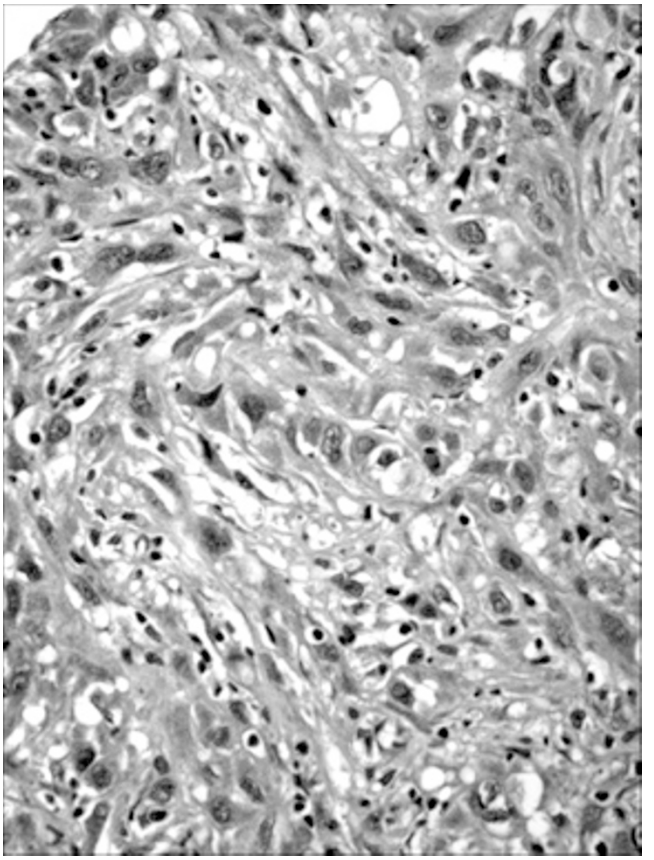
Background: Malignant pleural mesothelioma (MM) is an uncommon malignancy, which has a well-known association with environmental asbestos exposure. Aside from a few recurrent cytogenetic abnormalities, the molecular changes of MM have been incompletely described. Furthermore, although above baseline asbestos exposure is one of the only well-defined risk factors for the development of the disease, familial clustering of cases has suggested to some that genetic predisposition may play some role in MM oncogenesis. Here, we explore the status of p16 and the related cyclin dependent kinase 4 (CDK4) as well as the mismatch repair proteins in a large series of pleural MM.

Design: Fifty-nine cases of MM were identified and clinical histories obtained. The cases were classified as epithelioid (35/59), sarcomatoid (10/59), or mixed (14/59). A tissue microarray was constructed. Immunohistochemistry was performed with antibodies to MLH1, PMS2, MSH2, MSH6, p16, and CDK4. Presence or absence of staining in tumor cells and intensity was recorded.

Results: Patients included 44 men and 15 women and the mean age at diagnosis was 65 years. Forty percent (24/59) of patients had a documented history of asbestos exposure within their medical record and twenty percent (12/59) of patients had a noted first-degree relative with a previous epithelial malignancy. No loss of staining for MLH1, PMS2, MSH2, or MSH6 was observed in any cases. p16 loss was identified in all but 9 cases (83%) including 100% of sarcomatoid MM. CDK4 overexpression (both nuclear and cytoplasmic) was identified in 54 cases (92%) (figures 1 and 2).





Conclusions: Defects in the mismatch repair proteins do not appear to be involved in the oncogenesis of pleural MM. Alterations in p16 are common and most MM show overespression of CDK4, a protein typically regulated by p16.

1808 TTF-1, Napsin A, p63, TRIM29, Desmoglein-3 and CK5: An Evaluation of Sensitivity and Specificity and Correlation of Tumor Grade for Lung Squamous Cell Carcinoma vs. Lung Adenocarcinoma.

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Background: The current FDA-approved standard treatment for non-small cell lung cancer is Carboplatin/Taxol/Avastin; however, patients with lung squamous cell carcinoma (SqCC) should not receive Avastin due to a 30% mortality rate by fatal hemoptysis. Antibodies TTF-1 and p63 have been used to differentiate primary lung cancers; however, the need for a more sensitive and specific panel of antibodies to differentiate lung adenocarcinoma (LADC) from lung SqCC is of the utmost importance. In a pilot study, 14 antibodies were evaluated using an IHC method. Based on sensitivity and specificity, a six antibody panel was selected and tested on 132 lung cancer cases. Correlation of this antibody panel with tumor grade was determined.

Design: Formalin-fixed paraffin-embedded TMA tissues for lung cancers were obtained and processed in the usual manner for IHC analysis. The 6 antibody panel was applied to 65 cases of lung SqCC and 67 cases of LADC. Antibodies were optimized with a polymer detection system and visualized with DAB and/or Fast Red.

Results: A six antibody panel was evaluated for sensitivity and specificity (Table 1). Napsin A and TTF-1 provided 91% sensitivity (61/67) and 100% specificity for LADC. The combination of Desmoglein-3, CK5, p63 and TRIM29 provided 93.8% (61/65) sensitivity and 100% specificity for lung SqCC. In all cases, 8.3% (11/132) were unclassified and 7.6% (10/132) were reclassified by the IHC six antibody panel. 5.6% (5/89) of tumors grades 1-2 and 14% (6/43) of grade 3 cases were unclassified by IHC.

	TTF-1	Napsin A	p63	TRIM29	DSG-3	CK5
SqCC	4/65 (6%)	0/65 (0%)	61/65 (94%)	61/65 (94%)	54/65 (83%)	55/65 (85%)
LADC	51/67 (76%)	60/67 (90%)	11/67 (16%)	8/67 (12%)	0/67 (0%)	0/67 (0%)

Conclusions: Desmoglein-3, CK5 and Napsin A are highly sensitive and very specific markers for lung cancer and should be used in the initial screening of squamous vs. adenocarcinoma differentiation. TRIM29 and p63 positive staining in conjunction with TTF-1 and Napsin A negative staining were useful in certain cases of SqCC differentiation. Grade 3 lung cancers appears to be the more difficult to classify, therefore a more extensive panel, such as that described in this study should be considered.

1809 Histoarchitecture and Biochemical Profile of Collagen V in Skin and Lung Fibroblasts Culture from Patients with SSc Indicate a Defect in Fibrilogenesis.

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Background: The type V collagen (COLV) mutations is involved in collagen vascular diseases, such as systemic sclerosis (SSc), in which an unusual accumulation of this collagen was demonstrated (*Pathol Res Pract 200:681, 2004)*. In this context, our purpose was to analyze the tridimensional reconstruction (3D) and biochemical profile of COLV alpha-1 and alpha-2 chains in skin and lung fibroblasts culture from patients with SSc.

Design: We analyzed tridimensional reconstruction (3D), biochemical profile and RT-PCR of COLV alpha-1 and alpha-2 chains in skin and lung fibroblast of 5 and 14 patients with SSc without pulmonary hypertension, respectively. Five and 8 healthy control skin and lung fibroblasts were obtained from thorax region during mamoplasty and during lung. COLV 3D reconstruction was performed by confocal microscopy and COL V chain expression was analyzed by immunobloting and Real Time RT-PCR.

Results: The structure of COL V fiber in 3D reconstruction showed distorted and strongly thickened fibers in skin and lung fibroblasts from SSc patients compared with thin fibers pattern from the healthy controls. In skin fibroblasts was observed the biochemical profile of the COLV with the increase expression of the alpha-1 and alpha-2 chains related with controls (p=0.02, p=0.01, respectively). In SSc lungs fibroblasts we found increase in both, alpha 1 (1.32±0.34) and 2 (0.86±0.19) chains mRNA expression when compared with alpha 1 (0.50±0.10) and 2 (0.11±0.05) chains of control group. COL V chains from skin and lung fibroblasts presented alteration of molecular weight of the quoted chain.

Conclusions: The overexpression and the unusual organization of COLV fibers, besides the biochemical changes, suggest an interference with the fibrillogenesis process in skin and pulmonary fibrosis from SSc patients, reinforcing the participation of this collagen in pathogenesis of SSc and open new therapeutic perspectives for these patients.

Financial support: FAPESP, CNPq.

1810 Utility of Napsin A and TTF-1 Dual Stain on Paired Cytology and Surgical Specimens in Lung Carcinoma.

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Background: TTF-1 is a commonly used marker for diagnosing primary lung adenocarcinoma. Napsin A is an aspartic proteinase involved in maturation of surfactant protein B and is 80% positive in primary lung adenocarcinomas (ADC), and negative in squamous cell carcinoma (SCC) and small cell carcinoma (SmCC) of the lung. There is no literature on the expression of Napsin A on alcohol-fixed cytology smears.

