	Proximal Funisitis (n=24)	Proximal Arteritis (n=23)	Proximal Negative (n=26)*
Distal Funisitis (n=38)	16	5	17
Distal Arteritis (n=33)	6	18	9
Distal Negative (n=2)	2	0	0

Conclusions: Our results demonstrate that there is a significant improvement in detecting funisitis when the umbilical cord is sampled near the placental insertion site. The reason is uncertain, but we postulate that cytokine signaling from maternal neutrophils infiltrating the chorionic plate may stimulate fetal-mediated funisitis in addition to the fetal response to intra-amnionic infection.

1766 GATA-4 and FOG-2 Expression in Pediatric Ovarian Sex Cord-Stromal Tumors (SCST): An Italian Multi-Institutional Cooperative Study

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Background: GATA-4 is a transcription factor regulating cell differentiation and proliferation: in the ovary it controls the expression of α-inhibin and anti-Müllerian Hormone (MIH). FOG-2 activates or inhibits the transcriptional activity of GATA-4, counteracting the trans-activation of the MIH gene by GATA-4 in fetal ovary. A prognostic role of GATA-4 in adult ovarian Granulosa Cell Tumor has been reported. Pediatric SCST are less than 8-10% of all ovarian tumors, with the most frequent histotypes represented by Juvenile Granulosa Cell Tumors (JGCT) and Sertoli-Leydig Cell tumors (SLCT). We explored the potential pathogenetic and prognostic role of FOG-2 and GATA-4 in pediatric SCST.

Design: Histological slides from 15 SCST entered into TREP-study (an Italian multi-institutional study for rare tumors) were reviewed, and immunostains for GATA-4, FOG-2 and α-inhibin were performed. Clinical information were retrieved from TREP files.

Results: Clinico-pathologic features are summarized in the table. Mean age was 112 months (range 7-224); 1 tumor was bilateral, 12 were stage I, 2 stage II, 1 was metastatic at diagnosis. Mean follow-up was 26 months.

Table 1

Histology	FOG-2	GATA-4	α-inhibin	Follow-up
6 JGCT	5/6	1/5	3/6	6 I CR
6 SLCT	4*/6	2/4	5/6	4 I CR, 1 II CR(after metachronous ovarian tumor), 1DOD
FT/SST	2/3	1/1	0/3	3 I CR

*only Sertoli component. Legend: I/IICR=first/second complete remission, DOD=died of disease

All were treated by surgery, with adjuvant chemotherapy in 3. There were 6 JGCT, 6 SLCT, 2 Fibroma/Thecoma (FT) and 1 Sclerosing Stromal Tumor (SST). SLCT behaved more aggressively: 1 SLCT (FOG/GATA negative) was metastatic at diagnosis, and the patient is in I CR; one poorly differentiated SLCT with fatal outcome and 1 metachronous bilateral SLCT were GATA-4 and/or FOG2 positive.

Conclusions: Compared to adult GCT, JGCT express FOG-2, but are frequently GATA-4 negative, suggesting a possible switch to the phenotype of the fetal ovary. The favourable prognosis in most pediatric SCST does not allow to draw a conclusion on the prognostic role of FOG-2 and GATA-4. Therefore, absence of GATA-4 in JGCT may contribute to their favourable prognosis. In SLCT, GATA-4 and/or FOG-2 were expressed in more aggressive tumors. There was no relationship between FOG-2/GATA-4 and α-inhibin staining.

Pulmonary

1767 Are Cytology Blocks Adequate for EGFR Mutational Testing? An Institutional Experience

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Background: EGFR mutation status has been shown to predict response to anti-EGFR tyrosine kinase inhibitors in non-small cell lung cancer (NSCLC). In patients with advanced stage NSCLC, surgical resection is not part of routine care, therefore evaluation of mutational status is increasingly requested on biopsy or fine needle aspiration (FNA) specimens, in which the available material is limited. There are limited data on the suitability of cytology cell blocks for EGFR mutation testing. In this study we report our institutional experience with cytology cell block material for EGFR mutation testing.

Design: We retrospectively reviewed EGFR mutation analyses performed on 135 surgical (SP) and cytology cell blocks (CB) from Oct. 2007-Sept. 2009. One hundred fifteen (115) SP and 20 CB specimens were evaluated for L858R and exon 19 in-frame deletions (analytic sensitivity ~5%). Cytology cell block specimens were evaluated for overall specimen size (total cellularity) and percent tumor. Percent tumor was scored as <5%, 6-10%, 11-25%, 26-50%, >50%. Immunohistochemistry for TTF-1 and CK7 were used to assist in assessment of tumor percentage, when available. Demographic features such as gender and smoking status were evaluated, as EGFR mutations are more frequently seen in women and non-smokers.

Results: Of the 115 SP and 20 CB specimens, 19 (16.5%) and 7 (35%) were positive for EGFR mutation, respectively. The mutation rates were not statistically different between the surgical and cytologic specimens (p=0.065). Of the 20 CB, half were <2mm2; of the 7 cases with a mutation, 4 (57%) were <2mm2. Limited DNA (<25ng/uL) was obtained from 70% (14/20) of CB specimens, including 71% (5/7) of those which were mutation positive; additionally, 57% (4/7) of the positive FNA specimens had extremely low DNA yields (<6.25ng/uL). 20% (4/20) of all FNA specimens had <10% tumor, however all 7 of mutation positive cases had >10% tumor. There were no differences between the SP and CB specimens with regard to patient gender or smoking status (p=0.31 and p=0.27 respectively).

Conclusions: Targeted mutational testing was successfully performed in CB specimens, even when scant. These data indicate that CB specimens provide an alternative source for molecular evaluation of NSCLC, and that tumor percentage may be more important than DNA yield in determining the suitability of these specimens for testing.

1768 Distinguishing Primary from Metastatic Squamous Cell Carcinoma of the Lung in Patients with Known Head and Neck or Gynecological Tract Carcinoma

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Background: Determining if a lung squamous cell carcinoma (SCC) is a primary tumor or metastatic process in patients with concurrent or prior extrapulmonary SCC is challenging. In most cases, the prior SCC originates from the head and neck (H&N) or lower gynecological tract. Since a significant proportion of these tumors are HPV related, we used a panel of immunostains (IHC) for p16, p53, as well as *in situ* hybridization (ISH) for high risk HPV, to determine the relationship between the pulmonary and extrapulmonary tumors.

Design: Twenty-seven patients (14 males; mean age 58 years, range 42-77 years) with SCC of the lung as well as an extrapulmonary SCC were identified. Four extrapulmonary tumors were from the gynecologic tract (3 cervix, 1 endometrium) and 23 cases were from the H&N. All specimens were stained with p16 and p53 (for p16: diffuse staining, cytoplasmic and/or nuclear was considered positive; for p53: more than 5% of nuclei stained was considered positive). Negative cases for p16 were tested with ISH for HPV DNA. Pulmonary specimens with HPV-ISH or p16 status diverging from that of the extrapulmonary site were interpreted as primary SCC. When HPV-ISH or p16 status was positive in both sites, pulmonary SCC was considered to be metastatic. In cases with negative HPV-ISH and p16 status, divergent results for p53 were interpreted as primary SCC of the lung, while similar p53 results were considered inconclusive.