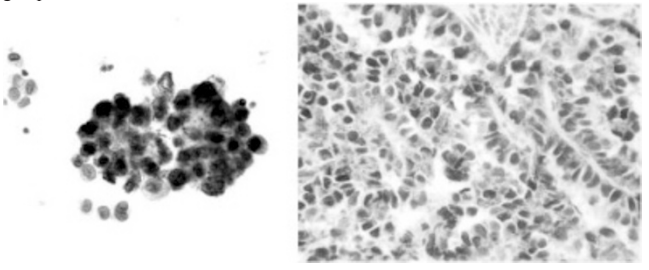
Design: 21 paired samples (alcohol-fixed cytology smears and subsequent biopsy/excision specimens) from patients with lung carcinoma (primary or metastatic) from January 2006 to December 2008 were stained using Lung Adeno-2™ (Napsin A + TTF-1) dual stain from BioCare Medical, CA.

Results: The dual staining results on biopsy/excision specimens demonstrated moderate positivity of Napsin A in 55% of primary lung ADC as compared to 73% TTF-1 strong positivity in these specimens.

Results of dual stain (Napsin A and TTF-1) on biopsy/excision specimens (21 patients)

Type of lung carcinoma	Positive TTF-1 (%)	Positive Napsin A (%)
ADC (n = 11)	8 (73)	6 (55)
a. Well to moderately differentiated (n = 5)	5 (100)	4 (80)
b. Poorly differentiated (n = 4)	2 (50)	1 (25)
c. ADC with sarcomatoid features (n = 1)	1 (100)	1 (100)
d. Mucinous ADC (n =1)	Negative (0)	Negative (0)
SCC (n = 5)	Negative (0)	Negative (0)
SmCC (n = 3)	3 (100)	Negative (0)
Poorly differentiated with neuroendocrine features (n = 1)	Negative (0)	Negative (0)
Metastatic ADC (n = 1)	Negative (0)	Negative (0)

Of the dual stain performed on 7 paired cytology and subsequent biopsy/excision specimens, Napsin A and TTF-1 was moderately to strongly expressed in 4/4(100%) primary lung ADC, and negative in poorly differentiated ADC (n=1), SmCC (n=1) and SCC (n=1). We are in the process of completing the remaining 14 cytology smears. A 100% concordance was found between cytology and surgical specimens in the latter group.



Conclusions: Napsin A and TTF-1 dual stain reactivity was robustly positive with a good concordance between alcohol-fixed cytology smears and formalin-fixed specimens of primary lung ADC.

1811 Histologic Classification of Thymic Epithelial Tumors Differs from Classification by Grade and Stage: A Study of 138 Surgically Resected Thymomas and Thymic Carcinomas.

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Background: Histologic classification of thymic epithelial tumors (TET) has evolved over the past few decades with much controversy and different concepts. The 2004 WHO provides a histologic classification of thymic epithelial tumors that is based primarily upon morphology. These tumors can also be classified according to histologic grade and stage that correspond to different degrees of clinical aggressiveness. These different approaches are frequently confused.

Design: With the purpose of comparing histologic classification of TET with grade and stage, 138 surgical resected TED were classified by the 2004 WHO classification. Tumors were staged according to the Masaoka system and survival analysis was used to consider tumor groups according to grade.

Results: Thymoma were classified as type A (12), AB (19), B1 (8), B2 (48), B3 (26), micronodular (1), and metaplastic (1). There were 23 thymic carcinomas. Tumors were grouped into three grades (GR): GR1 (A, AB, B1, micronodular, metaplastic; n=41; 25F, 16M; age: mean 61 yrs, 23-87 yrs), GR2 (B2 & B3; n=74; 37F, 37F; age: mean 55 yrs, 31-84 yrs) and GR3 (carcinoma; n=23, 8F, 15M; age: mean 58 yrs, 34-83 yrs). A higher percentage of patients with GR3 were Masaoka stage III or IV (17/23; 74%) compared to GR2 (44/74, 60%) and GR1 (5/39, 13%, p<0.001). 5 yr disease free survival (DFS) by Kaplan-Meier analysis with Tarone Ware comparisons was significantly reduced for GR2: 69% and GR3:36% compared to GR1: (77%, p<0.001). DFS was significantly worse for GR2 compared to GR1 (p=0.02) and G3 compared to GR2 (p=0.003). For Stage, in GR1 there were 36/41 Stage I/II (88%) and 5 Stage III/IV; GR2 there were 30/74 Stage I/II (41%) and 44 Stage III/IV (59%); GR3 there were 6/23 Stage I/II and 17 Stage III/IV. For all TET, survival according to stage was significant (p<0.001).

Conclusions: The 2004 WHO TET categories from type A to AB, B1, B2, B3 and thymic carcinoma represent increasing histologic grades with varying degrees of more aggressive behavior as measured by stage and prognosis. While there are tumors that may have similar grade, (i.e. the low grade type A, AB and B1 thymoma which are often low stage), the 2004 WHO categories have morphologic differences that remain the basis for maintaining a histologic classification separate from grading and staging.

1812 c-Met Amplification and Protein Expression in Non-Small Cell Lung Cancers.

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Background: c-Met is a transmembrane receptor tyrosine kinase that is occsionally amplified and/or expressed in patients with non-small cell lung cancer (NSCLC). Signaling through the hepatocyte growth factor/MET pathway has been shown to cause tumor growth, angiogenesis, and development of an invasive phenotype in several malignancies, including lung cancer. Here, we investigated the clinicopathologic characteristics of c-Met amplification and expression using dual-color chromogenic *in situ* hybridization (DISH) and a novel rabbit monoclonal antibody, respectively.

Design: For constructing tissue microarray, we used 2-mm tissue core specimens obtained from 906 patients (704 patients with adenocarcinoma [ADC]; 150, squamous cell carcinoma [SCC]; 43, sarcomatoid carcinoma [SaC]; and 9, large cell carcinoma [LCC]) who underwent surgical resection. The BenchMark® XT system (Ventana) was used for immunohistochemical analysis of c-Met (SP44; Ventana) and DISH (c-Met DNA Probe and Chromosome 7 Probe; Ventana). An immunohistochemically positive case was defined as strong complete homogeneous membrane staining in >30% of cells. We used the updated Colorado score (>40% of cells displaying >4 copies, c-Met to CEP7 ratio of >2, >4 spots gene, or ≥15 copies cluster in >10% of tumor cells) for c-Met amplification.

Results: The patient cohort included 332 women and 574 men. The mean follow-up time was 52.3 months, at which time, 631 patients were alive. c-Met expression was observed in 117/906 (12.9%) NSCLCs and was statistically correlated with the histological type ($P < 0.0001$) as follows: 108/704 (15.3%) ADCs, 1/150 (0.7%) SCCs, 8/43 (18.6%) SaCs, and 0/6 (0%) LCCs. c-Met amplification was observed in 92/844 (10.9%) NSCLCs and was statistically correlated with the histological type ($P < 0.0001$) as follows: 75/655 (11.5%) ADCs, 1/142 (0.7%) SCCs, 15/41 (36.6%) SaCs, and 1/6 (16.7%) LCCs. Half of c-Met amplified cases showed c–Met expression and that was statistically significant correlated ($P < 0.0001$). Analysis of all cases showed that c-Met expression did not correlate with overall survival (OS) and that c-Met amplification marginally correlated with OS ($P = 0.077$). However, when SCC cases were excluded, both univariate ($P = 0.0021$) and multivariate ($P = 0.020$) analyses revealed significant correlation between c-Met amplification and OS.

Conclusions: The subset of NSCLCs shows c-Met amplification. c-Met amplification/expression rate is significantly different among NSCLC histotypes, and it is particularly high ACC and SaC. In non-squamous NSCLC, c-Met amplification is significantly correlated with OS.

1813 Pulmonary Lesions Associated with BHD Syndrome.

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Background: Birt-Hogg-Dube (BHD) syndrome is a rare inherited disorder characterized by cutaneous lesions, increased risk of renal neoplasms and incidence of spontaneous pneumothorax. The characteristic skin lesion is the fibrofolliculoma; trichodiscomas and skin tags are also associated with the syndrome. Renal neoplasms observed in these patients include oncocytic hybrid tumors, chromophobe and clear cell carcinomas. Clinical evaluation of the patients have revealed that pulmonary lesions are commonly seen in this syndrome.

Design: Thirteen patients known to be members of BHD families were known to have lung lesions. We here report the pathologic features of the pulmonary lesions in 6 of these patients.

Results: They were 2 females and 4 males with a mean age of 55 years (range 32 -72 years). Two had significant smoking history. All patients had biopsy proven fibrofolliculomas. Two patients had renal tumors, one had a hybrid tumor and a chromophobe RCC, the other one has been kept under surveillance due to small and stable renal lesion. Four patients had history of prior spontaneous pneumothorax. Three presented with recurrent pneumothorax. Two asymptomatic patients were discovered to have pulmonary masses during routine radiologic studies.

All 6 patients had pulmonary changes consistent with emphysema. Two were centriacinar, one was mixed centriacinar and panacinar and three were panacinar emphysema. Five patients had subpleural blebs and cysts with a mean diameter of 2.75 cm ranging from 0.8 to 6.5 cm. They were located in the lower or middle lobes. The cysts had a delicate fibrous wall lined by a layer of pneumocytes. Two cases, with history of prior pneumothorax, had associated fibrinous and chronic pleuritis. Two patients had pulmonary malignancies; Adenocarcinoma Mixed Cell Type acinar, micropapillary and bronchioloalveloar, and Squamous Cell Carcinoma. The Adenocarcinoma was stage IA pT1aN0Mx, found before producing symptoms. The patient with Squamous Cell Carcinoma died of the disease. One patient had Necrotizing Granulomas with fungal organisms suggestive of Histoplasma Capsulatum, forming a nodule 2.5 cm in diameter, and pulmonary changes consistent with barotrauma.

Conclusions: Pulmonary cysts, bullae and ruptured blebs are a common finding in patients with BHD syndrome.

1814 Comparison of IHC, FISH and RT-PCR for the Detection of *EML4-ALK* Translocation Variants in Non-Small Cell Lung Cancer.

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Background: *EML4-ALK* gene fusions have been detected in 3-13% of non-small cell lung cancers and are associated with adenocarcinomas, lack of *EGFR* and *KRAS* mutations, and younger age. Patients with tumors harboring *EML4-ALK* fusions are candidates for targeted therapy with ALK inhibitors. An accurate diagnostic test for the detection of *EML4-ALK* gene fusions would be of great utility.

Design: Forty-eight formalin-fixed, paraffin-embedded lung adenocarcinoma specimens were selected from the surgical pathology files of the University of Utah. The study population was enriched for specimens with wild-type EGFR status (WT; n = 35, unknown; n = 13). Specimens were screened for the presence of *EML4-ALK* fusions by three methods: ALK IHC, ALK FISH, and RT-PCR (*EML4-ALK* variants 1 and 3a/b). Concordance between methods for the detection of *EML4-ALK* variants 1 and 3a/b was determined.

Results: ALK protein expression, as determined by IHC, was detectable in 18.8% (9/48) specimens. Only 2 of 9 (22.2%) ALK IHC positive specimens were confirmed positive for ALK rearrangement by FISH. RT-PCR determined that the two IHC +/FISH+ specimens both expressed *EML4-ALK* variant 3a/b. Of the remaining 7 IHC +/FISH – specimens, one was positive for variant 1 by RT-PCR. Nine additional IHC negative specimens expressed *EML4-ALK* variant 1 as determined by RT-PCR. Concordance between RT-PCR and FISH for *EML4-ALK* variant 1 was poor (11.1%) as only one of nine cases was called FISH positive by all three readers. The frequency of *EML4-ALK* variants 1 and 3a/b, as determined by RT-PCR, in our series of lung adenocarcinomas was 20.8% and 4.2%, respectively.

Conclusions: Concordance between all three detection methods (IHC, FISH, RT-PCR) for *EML4-ALK* variant 3a/b was 100%. Limited ALK protein expression and subjectivity in FISH scoring resulted in no concordance between all three detection methods for *EML4-ALK* variant 1. RT-PCR was the most sensitive and least subjective method for the detection of *EML4-ALK* variant 1 in lung adenocarcinoma. Enrichment for *EGFR* wildtype specimens in our study population likely resulted in our greater frequency (25%) of *EML4-ALK* positive lung adenocarcinomas.

1815 Are Bronchioloalveolar Carcinoma and Early Invasive Adenocarcinoma More Frequent in Elderly Caucasians Than in Young Asian Women?

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Background: Bronchioloalveolar carcinoma/adenocarcinoma-in-situ (BAC) and early invasive adenocarcinoma (BAC with <5 mm of invasion;EIA) have generally been described in Asian, never-smoking women <50 years of age. However, it has been our impression that most BAC and EIA patients in our practice are elderly and similar in age and gender ratio to patients with pulmonary mixed adenocarcinoma (MAC) (mean age 71 yrs; median age 70 yrs; 60% female; approximately 90% Caucasian).

Design: 77 consecutive single pulmonary lesions diagnosed as BAC (27) and EIA (50) were retrieved from among 1081 pulmonary adenocarcinomas excised at our hospital during a 5 year period. After slides were reviewed and diagnoses confirmed, EGFR and KRAS mutation analysis was performed on DNA extracted from each formalin fixed paraffin embedded tumor. Age, gender ratio, smoking history, tumor size, and frequency of EGFR (deletion in exon 19 and L858R mutation in exon 21) and KRAS mutations were tabulated and compared using the chi-square test.

Results: Results are shown in the table below.

Bronchioloalveolar Carcinoma and Early Invasive Adenocarcinoma: Demographics & Mutation Analysis

	Age Mean/ Range	Caucasian	Smoker	Female	Median Size	EGFR mutation	KRAS mutation
BAC	71/55-89 yrs	92.6%	69.2%	59%	1.1 cm	34.6%	7.7%
						del in EX19 (19.2%)	
						L858R in EX21 (15.4%)	
EIA	72/53-89 yrs	90%	65.2%	66%	1.6 cm	19.2%	20.5%
						del in EX19 (10.6%)	
						L858R in EX21 (8.5%)	

Chi-square test showed no significant difference ($p>0.05$) in age, gender ratio, ethnicity, smoking status, tumor size, or mutation results by diagnosis. Although EGFR and KRAS mutations are generally mutually exclusive, both an EGFR deletion in exon 19 and a KRAS mutation were identified in one EIA.

Conclusions: -In contrast to reports from Asia, the majority of our BAC and EIA patients were elderly Caucasians in their 7th-8th decades of life. A population-based study is needed to confirm whether this observation applies to BAC and EIA patients in the U.S. at large. -The proportions of EGFR and KRAS mutations in our patients are comparable to those reported in Asian patients. -As the rate of progression from BAC to EIA and to MAC is unclear and patients in the 7th and 8th decades of life frequently have comorbidities, the demographics of our patients raises a question as to whether selected individuals with ground glass opacity on chest imaging could be better managed conservatively with watchful waiting as is currently recommended for selected prostate cancer patients.

1816 Limited Role of Ki67 Proliferative Index in Predicting Overall Survival in Patients with Pulmonary Carcinoid Tumors.

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Background: The current WHO classification defines pulmonary carcinoid tumors (PC) as typical (TC) or atypical (ATC) based on mitotic index (2 per 10 hpf) and/or the presence of necrosis. Ki67 index (%) has been incorporated into the classification of GI carcinoid tumors but there are currently no established criteria for interpreting Ki67 index in PC.

Design: 101 PC diagnosed as TC (78) and ATC (23) by WHO criteria were retrieved from our surgical pathology files. Slides were reviewed, diagnoses were confirmed, and survival information was obtained. A representative section of each tumor and appropriate controls were immunostained with Ki67 antibody (Ventana Inc. Tucson AZ) and the percentages of positive tumor nuclei were determined using the Ariol SL-50 Image Analyzer and imaging software (Genetix Corp, Boston MA) in accordance with the manufacturer's recommendations. Kaplan-Meier methodology was used to determine rates of overall survival and Cox proportional hazard models were used to determine the prognostic value of histology-based diagnoses and Ki-67 indices. ROC curves were used to establish cut-points of various Ki67 indices on overall survival.

Results: The mean Ki67 indices were significantly different between TC (mean 3.8, range 0.5 to 22) and ATC (mean 17.9, range 0.5 to 56; $p=0.002$) although the frequency distributions of Ki67 indices in the two groups showed considerable overlap. There was no significant difference in the mean length of follow-up between the TC and ATC groups. Histological diagnosis and Ki67 index were each independently strong predictors of survival ($p<0.001$ and $p=0.003$, respectively). However, when considered together in multivariate analysis, histological diagnosis was a strong predictor of survival ($p<0.001$) while Ki67 index was not ($p=0.678$). ROC curve analysis indicated that Ki67 cutoffs of 5%, 4%, and 3% yielded specificities of 69%, 61%, and 44% and sensitivities of 71%, 76%, and 88%, respectively in predicting overall survival. However, none of these Ki67 cutoff values provided a significant difference in survival within either the TC or the ATC group.