Results: In 9 (33%) cases (3 cervical and 6 H&N), the pulmonary and extrapulmonary tumors had positive HPV status, and the lung SCCs were considered metastases, while 6 (22%) SCCs (all H&N) had different HPV status and the lung SCCs were determined to be primary in origin. In 3 cases, (2 H&N and 1 endometrium) both the extrapulmonary and pulmonary tumors were negative for HPV but with divergent p53, suggesting primary pulmonary origin. In 9 cases (all H&N) both sites were HPV negative and showed similar p53 staining; a definitive classification of primary or metastasis could not be determined by IHC alone. Overall, we were able to determine primary vs metastasis in 67% of the cases (100% gynecological and 61% H&N).

Conclusions: Our study supports a panel of immunostains for p16, p53 and HPV ISH as a useful tool to distinguish between primary and metastatic pulmonary SCC. These results raise the importance of markers that will distinguish pulmonary lung SCC from a metastasis, especially in HPV negative cases.

1769 Characteristic Morphology and Immunoprofile of Lung Adenocarcinoma with *KRAS* Mutations: Propensity for Solid Growth Pattern and Correlation with TTF-1 Expression

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Background: *KRAS* mutations in lung adenocarcinoma (AD) define a distinctive clinical subgroup of patients who are resistant to not only to *EGFR*-targeted therapies, but also to conventional chemotherapy. Compared to *EGFR*-mutant tumors, *KRAS* mutations are strongly associated with smoking, and are predictive of poor prognosis. However, whether *KRAS* mutations define a morphologically and immunophenotypically distinctive subtype of AD has not been established.

Design: 82 resected lung AD were analyzed for *KRAS* and *EGFR* mutations, and classified histologically based on the presence or predominance (>50%) of solid vs other (bronchioloalveolar, acinar, papillary, micropapillary) patterns. All tumors were evaluated by immunohistochemistry for TTF-1 as positive (any reactivity) or negative.

Results: Of 82 tumors, 27 (33%) harbored *KRAS* mutations, 17 (21%) *EGFR* mutations, and 38 (46%) neither *KRAS* nor *EGFR* mutations. *KRAS* mutations were strongly associated with solid-predominant growth pattern: 16/25 (64%) of solid-predominant tumors were *KRAS*-mutant compared to 11/57 (19%) of non-solid predominant tumors (P=0.0002). Even tumors with a minor solid component had more frequent *KRAS* mutations (21/45, 46%) than tumors without any solid component (6/37, 16%; P=0.004). In contrast, none of solid-predominant tumors had *EGFR* mutations (0/25) as compared to tumors with other patterns (17/57, 30%; P=0.001). TTF-1 was expressed in 26/27 (96%) of *KRAS*-mutant tumors, 17/17 (100%) of *EGFR*-mutant tumors, and 31/38 (82%) of non-mutant tumors. Presence of either *KRAS* or *EGFR* mutations was significantly associated with TTF-1 expression (P=0.02).

Correlation of KRAS and EGFR mutations with solid pattern in lung AD.

Histologic Type	*KRAS*-mutant	*EGFR*-mutant	*KRAS* and *EGFR* non-mutant
All types combined (n=82)	27 (33%)	17 (21%)	38 (46%)
Solid predominant (n=25)	16 (64%)	0 (0)	9 (36%)
Any solid (n=45)	21 (47%)	7 (15%)	17 (38%)

Conclusions: We find that solid growth pattern, particularly if predominant, is a characteristic histopathologic feature of *KRAS*-mutant AD. This pattern has been previously shown to correlate with aggressive behavior, consistent with the poor prognosis of *KRAS*-mutant tumors. Correlation with TTF-1 expression has been previously established for *EGFR*-mutant tumors, and we extend this observation to tumors with *KRAS* mutations.

1770 Expression of Squamous Markers in Lung Adenocarcinoma (AD): Clinicopathologic and Molecular Correlates, and Implications for Differentiation from Squamous Cell Carcinoma (SqCC)

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Background: The distinction of lung AD from SqCC has gained increased importance due to recent emergence of histology-based therapies. Immunohistochemistry (IHC) for squamous markers (p63 and high-molecular weight keratins [HMWCK]) in combination with TTF-1 has been suggested to aid in this classification. However, the precise rate, coexpression profiles, clinicopathologic and molecular correlates for these markers in AD have not been established.

Design: IHC for p63 (4A4), HMWCK (34βE12), and TTF-1 was performed on 185 unselected AD, including 88 consecutive resections and 97 tumors represented in tissue microarrays. Reactivity was scored as positive (POS) [>20%], focal (FOC) [≤20%], or negative (NEG) [0], and correlated with various clinicopathologic parameters and mutations in *EGFR* and *KRAS.*

Results: Of 185 AD, p63 was POS or FOC in 6% and 23%, and 34βE12 in 29% and 21%, respectively. While p63 was only rarely diffuse [>50% tumor cells] (n=3, 1.6%), AD with diffuse 34βe12 were common (n=39, 21%). p63 was strongly associated with TTF-1 coexpression: POS or FOC p63 was seen in 31% of TTF-1 POS AD vs only FOC p63 was seen in 9% of TTF-1 NEG AD (P=0.041). In contrast, 34βE12 was equally distributed in TTF-1 POS and NEG tumors. There was no association of p63 or 34βE12 with histologic patterns of AD, but there was a trend for p63 in bronchioloalveolar-predominant tumors. Neither p63 nor 34βE12 were associated with age, gender, smoking, stage, survival, and *EGFR* or *KRAS* mutations.

Coexpression profiles of squamous markers and TTF-1 in lung AD (n=185).

	TTF-1 POS or FOC n=163 (88%)			TTF-1 NEGTIVE n=22 (12%)		
	34βE12 POS	34βE12 FOC	34βE12 NEG	34βE12 POS	34βE12 FOC	34βE12 NEG
p63 POS	6 (3%)	1 (0.5%)	4 (2%)	0 (0)	0 (0)	0 (0)
p63 FOC	16 (9%)	9 (5%)	14 (8%)	1 (0.5%)	1 (0.5%)	0 (0)
p63 NEG	23 (12%)	22 (12%)	68 (37%)	7 (4%)	6 (3%)	7 (4%)

Conclusions: Reactivity for both p63 and 34βE12 is frequent in lung AD, therefore neither p63 nor 34βE12 alone can be used to distinguish AD from SqCC. However, no AD in this study had a 3-marker coexpression profile that overlapped with typical SqCC (TTF-1 NEG/p63 POS/34βE12 POS), suggesting the utility of a panel approach. Further studies are needed to elucidate the significance of p63 restriction to TTF-1 POS AD, and to determine whether there is a biological or clinical significance of squamous marker expression in lung AD.

1771 Immunohistochemical Analysis of Napsin A in 77 Lung Carcinomas and 509 Non-Pulmonary Malignancies

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Background: Napsin A is an aspartic proteinase recently reported to be expressed in the majority of lung adenocarcinomas (AD) with sensitivity superior to that of TTF-1. Napsin A is normally expressed only in type II pneumocytes and proximal renal tubules. In addition, expression in B-lymphocytes has been reported in animal models. Immunohistochemistry (IHC) for Napsin A has been proposed as a useful ancillary tool in the identification of lung origin in carcinomas of unknown primary. However, the specificity of Napsin A has not been fully evaluated.

Design: We performed IHC on tissue micro-arrays (TMA) constructed form of 50 lung AD, 23 lung squamous cell carcinomas, 4 lung neuroendocrine neoplasms, 160 colon AD, 81 gastric AD, 29 cervical AD, 52 endometrial adenocarcinomas, 57 breast adenocarcinomas, 50 clear cell renal cell carcinomas, 46 papillary renal cell carcinomas, and 34 marginal zone lymphomas. IHC was scored based on intensity of cytoplasmic staining as 1+ (faint), 2+ (moderate), or 3+ (strong). The scores of 2+ or 3+ were considered positive.