Conclusions: -Ki67 index is a significant predictor of overall survival in PC patients. -Histologic diagnosis utilizing WHO criteria is a significant predictor of overall survival in PC patients. -Taken together, histological diagnosis is a stronger predictor of overall survival and Ki67 index does not provide additional significant predictive information on overall survival within either TC or ATC patients.

1817 Limited Impact of Distinguishing between Bronchioloalveolar Carcinoma and Early Invasive Adenocarcinoma of the Lung in Frozen Sections.

AE Walts, RJ McKenna, AM Marchevsky. Cedars-Sinai Medical Center, Los Angeles, CA.

Background: Pathologists at our hospital are asked to distinguish between bronchioloalveolar carcinoma (BAC), early invasive adenocarcinoma (BAC with <5mm invasion;EIA) and mixed adenocarcinoma (MAC) of the lung at intraoperative frozen section (FS), information that is considered important to determine the extent of resection.

Design: 224 consecutive pulmonary resections diagnosed at FS as MAC (160), EIA (22), BAC (19), deferred benign vs. malignant (12), benign (9), and deferred BAC vs. EIA (2) were retrieved from our pathology files. Operations performed at FS were classified as wedge/segmental resection (WS) or more extensive resection (trisegmentectomy, lobectomy, bilobectomy) and correlated with FS diagnoses using the chi-square test. Our files were also searched for re-operations performed within 3 months of the FS and reasons for re-operation were investigated.

Results: 13 (68.4%) patients with FS diagnosis of BAC, 11 (50%) with FS diagnosis of EIA, and 36 (22.5%) with FS diagnosis of MAC underwent WS. Each of the 164 others underwent a more extensive resection. Comparisons of the proportions of WS showed

a significant difference in operation performed following FS diagnoses of benign, BAC, or EIA and MAC ($p<0.01$). No significant difference ($p>0.05$) was observed in the proportions of WS that followed FS diagnoses of BAC, EIA, benign, deferred benign vs. malignant, or deferred BAC vs. EIA suggesting that tumor size and location, respiratory function, and/or other factors were considered in determining the extent of resection in some cases. In 4 cases (3 benign vs. malignant deferrals and 1 EIA read as benign at FS), re-operation with completion lobectomy was performed 1-90 days after FS for underdiagnosis of BAC (1), of EIA (2), and of MAC (1). The prior wedge resection margins had been clear in 3 of the 4 cases. The completion lobectomies showed no residual tumor, a 1.7 cm MAC and a 7 cm MAC each apparently separate from the previously excised FS tumors, and a 0.9 cm residual MAC, respectively.

Conclusions: -Distinction between BAC and EIA in FS appears to have limited influence on the extent of lung resection in this case cohort. -Distinction of mixed adenocarcinoma (MAC) from the other diagnostic categories remains the most important decision to be made by pathologists at FS. -Deferrals or false negative diagnoses of malignancy at FS pose infrequent problems in surgical management as evidenced by the 4 (1.8%) patients who required re-operation.

1818 Evaluation of Napsin A, CK 5/6, p63, and TTF-1 in Adenocarcinoma (ACA) vs. Squamous Cell Carcinoma (SCC) of the Lung.

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Background: The distinction of lung ACA from other types of primary lung malignancies is clinically important because recent advances in treatment of lung cancer suggest that lung ACA in non smokers has a high response rate to Epidermal Growth Factor Receptors-Tyrosine Kinase inhibitors. In Addition certain antiangiogenic agents are contraindicated in SCC due to risk of bleeding. Studies have evaluated different panels of immunohistochemical markers in the distinction of ACA from SCC of the lung, with varying specificity and sensitivity. Accurate morphologic classification is further hindered because 70% of lung cancers are diagnosed on limited FNA or transbronchial biopsy specimens. Panels have included combinations of immunohistochemcal markers, TTF-1/ CEA for ACA, CK5/6, p63/ Desmoglein 3 for SCC. While TTF-1 has historically been the most specific marker for lung ACA, a relatively new marker, Napsin A (a functional aspartic proteinase involved in the maturation of pro-surfactant protein-B in type II pneumocytes), has recently been shown to be more sensitive and specific than TTF-1. A commercially available costly panel of 5 antibodies has been used in this endeavor. We wish to find the most cost effective and tissue preserving panel in reliably distinguishing lung ACA from SCC.

Design: A Total of 291 lung cancers were evaluated morphologically (ACA=197; SCC=66; 28 large cell carcinomas). Immunohistocmemistry for Napsin A, CK 5/6, p63, and TTF-1 was performed on a formalin fixed tissue microarray obtained from Toyama, Japan. Cases were scored as positive or negative against a negative control. Immunohistochemistry was performed at UTHSCSA IHC Laboratory. Antibodies used included Napsin A (Cell Marque, Prediluted), Cytokeratin 5/6 (Cell Marque, Prediluted), p63 (Biocare Medical, Prediluted), TTF-1 (Dako 1:80). All slides were treated for 30 minutes at 100 degrees and then allowed to cool down for 20 minutes using the Revel antigen retrieval solution (Biocare Medical). The slides were incubated in primary antibody for 1 hour followed by detection with Biocare Medicals Mach 2 universal polymer-HRP for 30 minutes and developed with DAB.

Results:

Sensitivity (SEN) and Specificity (SPEC) of Markers

MARKER	ACA (SEN.)	ACA (SPEC.)	SCC (SEN)	SCC (SPEC.)	LCC
Napsin A	83%	98%			Neg
TTF-1	20%*	100%			Neg
CK5/6			53%	96%	Neg
p63			95%	86%	total=2

* use of adsorbed slides may affect IM for TTF-1

A Panel of Napsin A and CK5/6 had a specificity of 98% and Sesitivity of 75%. When p63 was added to this panel the sensitivity increased to 99%.

Conclusions: The most cost effective tissue preserving panel in the diffential diagnosis of lung ACA vs SCC is a combination of Napsin A, CK5/6, and p63.

1819 Lung Carcinomas in Young Patients: A Clinicopathologic and Immunohistochemical Assessment of 140 Cases.

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Background: Although lung carcinomas demonstrate significant genetic diversity, underlying molecular abnormalities such as activating mutations of *EGFR* and the *EML4-ALK* fusion gene have been identified in some tumors. Recent studies have suggested that carcinomas harboring these abnormalities demonstrate distinct clinicopathologic features. Additionally, immunohistochemistry (IHC) with *EGFR* mutation-specific antibodies and ALK can accurately identify the abnormalities in lieu of molecular analysis. Given the increased incidence of lung cancer with age, these studies have predominantly evaluated tumors from older individuals. This study used histologic examination and IHC to evaluate the clinicopathologic features of lung carcinomas in patients 50 and younger.

Design: 140 lung carcinomas in patients 50 and younger were identified. Patient age, sex, and tobacco history were recorded. Each tumor was classified according to WHO guidelines. Tumor grade, size, stage, and large vessel invasion were evaluated in addition to lymph node status and other lung pathology. IHC for TTF1, p63, ALK, the E746-A750 *EGFR* deletion, and the L858R *EGFR* point mutation was performed.

Results: The 140 cases exhibited the following characteristics: age (range 31-50; mean 46; median 47), sex (75 female, 65 male), and tobacco history (111 smokers, 7 nonsmokers, 22 unknown). Histologic types were 90 adenocarcinomas (58 mixed, 16 acinar, 12 solid, 3 non-mucinous BAC, 1 mucinous BAC), 27 squamous, 17 large cell undifferentiated, 4 large cell neuroendocrine, 1 adenosquamous, and 1 sarcomatoid. Immunoreactivity with *EGFR* mutation-specific antibodies was seen in 10 adenocarcinomas, all of which demonstrated acinar and/or non-mucinous BAC morphology; focal solid growth was seen in only 1 case. The *EGFR* positive cases had an average smoking history of 29 pack years, while the negative cases had an average smoking history of 36 pack years. Immunoreactivity for ALK was identified in 1 tumor, which was from a non-smoker and demonstrated predominantly acinar histology with a minor solid component.

Conclusions: Lung carcinomas in young patients are typically adenocarcinomas. Within this group, the rate of *EGFR* mutations and the *EML4-ALK* fusion gene appears similar to that seen in studies consisting predominantly of older patients. This somewhat surprising finding is likely related to the high rate of tobacco use within our study. Similar to other studies, *EGFR* and ALK positive cases did not overlap, only rarely had a solid component, and were associated with a less extensive smoking history.

1820 Pathological and Radiographic Evaluation of Asbestos Exposure.

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Background: Japanese government compensates patients with asbestos-related lung carcinoma, and the evaluation for the compensation of the cases with lung carcinoma depends on the asbestos body counts in lung digests and radiographic features such as pleural plaque and fibrosis. However, there are few data on the relationship between the number of asbestos bodies in tissue sections and the level of asbestos exposure. Furthermore, there is no data on the relationship between radiographic findings of the patient and the level of exposure.