Results: Napsin A was positive in 37 of 50 (74%) lung AD, and 13 of 46 (28%) papillary renal cell carcinomas. All pulmonary squamous cell carcinomas, neuroendocrine neoplasms and all non-pulmonary malignancies were negative for Napsin A. Strong reactivity was also found in type II pneumocytes and renal tubular cells.

Conclusions: We confirm previous reports that Napsin A shows robust expression in the majority of lung AD and in a subset of papillary renal cell carcinomas. In addition, this study represents the largest screen of non-pulmonary carcinomas for Napsin A expression, revealing that no other tested extra-pulmonary malignancy had reactivity for Napsin A, confirming Napsin A as a highly specific marker of a lung or renal origin.

1772 p16 Expression in Squamous Cell Carcinoma in Multiple Organ Sites

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Background: p16 is a member the INK4 family of cell cycle regulatory proteins which keeps pRb, a tumor suppressor gene, in an unphosphorylated state thus maintaining its tumor suppressor activity. Following integration of the human papilloma (HPV) viral DNA into host genome, the viral E7 protein binds to and degrades pRb resulting in loss of tumor suppressor activity. This results in p16 upregulation by a positive feed back mechanism. HPV has been implicated in squamous cell carcinoma (SCC) in the anogenital and upper aerodigestive tracts. Its role in SCC of lung varies with location worldwide. Studies have implied the usefulness of p16 in discriminating between cervical SCC with lung metastases and primary lung SCC. We study the expression p16 in SCC in multiple sites to determine its usefulness as a marker in these sites.

Design: A total number of 56 cases of primary SCC were retrieved from our pathology archives and H&E stained slides were reviewed by 2 independent pathologists. The sites were lungs: 25 cases, anogenital area (cervix, vulva, anorectum): 8 cases, esophagus: 5 cases, skin: 7 cases and head/neck: 11 cases. Cases varied from well to moderately differentiated SCC. p16 immunohistochemical analysis was then performed on paraffin sections using an automated system (Ventana 1:200). Scoring 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.

Results: Of 25 cases of lung SCC, 40% were positive for p16. The proportion of positive cells was independent of degree of differentiation. All cases from the anogenital area were strongly positive, 40% of skin SCC were positive, 37.5% of head and neck SCC were positive and all cases of esophagus were negative (Table 1).

Table 1. SCC site and p16 immunoreactivity

		P16 immunoreactivity score			
SCC site	Number of cases	0	1+	2+	3+
Lung	25	15	1	5	4
Anogenital	8	0	0	0	8
Skin	7	5	1	0	1
Esophagus	5	5	0	0	0
Head & Neck	11	8	0	0	3

Conclusions: 1. p16 is expressed in SCC of the lungs, skin and head/neck areas implying a possible role of HPV in carcinogenesis of SCC in these sites. **2**. It is not a useful marker in discriminating between a cervical SCC with pulmonary metastasis and primary lung SCC. **3**. p16 is not expressed in SCC of esophagus and does not favor a carcinogenic role of HPV in esophageal SCC.

1773 Associations of C-MET Gene Copy with EGFR Mutation and Gene Copy in EGFR-Tyrosine Kinase Inhibitor (TKI) Untreated Non-Small Cell Lung Cancer (NSCLC)

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Background: C-Met is transmembrane receptor tyrosine kinase for HGF/SF, and known to be frequently amplified in NSCLC patients with secondary EGFR-TKI resistance. However, C-MET gene amplification rates have been variably reported, and the association of C-MET amplification with EGFR gene mutation and gene copy is not well known in EGFR-TKI untreated NSCLC patients. Here we investigated the clinicopathologic characteristics of C-MET amplification, EGFR mutation and gene copy number in EGFR-TKI untreated NSCLC.

Design: All 216 NSCLC patients (124 adenocarcinomas, 62 squamous cell carcinomas, and 30 others) were Korean, and FFPE tissues were obtained from surgically resected specimen. EGFR mutations (exon 18, 19, 20, 21) were analyzed using PCR based direct sequencing. EGFR and C-MET gene copy numbers were analyzed using *fluorescence in situ hybridization* (FISH) and estimated according to the University of Colorado Cancer Center (UCCC) criteria for EGFR and Cappuzzo scoring system for C-MET.

Results: EGFR mutations were observed in 32.4% (n = 70) and increased EGFR gene numbers (EGFR FISH-positive) were found in 31.5% (n = 68). EGFR mutation and increased gene copy were strongly associated with female ($p < 0.001$ and 0.002), non-smoker ($p < 0.001$ and 0.001), and adenocarcinoma histology ($p < 0.001$, and 0.02). Increased EGFR gene copy was associated with advanced stage ($p = 0.032$) and distant metastasis ($p = 0.002$). Increased C-MET gene copy (mean ≥ 5 per cell) was observed in 6.5% (n = 14, which included 6 adenocarcinomas, 4 squamous cell carcinomas and 4 others), and true amplification was observed in 2.3% (n = 5). Increased C-MET gene copy showed weak association with EGFR FISH-positive ($p = 0.041$) and advanced stage ($p = 0.047$), but was not associated with EGFR mutation, age, gender, smoking history and histology. Four patients (3 adenocarcinoma and 1 pleomorphic carcinoma) had both increased C-MET gene copy and EGFR mutation and were EGFR FISH-positive.

Conclusions: Increased C-MET gene copy was weakly associated with EGFR FISH positive and advanced stage in NSCLC. A small set of EGFR-TKI untreated NSCLC patients had both C-MET amplification and EGFR mutation.

1774 Clinicopathologic Characteristics of Malignant Mesotheliomas Arising in Patients with a History of Radiation for Hodgkin Lymphoma

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Background: Recent studies have reported an association between malignant mesothelioma and chest radiation for Hodgkin lymphoma (HL). The clinicopathologic characteristics of malignant mesotheliomas arising in these patients have not been established.

Design: We compared the clinicopathologic characteristics of nine malignant mesotheliomas from patients with a history of radiation for HL and no reported asbestos exposure (case group) with 12 malignant mesotheliomas from patients with a history of asbestos exposure (control group). Clinical features ascertained were sex, age at mesothelioma diagnosis, asbestos exposure, and dates of radiation for HL, diagnosis of malignant mesothelioma, and death. Tumors were classified as epithelioid, sarcomatoid, or mixed types according to WHO criteria. We reviewed an average of 14 H&E slides (range 2-25) from each tumor and recorded the presence of rhabdoid, clear cell, signet-ring cell, and myxoid morphologies, pleomorphism, necrosis, mitoses, and cytogenetic and molecular alterations.

Results: Median time from radiation for HL to mesothelioma was 24.4 years (range 13-36). Eight mesotheliomas after HL were epithelioid and one was mixed. Two cases had pleomorphic histology, one had a myxoid morphology, one had clear cells, and three had signet-ring cells. The cytogenetic and molecular alterations were numerous losses/deletions, including deletion of 22q, and deletion of the p16 gene. Patients with mesothelioma after radiation for HL were younger than the patients in the control group (median age 41 vs. 65, $p<0.0001$) and had a significantly longer median overall survival (31.5 vs. 11.2 months, $p=0.046$).

Conclusions: Patients with mesothelioma after HL are significantly younger and had a longer overall survival compared to patients in the control group. Continued studies are

needed to further define the clinicopathologic characteristics of patients with malignant mesothelioma with a history of radiation for HL.