Design: We studied the asbestos body counts by phase-contrast microscopy on lung digests of wet formalin-fixed lung in 175 cases with asbestos-related diseases (161 lung carcinomas, 5 mesotheliomas, 4 asbestosis, 3 interstitial pneumonia, 1 fibrosis, 1 unknown). We counted the asbestos bodies in 4 micron paraffin-embedded sections of lung tissues stained with iron in 113 cases with asbestos-related lung carcinoma, and compared them with the asbestos body counts in lung digests. We analyzed radiographic findings of 97 asbestos-related lung carcinomas.

Results: The distribution of the log of asbestos concentration was normal. There is statistically significant relationship between numbers of asbestos bodies in tissue sections and asbestos body counts measured by tissue digestion technique (correlation coefficient = 0.918). The number of asbestos bodies in the lung was significantly higher in the patients with pleural plaque detected by computed tomography (geometric mean (GM), 7,349/g dry lung) and those with pleural plaque and fibrosis (GM, 17,148/g dry lung). However, the number of asbestos bodies in the lung was significantly lower in the patients with pleural plaque detected during the thoracoscopy or surgery (GM, 202/g dry lung). The number of asbestos bodies in the lung of the patients with pleural plaque which occupies more than one fourth of thoracic circumference was higher than that of the patients with pleural plaque which occupies less than one fourth of thoracic circumference.

Conclusions: The number of asbestos bodies in the tissue section of the lung was related to the number of asbestos bodies in the lung in lung digests. The radiographic findings such as pleural plaque and fibrosis suggest the history of asbestos exposure, and the extent of pleural plaque helps to estimate the level of asbestos exposure.

1821 The Significance of Minimally Invasive Adenocarcinoma in the Classification of Pulmonary Tumors.

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Background: The classification of lung adenocarcinomas especially those with lepidic spread or bronchioloalveolar carcinoma (BAC) features is controversial. Currently, the determination of T stage (invasive size) in adenocarcinomas with lepidic spread is not well defined. Recently, a new classification system introduced a new category for adenocarcinomas known as minimally invasive adenocarcinoma (MIA) for small solitary adenocarcinomas with predominant lepidic growth and ≤5mm invasion. This distinction is made because patients in this category have 100% or near 100% disease specific survival after complete tumor resection.

Design: We retrospectively reviewed 88 resected adenocarcinomas of the lung and re-classified them as either acinar, solid, mucinous, colloid, papillary, enteric, or micropapillary. Tumors with peripheral lepidic spread (so-called BAC features) were identified, and only the invasive component measured. The tumor stage was compared before and after resizing the tumor using only the invasive component.

Results: There were 29 invasive tumors with lepidic spread (33%), 26 nonmucinous, and 3 mucinous. There were 3 non-invasive nonmucinous BACs. The lepidic extent ranged from 2 mm to 5 cm (mean 2.9 mm). When measuring only the invasive component for staging, 11/26 lepidic non-mucinous tumors were downstaged with 8 to minimally invasive tumors (<5 mm). 3 of 3 mucinous lepidic tumors were also reduced in T stage. Regional lymph nodes were positive in 0/3 non-invasive, 0/8 minimally invasive tumors, 2/29 invasive tumors with lepidic spread (7%), 9/39 of acinar and papillary carcinomas (23%), and 6/17 tumors with a solid growth pattern (35%).

Conclusions: In this series, approximately 1/3 of lung adenocarcinomas have significant lepidic spread. Measuring only invasive component as compared to measuring the entire tumor decreases the T stage in over 1/3 of the cases. Regional lymph node metastases were not present in minimally invasive tumors < 5mm in this series.

1822 Is the EGFR Mutation Distributed Heterogeneously within Tumors?

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Background: Some studies have shown heterogeneous distribution of the EGFR mutation in individual tumors. On the other hand, recent results of clinical trials, such as IPASS, WJTOG3405, and NEJ002, have demonstrated that clinical response to EGFR-TKIs was associated with the EGFR mutation, although most of the EGFR examinations were conducted using small biopsy specimens. The results suggest that the EGFR mutation status in a portion of a tumor represents that of the entire nodule, i.e., homogeneous distribution of the mutation.

Design: Distribution of EGFR mutation within tumors was examined on the three points as follows. 1. I speculated that if the EGFR mutation was heterogeneously distributed, a combination of two different hot spot mutations would be detected in some tumors, as the EGFR mutation occurs very frequently in Asian individuals. Therefore, I examined the frequency of simultaneous dual hot spot mutations using our mutation database. 2. Three small areas in each of 50 individual adenocarcinomas known to have the EGFR mutation were micro-dissected. In addition, three tumors with the EGFR mutation were divided into 100 segments. The mutational status was accessed in each sample. 3. A total of 100 pairs of primary adenocarcinomas with the EGFR mutation and corresponding lymph node metastasis, and 54 primary adenocarcinomas with the EGFR mutation and recurrent tumor pairs were examined for EGFR mutation status.

Results: In our database of 2781 primary lung cancers, the EGFR mutation at L858R or a deletion in exon 19 was detected in 862 tumors. None had simultaneous dual hot spot mutations, as all trans-sectional samples for each tumor examined showed an identical EGFR mutation. In addition, no discordant mutation patterns were found in the 154 paired samples.

Conclusions: The results in this study clearly demonstrated that heterogeneous distribution of the EGFR mutation within a tumor is extremely rare. However, it is possible that pseudo-heterogeneity occurs due to a combination of mutant allele specific imbalance (PLoS One. 2009;4:e4576) and heterogeneous distribution of EGFR amplification (Cancer Res 2008;68:2106), especially when a less sensitive method is used for detection. Namely, in some tumors, a mutant allele is specifically amplified, and amplification occurs specifically in a part related to invasive growth. Accordingly, the invasive part significantly over-represents the mutation signal relative to that in a non-invasive part. Such unbalanced mutation signals might cause pseudo-heterogeneity.

1823 Diagnostic Utility of PAX8, Napsin A and TTF-1 in Discriminating Metastatic Carcinoma from Primary Adenocarcinoma of the Lung.

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Background: TTF-1 and napsin A have been considered as useful markers for primary lung adenocarcinoma. However, studies have shown that they can also be expressed in extrapulmonary adenocarcinomas and that a small fraction of lung adenocarcinomas do not coexpress these two markers. The aim of this study was to determine if PAX8, TTF-1 and napsin-A can help segregate primary lung adenocarcinoma from metastasis.

Design: Total 102 metastatic carcinomas of the lung (98 resections and 4 biopsies) were retrieved from authors' institutions. The primary sites included breast (13), salivary gland (6), endometrium (5), ovary (7), endocervix (1), kidney (33), thyroid (5), urinary tract (3), prostate (1), liver (8), adrenal gland (2), and testis (3), pancreatobiliary (7), and colon (8). Tissue microarray of 120 lung adenocarcinomas was used for comparison. Immunohistochemistry was perforemd using antibodies against TTF-1, napsin A and PAX8. Nuclear staining for TTF-1 and PAX8 and cytoplasmic staining for napsin A were considered positive. Immunostaining was graded as weak, moderate or strong and the percentage of positive cells was recorded. A p-value of <0.05, as determined by Fisher's exact test, was considered statistically significant.

Results: Nine of 102 (8.8%) metastatic carcinomas (2 endometrial, 3 colonic, 1 prostatic, 1 salivary adenoid cystic and 2 renal cell carcinomas) showed weak to strong TTF-1 nuclear staining in 5% to 60 % of the tumor cells. The frequency of positive TTF-1 immunostaining in metastatic carcinomas was significantly lower than that reported in primary lung adenocaricnoma by our lab in the past (p<0.01). All metastatic carcinomas were negative for napsin A. PAX8 was strongly positive in 44 (43.1%) of 102 metastatic carcinomas (5/5 endometrial, 1/1 endocervical, 4/6 ovarian, 1/3 urothelial, 3/3 papillary thyroid, 1/1 Hurthle cell and 29/33 renal cell carcinomas). All 120 lung adenocarcinomas were negative for PAX8.

Conclusions: A small fraction of metastatic carcinomas to the lung were TTF-1 positive, which can be confused with lung primaries. However, the metastatic carcinomas are typically negative for napsin A and some of the metastatic carcinomas from kidney, thyroid, ovary, endometrium and urothelium show positive staining for PAX8, a marker that is negative in primary lung adenocarcinomas. These findings indicate that combined use of PAX8, TTF-1 and napsin A is reliable to separate a lung primary from a metastasis

1824 Most ALK-Positive Lung Adenocarcinomas Exhibit Characteristic Histology.

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Background: *EML4-ALK* translocation occurs in 1—5% of all lung adenocarcinomas. Histological identification of ALK-rearranged tumors holds potential impact on the clinical management, because these tumors may respond to ALK inhibitor treatment. Although previous studies suggested that the histology of *ALK*-rearranged adenocarcinomas may be characteristic, there is no large-scale comprehensive morphological analysis with surgically resected materials. In this study, we examined the largest cohort of ALK-positive tumors to delineate the morphological aspect of this subgroup of lung cancer.