1775 Reproducibility of a Proposed Grading System for Lung Adenocarcinoma and Correlation with Molecular Alterations

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Background: We previously described a prognostically significant grading system for lung adenocarcinoma that incorporated the percentage of solid growth pattern and the degree of cytologic atypia. The purpose of the current study was to assess interobserver reproducibility of our proposed grading system and correlate grade with molecular alterations.

Design: We studied two cohorts (104 and 197 patients) with lung adenocarcinomas treated at two institutions. All H&E slides of tumors were evaluated for the percentage of solid growth pattern and the degree of cytologic atypia. Based on previous analyses, tumor architecture was scored based on the percentage of solid growth pattern as 1=≤80% solid growth or 2=>80% solid growth, and cytologic atypia was scored as 1=mild or moderate atypia with uniform nuclei or 2=severe atypia with bizarre, enlarged nuclei of varied sizes. The grading score was computed as the sum of the architecture and cytologic atypia scores (2=well differentiated [WD], 3=moderately differentiated [MD], 4=poorly differentiated [PD]). For cohort A, three pathologists reviewed the slides independently. For cohort B, the EGFR and KRAS mutation status was recorded. We calculated the interobserver reproducibility for cohort A and correlated the grade with the molecular alterations in cohort B.

Results: For cohort A, the percentage overall agreement for assessment of solid growth pattern was 88.5%, for cytologic atypia it was 82.1% and for grade it was 66.7%. In cohort B, 32 cases (16.2%) had EGFR mutations, 68 (34.5%) had KRAS mutations, and 97 (49.2%) lacked both EGFR and KRAS mutations. Grade was significantly correlated with molecular alterations (p=0.0003). For tumors with EGFR mutations, 19 cases (59.4%) were WD, 13 (40.6%) were MD, and none were poorly differentiated. For tumors with KRAS mutations, 43 cases (63.2%) were WD, 14 (20.6%) were MD, and 11 (16.2%) were PD. For cases lacking EGFR and KRAS mutations, 33 cases (34.0%) were WD, 47 (48.5%) were MD, and 17 (17.5%) were PD.

Conclusions: Our results indicate that there is good interobserver agreement among pathologists for this grading system. In addition, the differentiation based on this grading system correlates with molecular alterations.

1776 Epidermal Growth Factor Receptor Amplification and KRAS Mutation in Pre-Invasive Versus Invasive Lung Adenocarcinoma

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Background: Two common molecular alterations in lung adenocarcinoma are KRAS mutation and epidermal growth factor receptor (EGFR) alterations including polysomy, amplification and mutation. While certain mutations are associated with improved survival and response to tyrosine kinase inhibitors, it has been suggested that amplification is associated with tumor progression and invasion. Here we investigate molecular alterations in bronchioloalveolar carcinoma (BAC) versus invasive lung adenocarcinoma.

Design: 187 of cases of lung carcinoma from 2007-2009 (185 adenocarcinoma or BAC) were selected for inclusion on the basis of having had KRAS mutation and/or EGFR amplification testing done. The cases were reviewed and categorized according to predominant histologic subtype (BAC, acinar, micropapillary, papillary, solid, mucinous, adenosquamous and squamous). The histologic subtypes were then compared for differences in molecular alteration (KRAS mutation type, EGFR amplification, high polysomy and low polysomy). The cases were also compared for molecular alteration without reference to histology.

Results: None of the predominant BAC cases (0 of 17) have EGFR amplification, while 22% of the invasive adenocarcinomas have EGFR amplification (28 of 130), (p = 0.033). KRAS mutations were seen in 21% (3 of 14) of the BACs, and 34% (51 of 150) of the invasive adenocarcinomas (p = 0.48). Consistent with previous reports, 6 of 7 of mucinous type adenocarcinomas have KRAS mutations while none have EGFR amplification. Overall, EGFR amplification and KRAS mutation are virtually always mutually exclusive with only 1.3%(2 of 157) having both (p = 0.032); This mutually exclusive relationship with KRAS mutation is also seen when high polysomy is included with EGFR amplification (p = 0.000011).

Conclusions: KRAS mutation and the EGFR alterations of amplification and high polysomy are frequently mutually exclusive. In BAC and predominant BAC, EGFR amplifications were not seen, in contrast to being present in 22% of invasive adenocarcinomas. In pre-operative assessment of adenocarcinoma, an EGFR amplification is an indication that the tumor has acquired invasiveness.

1777 EGFR and KRAS Mutations in Non-Small Cell Lung Cancers: A Pilot Study from Turkey

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Background: Mutation profile of the MAPK pathway in non-small cell lung cancers (NSCLC) shows wide variations because of many factors including geographic and ethnic background. There is no data denoting EGFR and KRAS mutations of NSCLC cases about Turkey in the literature. We aimed to screen the frequency and the types of EGFR and KRAS mutations in a sample group of NSCLC cases in Turkey, and we compared our data to the available literature to realize weather geographic and ethnic factors has any implication.

Design: Fourteen adenocarcinomas (AC), 11 squamous cell carcinomas (SCC) were screened for EGFR exon 19 and 21, and KRAS exon 2. Pure tumor tissues from formalin fixed paraffin embedded tumor tissues were used for DNA extraction. After PCR amplifications, sequence data were analyzed and compared to the literature findings according to frequency, site and type of mutations detected.

Results: We found 11 (44%) cases with EGFR mutations (exon 19; n=8 and exon 21; n=5) and 6 (24%) cases with KRAS mutations in NSCLC cases. For EGFR exon 19, we detected frame or frame shift deletion type mutation in all cases. For EGFR exon 21, 3 cases were found with L858R mutation (CTG>CGG) and in 2 cases deletion type mutations were detected. While all 5 cases were revealing codon 12 mutations (3 patients G>T; 1 patient T>C; 1 patient G>A), only one case was detected with codon 13 mutation (G>T) in KRAS gene. As the histological types, in AC group, 5 cases with EGFR (exon 19; n=3 and exon 21; n=3) and 3 cases with KRAS exon 2 mutations were detected. Mutations in the SCC group were distributed as 5 exon 19 and 2 exon 21, and 3 KRAS mutations. Additionally, KRAS and EGFR mutations were observed together only in 3 patients (2AC and 1SCC), and EGFR 19 and 21 mutations were detected together only in 2 cases (1AC, 1SCC).

Conclusions: The mutation frequency and the site profiles of KRAS in our small series of Turkish non-small cell lung cancer patients were found quite similar to those seen in the western countries. However, the EGFR mutations were distinctive in terms of frequency, than the westerns, and were quite similar to that of East Asian.

1778 Accurate Classification of Non-Small Cell Lung Carcinoma Using a Novel MicroRNA-Based Approach

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Background: Advances in targeted lung cancer therapy now demand accurate classification of non-small cell lung cancer (NSCLC). MicroRNAs (miRNAs) are recently discovered short, non-coding genes that play essential roles in tissue differentiation during normal development and tumorigenesis. For example, hsa-miR-205 is a miRNA that is highly expressed in lung squamous cell carcinomas (SqCCs) but not in lung adenocarcinomas (ACs). The differential expression of miRNAs could be exploited to distinguish these tumor types.

Design: 102 resected NSCLCs were classified as SqCC or AC based on their histologic features and immunohistochemical profiles. Corresponding pre-operative biopsies/aspirates that had been originally diagnosed as poorly differentiated NSCLCs were available for 21 cases. A qRT-PCR diagnostic assay which measures the expression level of hsa-miR-205 was used to classify the carcinomas as SqCC or AC based solely on expression levels. The two sets of diagnoses were compared.

Results: Using standard pathologic methods of classification (i.e. microscopy and immunohistochemistry), 52 resected lung carcinomas were classified as SqCCs and 50 as ACs. There was 100% concordance between the diagnoses established by conventional and miRNA-based methods. MiRNA profiling also correctly classified 20 of the 21 preoperative biopsy specimens.

Conclusions: MiRNA profiling is a highly reliable strategy for classifying NSCLCs. Indeed, classification is consistently accurate even in small biopsies/aspirates of poorly differentiated tumors. Confirmation of its reliability across the full range of tumor grades and specimen types represents an important step towards broad application.

1779 A Role for HPV Analysis in Distinguishing Second Primary Tumors from Lung Metastases in Patients with Head and Neck Squamous Cell Carcinoma

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Background: For patients with head and squamous cell carcinoma (HNSCC), a squamous cell carcinoma in the lung may represent either a primary lung cancer or a metastasis. Although the distinction influences patient prognosis and therapy, it is far from straightforward using standard clinical and histologic parameters. Human papillomavirus (HPV) is an etiologic agent for an important subgroup of HNSCCs, but not for primary lung carcinomas. Detection of HPV could be useful in establishing tumor origin and clarifying other important relationships for patients with HNSCC who develop a squamous cell carcinoma in the lung.

Design: We performed HPV in-situ hybridization on 46 squamous cell carcinomas involving the lungs of patients with a prior history of HNSCC. HPV status was correlated with certain clinical features including anatomic site of the HNSCC and disease free interval.

Results: HPV was detected in 5 of 46 (11%) cancers in the lungs. When stratified by anatomic site of the prior HNSCC, HPV was more common in patients with oropharyngeal carcinomas than in those with non-oropharyngeal carcinomas (25% vs. 0%, p = 0.01). The presence of HPV was confirmed in the corresponding oropharyngeal carcinomas (3 of 3, 100%). Although lung carcinomas in patients with HNSCC are generally believed to represent second primary tumors if they occur more than 3 years after treatment of the HNSCC, 4 of the HPV-positive lung metastases were discovered beyond this time frame. Indeed, 2 patients with HPV-positive oropharyngeal carcinomas developed lung metastases after a disease free interval of 8 years.

Conclusions: For patients with carcinomas of the oropharynx who develop squamous cell carcinomas in their lungs, HPV analysis may be helpful in clarifying tumor relationships. These relationships may not always be obvious on clinical grounds as HPV-related HNSCC may metastasize long after treatment of the primary tumor. Although not previously appreciated, long periods of quiescence is in keeping with the improved 5 year-survival rates associated with HPV-related HNSCC and underscores a need for long term patient follow-up.

1780 Age-Related *CDKN2A (p16)* Promoter Methylation and Outcome in Surgically Treated Stage I-II Non-Small Cell Lung Cancer Patients

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Background: Non-small cell lung cancer (NSCLC) is a disease that infrequently occurs in younger individuals (< 40 years of age). Currently, no biological indicators accurately differentiate NSCLC in these patients from those that occur in older individuals. To explore epigenetic influences, promoter methylation (silencing) at multiple CpG dinucleotides of selected tumor suppressor genes was analyzed in stage I and II NSCLC patients.

Design: Methylation was quantified at specific CpG sites in p16, MGMT, DAPK, RASSF1, CDH1, LET7-3-a, NORE1(RASSF5), and PTEN promoters in assessable tumor tissue from 196 surgically treated NSCLC patients by pyrosequencing. Molecular and clinical characteristics with time to recurrence (TTR) and overall survival (OS) were evaluated.

Results: Methylation levels of specific promoter sites in DAPK and CDH1 were significantly higher in patients with age at diagnosis over 50 years (n=167) than in those patients with diagnosis at 50 years or younger (n=29). Methylation levels at all 7 potentially hypermethylated cytosine positions tested in the p16 promoter (-64 to -40; A of ATG = +1) were significantly higher in the >50 group than in the ≤50 group (p=0.001 – 0.012). The results were more significant using a cutoff of 40 years or younger (p<0.001). In Kaplan-Meier analysis, patients with age at diagnosis ≤40 years had shorter median TTR than those over 40 years (18.3 mos vs 114 mos, Log rank p=0.018). When p16 was hypermethylated at cytosine position -49, patients with diagnosis ≤ 50 years had significantly shorter TTR (9.9 mos vs 78.8 mos; p=0.098) and significantly shortened OS (14.9 mos vs 125 mos; p=0.008). No such effect was seen in patients >50.

Conclusions: Although P16 methylation had the highest frequency in patients > 50 years, it was associated with a worse outcome in patients ≤ 50 years who had hypermethylation at cytosine position -49. Overall, these data support an influence of hypermethylation in the p16 promoter and outcome in young patients (≤50 years) after surgical resection for stage I and II NSCLC.

1781 Assessment of *EGFR* Mutation Status in Lung Adenocarcinoma by Immunohistochemistry Using Antibodies Specific to the Two Major Forms of Mutant EGFR

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Background: Somatic mutations within the tyrosine kinase domain of EGFR have emerged as the most reliable predictors of response to EGFR tyrosine kinase inhibitors (TKI) in patients with lung adenocarcinoma. Deletions in exon 19 and the L858R substitution in exon 21, accounting for approximately 90% of all mutations, are the best characterized sensitizing mutations. At present, mutations are detected by molecular methods but alternative assays that could be performed more widely in clinical laboratories remain of substantial interest.

Design: We evaluated two mutation-specific monoclonal antibodies for the detection of *EGFR* mutations by immunohistochemistry (IHC), generated respectively against the L858R mutant and the exon 19 mutant with the common 15bp/5AA deletion (Cell Signaling Technology). IHC staining performed on 218 paraffin-embedded lung adenocarcinomas was assessed on a 0 to 3+ scale, and positivity cutoffs of 1+ and 2+ were compared. All cases were studied by standard molecular methods for these two mutations and selected cases were also studied using higher sensitivity molecular assays. These molecular studies confirmed 21 cases with EGFR L858R and 55 cases with EGFR exon 19 deletions, both reflecting enrichment of these mutations for this study.

Results: Both antibodies showed a cytoplasmic and membranous staining which was usually homogeneous in cases with moderate to strong staining, but was more heterogeneous in cases with faint staining. The EGFR L858R mutant antibody showed a sensitivity of 95% and a positive predictive value (PPV) of 99% with a positivity cutoff of 1+ and a sensitivity of 76% and a PPV of 100% with a positivity cutoff of 2+. The EGFR exon 19 mutant-specific antibody showed reduced sensitivity for exon 19 deletions other than 15bp. A positivity cutoff of 1+ resulted in a sensitivity of 85% and a PPV of 99% while a 2+ cutoff gave a sensitivity of 67% and a PPV of 100%.

Conclusions: These two mutant specific antibodies could be incorporated into the routine IHC work-up of lung adenocarcinomas to reduce the molecular testing volume for *EGFR* mutations while allowing faster treatment initiation for some patients with sensitizing *EGFR* mutations.