Design: We collected 50 cases of surgically resected lung adenocarcinoma that had shown strong reactivity in the highly sensitive ALK immunohistochemical test. RT-PCR and/or FISH analyses were performed for 32 selected cases, all of which were confirmed to harbor *EML4-ALK* translocation or *ALK* rearrangement. All the glass slides were reviewed, and histological findings were compared with those of 100 consecutive surgical cases of lung adenocarcinomas that were immunonegative for ALK. All the tumors had been extensively sampled. Student t-test and χ^2 test were used for statistical analyses, and $p < 0.01$ was considered significant.

Results: The following histological features were significantly more frequent in ALK-positive tumors than ALK-negative tumors: predominant acinar pattern (42% vs 11%), at least focal solid pattern (60% vs 33%), cribriform formation(78% vs 16%), abundant extracellular mucus (66% vs 10%), signet-ring cells (64% vs 1%), and any mucous cells (including goblet cells and signet-ring cells) (82% vs 4%). In contrast, ALK-positive tumors showed significantly less frequent occurrence of predominant lepidic pattern (2% vs 31%), at least focal lepidic pattern (26% vs 83%), and nuclear pleomorphism (8% vs 45%). Two frequently observed constellations of findings in ALK-positive tumors were cribriform structure associated with abundant extracellular mucus (58%) and solid growth admixed with clusters of signet-ring cells (34%). Most (82%) ALK-positive tumors contained at least focal areas exhibiting either or a combination of these 2 constellations, while these patterns were rare (1%) in ALK-negative tumors.

Conclusions: ALK-immunopositive tumors are histologically distinguishable from ALK-negative tumors in most instances if adequately sampled. Characteristic histological features, which may be present only focally, should serve as clues to prompt immunohistochemistry and confirmatory genetic testing for *ALK*-rearrangement.

1825 Validation of the IASLC/ATS/ERS Lung Adenocarcinoma (ADC) Classification and Use of Comprehensive Histologic Subtyping (CHS) for Architectural Grading in 432 Japanese Patients.

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Background: A new lung adenocarcinoma (ADC) classification is being proposed by the International Association for the Study of Lung Cancer (IASLC), American Thoracic Society (ATS) and European Respiratory Society (ERS). Moreover we have reported how CHS proposed in the new classification can be used for architectural grading system of early stage resected lung ADC. The purpose of this study is to validate the proposed histological classification and the histological grading system of lung ADC in Japanese patients (pts).

Design: 432 lung ADC pts who had undergone resection in Kyoto University hospital from 2001 to 2009 were retrospectively reviewed. Comprehensive histologic subtyping was used to estimate the percentage of each histologic subtype and to identify the predominant subtype. Tumors were classified according to the new ADC classification with predominant subtypes for overtly invasive tumors and our proposal histological grading system [Grade1: composed of ADC in situ (AIS), minimally invasive ADC (MIA), lepidic ADC (LP), Grade2: composed of papillary ADC (PP) and acinar ADC (AP), Grade3: composed of solid ADC (SP) and micropapillary ADC (MP)]. Survival analysis was performed using Kaplan Meier method.

Results: There were 206 females (48%) and 226 males (52%) with a median age of 65.5 years (23-88 years) and 250 Stage 1A pts, 88 Stage 1B pts, 30 Stage 2A pts, 13 Stage2B pts, 26 Stage3A pts, 4 Stage3B pts and 15 Stage4 pts. 5-yr disease free survival (DFS) rates of the group classified by the new classification were shown below: 100% for AIS (n=17) and MIA (n=35), 88.8% for LP (n=33), 67% for PP (n=180), 59% for AP (n=56), 61% for SP (n=71), 17% for MP (n=19). Moreover 5-yr DFS for mucinous ADC (n=15) and others (n=6, including colloid ADC) were 79.5% and 44.4%, respectively. The 5-yr DFS for patient with Grade1 was significantly better than Grade2 and Grade3 (90%, 65% and 51%, respectively (p<0.001).

Conclusions: The new classification of lung ADC identifies histologic categories with prognostic differences that may be helpful in identifying candidates for adjunctive therapy. AISs and MIAs had 100% 5-year survival confirming previous report. In contrast mucinous carcinomas appear to have worst prognosis. Moreover the new histological grading system could be used to select patients with higher risk of recurrence and to provide valuable information for clinicians to manage postsurgical therapy.

1826 Malignant Mesothelioma Expresses Melan-A (A-103), a Possible Diagnostic Pitfall.

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Background: Malignant mesothelioma, with its numerous morphologic subtypes, may well enter into the differential diagnosis of melanoma, clear cell (sugar) tumor, adrenal cortical, and sex cord stromal tumors. The immunohistochemical patterns of expression of these tumors partly overlap since even keratins can be expressed in all of them. Expression of melanocytic marker Melan-A (A103 clone) has also been documented in adrenal cortical neoplasms, sugar tumor, and sex-cord stromal tumors. However, to the best of our knowledge, expression of Melan-A (A103) has not been documented in malignant mesothelioma. This study analyzes the possible expression of A103 in mesothelioma and compares it with other melanocytic markers.

Design: 12 specimens with the diagnosis of malignant mesothelioma (epithelioid type) were collected from the files. These were from 2 females and 6 males (in 2 cases more than one metastatic site was evaluated), ranging from 40 to 73 years of age. Sites of disease included: lung/pleura, mediastinum, small bowel/mesentery, peritoneum,

and uterus/ovaries. 5-micron sections of paraffin embedded tissue were utilized for immunohistochemical analysis with anti-MART-1 (clone Ab3; Thermo), Melan-A (clone A103; Labvision), and anti-Tyrosinase (clone T311; Leica).

Results: Table 1 summarizes IHC findings. All cases (12/12) showed strong positive labeling for Melan-A (A103); 4 cases had at least focal expression of tyrosinase. MART1 was negative in all cases examined.

case #	MART-1	Melan-A (A 103)	Tyrosinase
1	0	4+	Rare cells +
2	0	4+	Rare cells +
3	0	4+	0
4	0	3+	N/P
5	0	4+	N/P
6	0	3+	3+
7	0	4+	Rare cells +
8	0	3+	3+
9	N/P	3+	1+
10	N/P	3+	Rare cells +
11	N/P	4+	2+
12	N/P	3+	Rare cells +

Grading: 2-10%=1+; 10-40%=2+; 40-70%=3+; 70-100%=4+; N/P: not performed

Conclusions: Expression of Melan-A (A103) or tyrosinase can be seen in epithelioid mesothelioma. These findings warrant caution in the diagnostic use of Melan-A (A103) or tyrosinase in differentiating malignant mesothelioma form metastatic melanoma, clear cell tumor, adrenal cortical, and sex cord stromal tumors.

1827 Expression of Napsin A in Carcinomas from Various Organs.

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Background: Napsin A has been recently reported as a highly sensitive and specific marker for identifying a primary adenocarcinoma of the lung. Its expression has also been demonstrated in papillary and clear cell renal cell carcinomas. In this study, we investigate the expression of napsin A in carcinomas from various organs using a single immunostaining system (Ventana XT).

Design: We immunohistochemically evaluated the expression of napsin A (Cat No. 760-4446; rabbit polyclonal; prediluted; Ventana) on 862 cases of carcinoma from various organs on tissue microarray sections. The staining intensity was graded as weak or strong. The distribution was recorded as negative (<5% of tumor cells stained), 1+ (5-25%), 2+ (26-50%), 3+ (51-75%), or 4+ (>75%).

Results: The immunostaining results are summarized in Table 1. Weak nuclear staining was also observed in 6 of 15 seminomas and 3 of 8 embryonal carcinomas in addition to cytoplasmic granular staining.