1782 Molecular Profiles of EGFR and KRAS Mutations in Non-Small-Cell Lung Carcinoma: A Survey of 344 Cases

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Background: Activating mutations of the epidermal growth factor receptor (EGFR) predict clinical response to EGFR inhibitor (EGFRi) therapy in non-small-cell lung carcinoma (NSCLC) patients, whereas mutations of the Kirsten rat sarcoma viral oncogene (KRAS) predict primary resistance to EGFRi therapy. EGFR and KRAS mutations are generally mutually exclusive, with rare reports documenting coexistent mutations. There is also limited data about the coexistence of multiple EGFR mutations in a single tumor sample. For instance, the development of the secondary resistance mutation T790M has been identified in NSCLC with the primary sensitive mutation L858R following treatment with EGFRi.

Design: 334 randomly selected NSCLC cases were analyzed for EGFR and KRAS mutations. Samples tested included surgical biopsy and cytology fine needle aspiration specimens. For EGFR analysis, exons 18-21 were assessed using Sanger sequencing. For KRAS analysis, codons 12, 13, and 61 were assessed using Pyrosequencing.

Results: EGFR mutations were identified in 66 of 327 (20.2%) samples and were distributed as follows: 5 (6.7%) in exon 18, 29 (38.7%) in exon 19, 13 (17.3%) in exon 20, and 28 (37.3%) in exon 21. These mutations included 26 (34.7%) in-frame deletions in exon 19, 4 (5.3%) in-frame insertions in exon 20, and 45 (60%) point mutations in exons 18, 19, 20, and 21. Multiple EGFR mutations coexisting in the same tumor sample were rare. KRAS mutations were identified in 66 of 327 (20.2%) samples and were distributed as follows: 55 (83.3%) in codon 12, 6 (9.1%) in codon 13, and 5 (7.6%) in codon 61. 320 samples were analyzed for both EGFR and KRAS mutations: 195 (60.9%) had no mutations, 60 (18.6%) had EGFR mutation, 61 (19.1%) had KRAS mutation, and 4 (1.3%) had both EGFR and KRAS mutations. The coexistent EGFR and KRAS mutations were: KRAS G13C + EGFR Y801A, and KRAS G12C + EGFR V726M or R748G or K875R.

Conclusions: EGFR and KRAS mutations rarely (~1%) coexist in cases of NSCLC. Similarly, multiple EGFR mutations rarely coexist in these tumors. The clinical relevance of coexisting EGFR and KRAS mutations and that of multiple mutations in EGFR to clinical response to EGFRi therapy needs further evaluation, which may lead to a better understanding of the mechanisms explaining therapeutic response to patients with NSCLC treated with EGFRi.

1783 Mutational Analysis of *EGFR, KRAS, BRAF, PIK3CA,* and *ERBB2* in Non-Small-Cell Lung Carcinomas (NSCLC)

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Background: Despite advances in the management of EGFR-mutant NSCLC with targeted therapy, prognosis for the majority of patients, whose tumors lack EGFR mutations, remains poor. Nevertheless, the success of EGFR therapy suggests that targeted therapy directed against other molecular alterations, where appropriate, may lead to similarly successful outcomes. Analysis of multiple oncogenes, rather than EGFR alone, was performed on a set of lung cancers to classify and quantitate a variety of molecular abnormalities therein.

Design: Paraffin-embedded, formalin-fixed tumor samples from 54 consecutive nonsmoking patients with advanced-stage non-squamous NSCLC were dissected and analyzed by PCR-Sanger sequencing for mutations in selected exons and splice sites of *EGFR*, *KRAS*, *BRAF*, *PIK3CA*, and *ERBB2*.

Results: Mutations were found in 24 (44%) of samples; none of the samples had more than one mutation. Mutations in *KRAS* were most common, seen in eight patients (14.8%), followed by *EGFR* in seven (13%), *BRAF* in five (9.3%), *ERBB2* in three (5.6%), and PIK3CA in one (1.9%). Two novel mutations were identified: a 7-bp deletion (2284_90del7) affecting the *EGFR* exon 20 splice site, and an in-frame deletion of *BRAF* codon 469.

Conclusions: Mutually-exclusive oncogene mutations were seen in 44% of these NSCLCs. Many of these oncogenes are suitable candidates for directed therapies. Further studies, including prospective clinical trials, are warranted to determine if molecular classification of NSCLC beyond *EGFR* sequencing can stratify patients into treatment cohorts with improved outcomes.

1784 Expression of hMLH1 and hMSH2 Proteins in Non-Small Cell Lung Cancer and Its Clinicopathologic Significance – From the Viewpoint of Forthcoming New TNM Classification

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Background: The inactivation of mismatch repair (MMR) system is one of the important mechanisms in the carcinogenesis of colon cancer. But in non-small cell lung cancer (NSCLC), it is not fully studied although microsatellite instability (MSI) which is resulted from loss of MMR system reported up to 68%. Moreover its clinicopathologic significance seems to be controversial. Thus we investigate the expression of DNA MMR proteins, hMLH1 and hMSH2 in NSCLC and evaluate their clinicopathologic significance. Pathological stages were compared between present TNM stage and forthcoming new TNM stage proposed by the International Association for the Study of Lung Cancer (IASLC).

Design: Consecutive 293 cases of surgically resected NSCLC specimen were enrolled in this study. Immunohistochemical analysis for hMLH1 and hMSH2 were performed and correlated with the clinicopathological characteristics. Statiscal analyses were performed using the χ^2 analysis and the Kaplan-Meier method.

Results: The frequency of loss of hMLH1 and hMSH2 proteins were 27.6% (79/286) and 8.7% (25/288), respectively. Loss of MMR proteins were correlated with present and new TNM stage classification. Loss of hMLH1 was correlated with newly revised TNM stage (p=0.043) as well as old TNM stage (p=0.031) including T stage (p=0.047) and N stage (p=0.037). Furthermore in hMSH2, it was more closely correlated with proposed TNM stage (p=0.001) similar to present TNM stage (p=0.001) including T stage (p=0.003) and N stage (p=0.006). Also, expression of hMSH2 tended to be associated with disease free survival (p=0.073).

Conclusions: Less correlation in hMLH1 compared to hMSH2 may be explained by differences of the mechanism of the inactivation of MMR system. There were no significant differences between loss of MMR proteins and other clinicopathologic factors. In conclusion, the inactivity of MMR system is correlated with advanced TNM stage in both current and proposed TNM system, more prominent in hMSH2. This is the largest study to show correlation between loss of MMR proteins and poor prognosis in the NSCLC.

1785 Therapeutic Oral Iron Supplementation Associated Mucosal Injury of the Upper and Lower Respiratory Tract

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Background: Mucosal injury of the upper gastrointestinal tract due to therapeutic oral iron supplementation is a well-described yet under-recognized entity. Although iron pill aspiration is well documented to cause massive necrosis and stricture of the airways, injury to the bronchial/upper respiratory tract mucosa secondary to aspiration of therapeutic oral iron ingestion has rarely been described.

Design: We describe the mucosal injury pattern in bronchial and hypopharyngeal biopsies of two elderly patients (84 y.o. woman and 8.4 y.o man) who were both receiving therapeutic oral iron supplementation. The patients had no known aspiration events. Patient 1 is an 84 y.o. woman with iron-deficient anemia who developed hemoptysis. Patient 2 is an 84 y.o man with past medical history of prostate cancer and basal cell carcinoma who presented to with an ulcerated and protuberant hypopharyngeal mass suspicious for a neoplasm.

Results: Endoscopy demonstrated friable mucosa and maculopapular lesions in the right mainstem bronchus and in the hypopharynx, respectively. Biopsies of both lesions showed mucosal ulceration with deposits of brown fibrillar material in the epithelium and submucosa with associated fibrohistiocytic reaction. Both patients had been confirmed to be taking oral iron supplements during the weeks prior their presentation. Following modifications in their medications both patients remained asymptomatic. Bronchial washings were also availble in one of the cases and demonstrated numerous crystalline particles within macrophages or free floating. Iron stains were positive in both the biopsy materials and the washings.

Conclusions: Aspiration of therapeutic oral iron supplements result in mucosal injuries that are histologically similar to the mucosal injury seen in the upper gastrointestinal tract with "iron pill" gastritis, which is an under-recognized pathologic diagnosis with important therapeutic implications for patients. These lesions can mimic neoplastic processes in the airways, particularly due to their ulcerative, friable and occasionally protuberant gross appearance. The presence of extracellular crystalline iron in the airway lumen as well as in the mucosa, associated with varying degrees of ulceration and inflammation, confirms the diagnosis of iron-induced injury even in patients with no known aspiration events.

1786 Neoangiogenesis in Pulmonary Adenocarcinoma: Stratification by Size of Invasive Component

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Background: Adenocarcinoma (AdCA) comprises ~40% of lung cancers, for which stage I-II patients undergo resection with curative intent; however, most patients present with metastatic disease, and prognosis is grim. Increased expression of vascular endothelial growth factor (VEGF) and its receptors (VEGFR) imparts a poor prognosis, as does increased CD105 microvascular density. Cases of bronchioloalveolar carcinoma (BAC) with a <5mm invasive AdCA center behave indolently. Those with invasive centers 5-10mm or >10mm behave aggressively. In this study we determined if the size of the invasive component and metastases correlated with angiogenesis.

Design: An IRB-approved 18-year retrospective search of our database identified 55 lung resection specimens with combined AdCA and BAC. Of non-metastatic cases, the largest diameter of the invasive AdCA component was measured, and cases were sub-categorized as <5mm, 5-10mm, or >10mm. Microarrays were created, including invasive center, peripheral BAC, uninvolved lung, and in metastatic cases, positive lymph node. Arrays were stained with H&E to verify histology of each 1 mm core and with immunohistochemical (IHC) stains VEGFR2, VEGFR2-3, and CD105. The intensity and distribution of each IHC stain for each core was graded and statistical analyses were performed.

Results: Both the invasive AdCA and BAC components of all tumors showed increasing VEGFR2 staining with increasing diameter of invasive center, with highest staining in metastatic tumors (p=<0.001 to 0.65). In metastatic tumors, there was no difference between the primary AdCA and its corresponding metastases. Within each group, there was no significant difference between the invasive AdCA and the BAC components. Staining for VEGFR2-3 demonstrated a slight trend toward increased expression with increasing diameter of invasive center, but to a lesser degree. CD105 staining was not statistically significantly different.

Conclusions: The expression of VEGFR2 in lung AdCA with BAC increases as the size of the invasive center increases, with the highest expression in metastatic tumors. This finding supports use of antiangiogenic drugs in AdCA, and suggests that the size of the invasive component may be a predictor of response to targeted therapy. The similar expression of VEGRF2 in primary and metastatic AdCA also suggests that tumor at both sites would be responsive to antiangiogenic agents.

1787 Signet Ring Cell Features (SRCF) in Lung Adenocarcinoma: A Cytologic Feature or a Histologic Subtype?

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Background: The 2004 WHO Classification recognizes SRC adenocarcinoma as an uncommon histologic variant of lung adenocarcinoma. The possibility of a molecular association was recently suggested between SRCF and EML4-ALK mutations. However, few studies have explored the pathologic spectrum and clinical significance SRCF in various adenocarcinoma subtypes.

Design: We reviewed 569 lung adenocarcinomas for the presence of signet ring cell features (SRCF) and recorded their presence in various histologic adenocarcinoma subtypes. The relationship of SRCF to clinical and pathologic features was analyzed using SPSS version 17 including crosstables with Chi-square statistics as well as survival using Kaplan Meier and Cox regression analysis.

Results: SRCF of at least 5% were found in 47 (8.3%) cases with 25F and 22M & mean age: 66 yrs (32-83). There was no significant difference in age or sex compared to non-signet ring cell cases (NSRC) cases. SRCF were found in 14/246 (5.7%) of acinar, 7/155 (4.5%) of papillary, 3/14 (21.4%) of micropapillary, 21/89 (23.6%) of solid and 2/10 (20%) of colloid predominant histologic subtypes. Only the association with SRCF and the solid subtype was significant (p<0.001). SRCF were not seen in tumors with predominant mucinous or non-mucinous BAC. SRCF was associated with a worse, 58% 5-yr disease free survival compared to 83% for NSRC cases (p<0.001). However, in multivariate analysis stratified for stage, including major histologic types and sex, only solid versus other histology and not SRCF was an independent prognostic predictor for poor survival. TTF1 was positive in 6/13 (46%).

Conclusions: In summary SRCF in lung adenocarcinoma occurs most often in association with the solid subtype but it can also be seen less often in the acinar, papillary and micropapillary patterns. The association of SRCF with poor prognosis appears to be due to its strong association with the solid subtype and it is not an independent prognostic factor. Consideration should be given to recognizing SRCF as a cytologic change that can occur in multiple histologic types, rather than a distinct adenocarcinoma subtype.

1788 Cytological, Histological, and Immunohistochemical Findings of Pulmonary Carcinomas with Basaloid Features

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Background: Pulmonary basaloid carcinoma (BC), a variant of large cell, non-small cell carcinoma (NSCC), and basaloid squamous cell carcinoma (BSQCC) can show features similar to small cell carcinoma (SCC) and large cell neuroendocrine carcinoma (LCNEC). Distinction from SCC, especially on FNA, is therapeutically relevant. We describe cytological, histological, and immunohistochemical features of BC and BSQCC.

Design: Cytologic features were documented in the cytologic preparations. Similar features and as well as architecture were evaluated in resections. Immunohistochemical results were recorded. Histologically confirmed BC (n=3) and BSQCC (n=3) were included. 5 SCC FNAs, 4 with histologic follow-up, were compared.

Results: BC/BSQCC FNAs: mostly tightly cohesive clusters (n=4) or single cells and in clusters (n=2) with a predominance of clusters. Cytologic features were similar: palisading (n=6), crush artifact (n=6), hyperchromasia (n=5), nuclear molding (n=6; focal/rare in 4/6), nucleoli, usually pinpoint (n=3), scant cytoplasm (n=6), necrosis (n=5), apoptosis (n=4), squamous differentiation (n=1). BSQCC tended to have occasional larger cells, including keratinizing cells in 1 case. Histologic sections: neuroendocrine architectural patterns, including organoid arrangements (n=5); palisading (n=5). SCC FNAs: cells predominantly single cells (n=3) or clusters and single cells (n=2), rare palisading (n=4), crush (n=5), hyperchromasia (n=5), nuclear molding (n=5; prominent in 3/5), absent/rare inconspicuous nucleoli (n=5), scant cytoplasm (n=5), necrosis (n=5), apoptosis (n=5). SCC histologic sections: solid sheets (n=3); organoid (n=1); palisading (n=1).