Table 1. Summary of immunostaining results

Tumor	Positive cases % (N)
Adenocarcinoma, lung	93% (50/54)
Squamous cell carcinoma, lung	5% (2/42)
Papillary renal cell carcinoma	69% (18/26)
Clear cell renal cell carcinoma	38% (20/53)
Adenocarcinoma, colon	29% (11/38)
Seminoma	50% (15/30)
Embryonal carcinoma	33% (8/24)
Yolk Sac tumor	50% (6/12)
Papillary carcinoma, thyroid	18% (8/45)
Adenocarcinoma, esophagus	17% (5/30)
Adenocarcinoma, stomach	6% (1/18)
Adenocarcinoma, pancreas	5% (3/56)
Urothelial carcinoma, bladder	5% (2/40)
Adenocarcinoma, prostate	0 (0/100)
Cholangiocarcinoma	0 (0/11)
Hepatocellular carcinoma	0 (0/18)
Ductal carcinoma, breast	0 (0/110)
Lobular carcinoma, breast	0 (0/53)
Follicular carcinoma, thyroid	0 (0/36)
Medullary carcinoma, thyroid	0 (0/10)
Adenocarcinoma, endocervix	0(0/17)
Papillary serous carcinoma, ovary	0 (0/15)
Adrenal cortical neoplasm	0 (0/24)

Conclusions: Napsin A is a sensitive and specific marker for differentiating lung adenocarcinoma from lung squamous cell carcinoma. However, napsin A is not an entirely specific maker for identifying a lung primary since it also expressed in a high percentage of renal cell carcinomas and germ cell tumors, and a significant number of carcinomas from the colon, thyroid and esophagus.

1828 Phenotypic Correlates of *ERBB2, BRAF* and *PIK3CA* Mutations in Non-Small Cell Lung Cancer.

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Background: A subset of non small cell lung carcinomas (NSCLC) contains mutations in *EGFR/ERBB1* or in downstream oncogenes. *EGFR* and *KRAS* mutations are the most common, occurring in ~30% of NSCLC, but similar, less frequent, mutations occur in *ERBB2*, *BRAF* and *PIK3CA*. While *EGFR*-and *KRAS*- mutant NSCLC are more frequently associated with bronchioloalveolar (BAC)/papillary and solid histologies, respectively, the histologic features associated with NSCLC bearing *ERBB2, BRAF* or *PIK3CA* mutations have not been fully described.

Design: 407 cases of NSCLC were tested for *EGFR, KRAS, ERBB2, BRAF* and *PIK3CA* mutation by PCR-Sanger sequencing from 8/2009 – 8/2010. H&E slides from NSCLC with a mutation in *ERBB2*, *BRAF*, or *PIK3CA* were reviewed, to examine correlations between genotype and morphology.

Results: 35/407 cases (8.6%) contained one of the selected non-*EGFR/KRAS* mutations, 14 (3.4%) *ERBB2*, 15 (3.7%) *BRAF*, and 6 (1.5%) *PIK3CA* mutations. H&E slides were available for 25 cases (10 *ERBB2*, 9 *BRAF*, 6 *PIK3CA* mutants). Non-*EGFR/KRAS* mutations were mutually exclusive though one tumor contained *PIK3CA* and *EGFR* mutations. Table 1 shows the histologic features of each genotype: *ERBB2*-mutant tumors tended towards acinar/papillary histology, and *BRAF*-mutant tumors tended towards high grade/solid histology, often (4/9) with signet ring cells, while *PIK3CA* mutations occurred in tumors with adenosquamous, mixed subtype adenocarcinoma, mucinous BAC and sarcomatoid histologies. Other morphologic features, including inflammation and desmoplasia, did not predict genotype.

	Mutated gene		
	ERBB2	*BRAF*	*PIK3CA*
	(n=10)	(n=9)	(n=6)
Adeocarcinoma	10	9	5
Squamous cell carcinoma	0	0	0
Adenosquamous carcinoma	0	0	1
Patterns present:			
Acinar	6	5	3
BAC	0	3	1
Mucinous BAC	0	0	1
Papillary	6	4	3
Micropapillary	2	2	1
Solid	3	5	3
Sarcomatoid	0	0	1
Grade:			
Low	0	1	0
Intermediate	8	4	4
High	2	4	2
Other features:			
Clear cell change	2	2	1
Signet ring cells	0	4	1

Conclusions: In this small series, several genotype-phenotype trends were noted, including similar histologies in *ERBB2*-mutant and those reported in *EGFR*-mutant tumors, and in *BRAF*-mutant and those reported in *KRAS*-mutant tumors. *PIK3CA* mutations were associated with the broadest range of histologies, consistent with prior reports. Our findings, while preliminary, suggest that tumor morphology may predict specific ERBB signaling pathway alterations.

1829 Increased Expression of Collagen V and IL-17 during Usual Interstitial Pneumonia.

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Background: Usual interstitial pneumonia (UIP)/idiopathic pulmonary fibrosis (IPF) is a progressive fibrosing disease that is a major cause of pulmonary failure in this country. The etiology of UIP is unknown but it is thought to be an autoimmune disorder. Type V collagen [Col(V)] is considered as a sequestered antigen in the normal lung, and immunity to unmasked Col(V) enhanced by cytokine IL-17 contributes to chronic rejection in allograft lungs. We investigated the expression of Col(V) and IL-17 in patients with UIP to understand whether or not they may contribute to the etiology of that disorder as well.

Design: Col(V) and IL-17 protein expression were studied by immunohistochemical stains in 9 cases of UIP, as well as in 3 cases of normal lung. The mRNA expression level of *col(V)* was also determined by real-time RT-PCR using formalin-fixed paraffin-embedded tissue.

Results: We found that *col(V)* mRNA expression is increased by 2.64 ± 1.35 folds in the lungs of patients with UIP as compared with that in normal lungs (p=0.02). Col(V) protein expression as determined by immunohistochemistry analysis is also increased in the lungs of UIP patients. Scattered IL17-expressing cells are present in the lungs with UIP, but absent in normal lungs.

Conclusions: Collagen V and IL-17 expressions are both increased in the lungs of patients with UIP, suggesting a possible role for these molecules in the pathogenesis of UIP.

1830 Prognostic Value of Ki-67 Proliferative Index in Diffuse Malignant Mesothelioma.

W Zhang, M McIntire, R Bueno, J Godleski, L Chirieac. Brigham and Women's Hospital, Boston, MA; Caris Life Science, Newton, MA.

Background: The rate of cell proliferation as assessed by Ki-67 immunohistochemistry has been studied as a prognostic indicator in numerous malignant neoplasms and shown to correlate with tumour grade and patient outcome. The clinical significance of Ki-67 expression in diffuse malignant mesotheliomas has not been described in detail. In this study, we investigated the proliferative index by Ki-67 immunohistochemistry in a large cohort of patients and correlated the findings with the clinicopathologic characteristics.

Design: We studied 204 patients with diffuse malignant mesotheliomas (125 epithelioid, 8 sarcomatoid, 71 biphasic type) and surgical resection performed at Brigham and Women's Hospital between 2001 and 2008. Paraffin embedded tumor samples were used to construct tissue microarrays. We evaluated the Ki-67 proliferative index by immunohistochemistry as a percentage of Ki-67 positive tumor cells in each tumor. Patient age, sex, tumor type, TNM stage, lymphovascular invasion and number of positive lymph nodes were recorded and correlated with Ki-67 proliferative index.

Results: In our study, 166 patients (81%) were men and 38 (19%) were women with mean age 63.2 (range 34-84). T category was 1 in 4% of patients, 2 in 24%, 3 in 43% and 4 in 29%. The median Ki-67 proliferative index was 25% (range 0-95%). A high Ki-67 proliferative index was significantly correlated with positive metastatic lymph nodes (p=0.02), lymphovascular invasion (p=0.02) and number of positive lymph nodes (p=0.0005).

Conclusions: Ki-67 is a useful marker of cell proliferation in diffuse malignant mesotheliomas. A high Ki-67 proliferative index predicts a poor prognosis and correlates

with positive lymph nodes, lymphovascular invasion and high number of positive lymph nodes in patients with diffuse malignant mesothelioma.

1831 Abstract moved to 327A

1832 Prognostic Significance of Tumor Chronic Inflammatory Infiltrate in Diffuse Malignant Mesothelioma.

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Background: Diffuse malignant mesothelioma is well known to be an aggressive disease. Therefore, identification of histopathologic factors with prognostic value is important for selection of therapeutic modalities. Recent studies in other malignancies have shown that the tumor inflammatory infiltrate has important prognostic value. The aim of the present study was to investigate whether the tumor stromal inflammatory infiltrate represented by CD3 positive cells is a prognostic factor in patients with diffuse malignant mesothelioma.

Design: We studied 204 patients with diffuse malignant mesothelioma (125 epithelioid, 8 sarcomatoid, and 71 biphasic type) who had surgical resection performed at Brigham and Women's Hospital between 2001 and 2008. Paraffin embedded tumor samples were used to construct tissue microarrays. CD3 positivity was evaluated and graded in each tumor as absent (<1%), moderate (<50%), or severe (>50%). Patient age, sex, tumor type, TNM stage, lymphovascular invasion and number of positive lymph nodes were recorded and correlated with CD3 positivity.

Results: In our study, 166 patients (81%) were men and 38 (19%) were women with mean age 63.2 (range 34-84). T category was 1 in 4% of patients, 2 in 24%, 3 in 43% and 4 in 29%. We found that a higher number of CD3 positive lymphocytes were correlated with fewer mediastinal lymph node metastases (p=0.002).

Conclusions: Our results suggest that the degree of chronic inflammatory infiltrate in diffuse malignant mesothelioma has prognostic value. Chronic inflammatory infiltrate may represent an important parameter in the histopathologic assessment of diffuse malignant mesothelioma. An increased number of CD3 positive lymphocytes are associated with fewer lymph node metastases. Stromal chronic inflammatory infiltrate should be considered in the planning of the management of patients with diffuse malignant mesothelioma.

1833 IMP-3 and Ki-67 Expression in Neuroendocrine Tumors of the Lung.

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Background: Insulin-like growth factor-II mRNA-binding protein 3 (IMP3) plays a key role in multiple cancers. Ki-67 is a nuclear protein involved in cell proliferation. IMP3 has been shown to have higher expression in high grade tumors and to be a poor prognostic marker for some others. Ki-67 has been reported as a helpful biomarker in differentiating carcinoid tumors from small cell carcinomas (SCC) of the lung. In this study, we explore for the first time the usefulness of IMP-3 in discriminating pulmonary carcinoid tumors from SCC. To our knowledge, this is the first study to evaluate the expression of both IMP3 and Ki-67 in pulmonary neuroendocrine tumors.

Design: Sixty-seven tissue micro-array cases were retrieved from the surgical pathology files of a large academic institution. These included 40 cases of carcinoid tumors (39 typical and 1 atypical) and 27 SCC. All cases were stained with antibodies against IMP3 and Ki-67 proteins, and evaluated independently by 2 observers. IMP3 expression was divided into 2 categories: negative (absent or weak cytoplasmic staining) and positive (moderate or strong cytoplasmic staining with membranous accentuation). Ki-67 expression was scored as negative or low (0-20% positivity) or high (> 20 %).

Results: IMP-3 was expressed in 60% (16/27) of SCC cases. KI-67 expression was high in 48% (13/27) of SCC cases. Combined, IMP-3 and KI67 were expressed in 30% (8/27) of SCC cases. In carcinoid tumors, IMP-3 was expressed in only 5% (2/39) cases, while Ki-67 expression was expressed in 2.5% (1/39) cases. The atypical carcinoid case was positive for both IMP-3 and ki-67. None of the typical carcinoid cases showed positivity for both markers.

Conclusions: In this study, IMP-3 is shown to have higher sensitivity than KI-67 in SCC (60 vs. 48%) and slightly lower specificity (95 vs. 97.5%). However, the combined sensitivity of both markers in SCC was 30%. Our data suggest that IMP-3 is preferentially expressed in SCC than in typical carcinoids. Our findings further suggest that neuroendocrine tumors with equivocal features of SCC but expressing both IMP3 and Ki-67 can be safely classified as SCC.

Quality Assurance

1834 Effect of Fixatives and Duration of Fixation on Expression of MSI Markers.

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Background: Tumors with abnormal DNA mismatch repair (MMR) genes are described as having microsatellite instability (MSI) and have also been shown to have distinctive clinical and pathologic features including tumor response to chemotherapeutic agents and patients' survival. Hence, immunohistochemical expression of MMR gene products is used to screen for MSI status of tumors for therapeutic decisions and determination of prognosis. This study investigates the optimal tissue processing and fixative conditions for immunohistochemical assay of MMR proteins.

Design: Benign samples of 10 routine tonsillectomy specimens and 5 colectomy specimens were fixed separately in 10% neutral buffered formalin (NBF) solution and dissect aid (DA). Matched samples from each fixative were processed for routine paraffin embedding after fixation for 1, 7, 14, 21, 42, 84, 96, and 112 days. Section of each block was immunostained for MLH1, MSH2 and MSH6. Immunoreactivity was scored in a blinded fashion using a semi quantitative score of 0, 1, 2, 3, and 4. The fixatives' code was then broken.

Results: In tonsils, MLH1 expression in germinal centers was comparable in both fixatives for the first 4 weeks; and thereafter begins to fade out in fixative A samples – with even lower scores in the inter-follicular areas and in the overlying squamous mucosa. For fixative B, MLH1 and MSH6 expression were 4+ in all samples and in all compartments of the tonsil throughout the 16 weeks of tissue fixation; MSH2 expression was 3- 4+ but begins to fade out after 4 weeks. Fixative A samples expressed 1-2+ MSH6 only in the germinal centers and MSH2 was undetectable in most cases (8/10) – even with as little as 24 hours of fixation in fixative A.

In the colon, all fixative B samples stained strongly positive for MLH1, MSH2 and MSH6 and the strong staining reaction was maintained throughout the study. In comparison, fixative A samples' MLH1 scores were consistently at least 1+ lower than corresponding scores for fixative B samples. MSH2 and MSH6 had negative staining reaction for all colon samples processed in fixative A – with as early as 24 hours fixation.

Conclusions: The effect of tissue fixatives on levels of MSI markers is tissue dependent and also varies with the gene product examined. In the tonsil, the overlying squamous mucosa and interfollicular lymphoid cells are more negatively affected than the germinal center cells fixed in fixative A (DA). Compared with MLH1, MSH2 and MSH6 are more susceptible to the adverse effects of fixative A in all the tissue types studied. Fixative B (NBF) is the preferred fixative for MSI immunohistochemical assays.

1835 Quality Control in Microsatellite Instability Testing.

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Background: Microsatellite instability (MSI) analysis and immunohistochemistry (IHC) for DNA mismatch repair (MMR) gene products are well-accepted methods in the evaluation of cancers for Lynch Syndrome (LS). Additionally, MSI in sporadic colon cancer has important prognostic and therapeutic implications. The concordance between PCR-based MSI analysis and IHC is typically high. However, whether in a large molecular diagnostics lab or a small community practice, problem cases will arise and are not well-described in the literature. We summarize our findings to document the frequency and outcomes of such cases.

Design: The records of 736 patients who underwent MSI analysis and/or IHC from 2002 to 2010 were reviewed. Patients with both MSI and IHC analysis (n=628) were subsequently studied in detail. Discordance was defined as a discrepancy between the result of MSI and IHC. Problem was defined as a case with indeterminate or questionable IHC for one or more of the MMR genes. All discordant/problematic results were re-reviewed by two pathologists.

Results: Discordances (13) and problems (10) were identified in 23/628 (3.7%) of the cases. Most (12/13) discordances were detected in MSI-high cancers with positive IHC for the four MMR proteins, MLH1, MLH2, MSH6 and PMS2. Following genetic counseling, 67% of those who underwent germline MMR testing were identified to have MMR mutations. The ten problematic cases were grouped as selection error (2), pathologist error in IHC interpretation (6), or unusual pattern of IHC expression (2). Selection error cases involved patients with multiple synchronous MSI-high and MS-stable colon cancers. Pathologist error cases involved difficulties with MSH6 IHC interpretation and overlooking a lack of positive internal control staining. One unusual staining pattern identified included a case with heterogeneous MLH1 and PMS2 staining, later explained by methylation of *MLH1*. The second unusual pattern case showed expression of MLH1 and MSH2 with complete loss of MSH6 and PMS2 in a colorectal tumor that was MSI-high.

Conclusions: Through this quality control review, several recurring sources of problems in laboratory testing for DNA MMR genes were identified including selection and interpretation error and unusual IHC patterns. Many of these problems are correctable via pathologist education. Recognition of discordant cases and referral for germline mutation analysis is important, as a relatively high percentage of these patients will have germline MMR mutations.

1836 A 383% Increase in Testing Efficiency in a Diagnostics Molecular Lab: LEAN Work Design and Continuous Process Improvements Are Critical for Maintaining Steady Expansion of Services and Short Turn-Around Times.

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Background: With new cancer biomarkers and targeted therapies growing in number, the demand for molecular oncology lab services has been increasing. In 2006, faced with the necessity to bring on new tests and increase revenue for sustainability, our laboratory made an effort to aggressively eliminate non-value added waste and promote LEAN work practices. Standard activities, direct connections and pathways, and continuous improvement and worker empowerment were focused on. The aim of this study was to evaluate effectiveness and sustainability of LEAN in a laboratory environment with monitoring of total volumes and turn around time (TAT) for clinical tests as the ultimate indicator of lab efficiency and customer satisfaction.

Design: Management and staff received LEAN training in May of 2006 as part of a department-wide effort. All processes, inventories, and customer-supplier connections were evaluated. Specimen delivery, test ordering, reporting of results, inventory, and assay validations were standardized. Teaching modules were developed to educate