Immunostaining Profile of BC, BSQCC, and SCC

	CK7	p63	HMWCK	TTF-1	Synaptophysin	Chromogranin	CD56
BC							
1	+**	+	-	-	-	-	-
2	+			-	-*	-	+
3	+	+	+	-	-	-	+
BSQCC							
1	-*			-*	-*		
2	-		+	-	-		
3	+			-	-		
SCC							
1	+**			+**	-**	-**	+
2	-			+* (- on histo)	focal +* (- on histo)	-**	
3	+			+	focal weak+	+	
4	-			+	+	weak+	
5	+*	weak +*	-*	+*	+*	equivocal*	-*

*Immunostain on FNA; **Immunostain on both FNA and histology

Conclusions: BC and BSQCC show overlapping features with SCC and LCNEC in cytological and histological specimens. Unlike SCCs, BC/BSQCC lack prominent nuclear molding, show greater numbers of tightly cohesive clusters, and demonstrate palisading of cells along the periphery of nests. p63 (+), HMWCK (+), and TTF-1 (-) are helpful in distinguishing BC/BSQCC from SCC/LCNEC.

1789 Clinical Utility of Immunohistochemistry for the Diagnosis of Lung Squamous Cell Carcinoma in Biopsy and Cytologic Specimens

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Background: Oncologists are currently interested in distinguishing squamous cell carcinoma (SCC) from 'non-squamous' (non-SCC) lung cancer as histologic subtype is associated with efficacy and toxicity of emerging targeted or combination therapy regimens. SCC generally express CK5/6 and p63 while non-SCC stain for TTF-1 and Napsin-A. Desmoglein-3 (DSG3) is a recently described marker for SCC particularly for the lung.This study aims to evaluate the clinical utility of immunohistochemistry (IHC) using these antibodies in the distinction between SCC and non-SCC in small biopsy specimens.

Design: A total of 47 patients with resected lung SCC who had previous positive bronchial or core biopsy or fine needle aspiration (FNA) were identified from the files of 2 institutions. There were 13 bronchial biopsies, 23 core biopsies and 11 FNA. Formalin-fixed paraffin-embedded tissue sections of the biopsies or cell block were stained with p63 (Biocare), CK 5/6 (Cell Marque), DSG3 (Abcam), TTF-1 (Cell Marque)

and Napsin-A (Leica). Double immunostaining technique was used for p63/CK 5/6 and TTF-1/Napsin-A. 20 lung adenocarcinoma biopsies were stained for comparison. The extent of staining was evaluated as 1+ to 3+ (<25%, 25-50% and >50%) and the intensity as 0 to 3+.
Results: Biopsies and FNA were diagnosed as SCC based on histomorphology in 97% and 72% of cases, respectively. The use of IHC identified all SCC in all types of biopsy material based on positivity with at least one squamous marker. p63 was the most sensitive antibody for SCC (98%) followed by DSG3 (94%) and CK 5/6 (85%). DSG3 membranous staining tended to be variable and patchy compared with p63 or CK 5/6. Four cases of SCC (8.5%) expressed TTF-1 while none expressed Napsin-A. Of the 20 adenocarcinoma biopsies, 8 (40%) showed rare faint p63 staining. None of the adenocarcinoma cases showed staining for CK 5/6 or DSG3. Sensitivity and specificity for the diagnosis of SCC using DSG3, CK 5/6 and p63 were 94% and 100%, 85% and 100% and 98% and 60%, respectively.
Conclusions: Immunohistochemistry on small biopsy material, particularly on FNA with adequate cell block, can provide a definitive diagnosis of SCC. Although p63 is the most sensitive, DSG3 and CK 5/6 have higher specificities for diagnosing SCC. A panel of 2 squamous markers (DSG3 and CK 5/6) and 2 adenocarcinoma markers (TTF-1 and Napsin-A) is useful in distinguishing SCC from non-SCC in small biopsy specimens.

1790 2009 Pandemic Influenza A/H1N1 Viral Infection: Clinical and Pulmonary Pathological Findings in 34 Fatalities

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Background: In June 2009, World Health Organization (WHO) declared an influenza pandemic due to a novel swine-origin influenza A/H1N1 virus. By September 25, 2009, WHO reported 43,771 confirmed infections with 593 deaths in United States. We describe 34 deaths with confirmed influenza A/H1N1 virus infection investigated by New York City Office of Chief Medical Examiner and consultation service of a co-author.
Design: Autopsies were performed on all decedents. Clinical information was obtained from medical records and/or medicolegal investigation. Nasopharyngeal and tracheal swabs were analyzed for H1N1 viral RNA by real-time reverse-transcriptase polymerase chain reaction (rRT-PCR). Histopathologic and microbiologic evaluation was performed using H&E, B&H tissue Gram and GMS stains. Formalin fixed, paraffin embedded (FFPE) autopsy tissue sections were analyzed for influenza A virus matrix gene and H1N1 hemagglutinin gene by rRT-PCR. H1N1 viral antigen tissue localization was detected by immunohistochemistry (IHC).
Results: Majority (62%) of these decedents were 25-49 years of age. Tracheitis, bronchiolitis and diffuse alveolar damage (74%) were noted in most. Influenza viral antigen was observed by IHC, most commonly in the epithelium of tracheobronchial tree, but in some, also in alveolar epithelial cells, submucosal glands and macrophages. Histologic and microbiologic evidence of bacterial pneumonia was detected in 55% (most commonly *streptococcus pneumoniae*). Frequent symptoms were fever>38°C (94%), cough (91%) or dyspnea (73%). Comorbidities included obesity (72%), cardiorespiratory diseases, immunosuppressed states and diabetes mellitus with 91% having one or more.
Conclusions: Pulmonary pathological findings in fatal disease caused by the novel pandemic influenza virus are similar to those identified in past pandemics. Bacterial co-infections of the respiratory tract are common. Pre-existing obesity, cardiorespiratory disease and other comorbidites were present in majority.

1791 Molecular Epidemiology of *EGFR* and *KRAS* Mutations in Lung Adenocarcinomas Based on Clinical Testing of 2787 Consecutive Cases

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Background: Almost 50% of lung adenocarcinomas harbor *EGFR* or *KRAS* mutations but the etiology of most of these mutations is unclear, except for the subset of *KRAS* mutations known to be smoking-associated (G12C, G12V). Associations between types of somatic mutations and demographic and epidemiologic data can provide clues to distinct etiology or biology. Here, we took advantage of our clinical testing data on over 2700 lung adenocarcinomas studied since 2004 to examine correlations between types of *EGFR* or *KRAS* mutations and demographic data in unprecedented detail.
Design: Of 2787 cases tested clinically for the two major *EGFR* mutations (exon 19 deletions and the L858R mutation in exon 21) using sensitive mutation-specific PCR assays, 522 (19%) were mutant, including 320 exon 19 deletions (61%) and 202 L858R mutations (39%). Routine testing for *KRAS* mutations (codons 12 and 13) by PCR-sequencing (after microdissection if needed) started in 2006 and in 2200 cases received since, we detected 553 (25%) mutations, including G12C (37%), G12V (22%), G12D (18%), G12A (10%), and other G12 and G13 mutations (13%). We examined correlations with demographic data using the Fisher exact test or unpaired t-test.
Results: Comparison of *EGFR* and *KRAS* mutant cases showed differences in sex ratio (p=0.001; favoring males for latter) but not age. Among *EGFR* mutant cases, men showed a higher ratio of exon 19 to L858R than women (2.4 vs 1.4; p=0.007). Patients with *EGFR* L858R tended to be older than exon 19 mutants (average age 68 vs 64; p=0.0001), reflected by an exon 19 to L858R ratio of 3.5 under age 50 (p=0.008) and of 1.1 in patients 70 and over (p=0.002). Among *KRAS* mutant cases, compared to other *KRAS* mutations types, the sex ratio for G12C was skewed more towards women (p=0.006) and G12D, the *KRAS* mutation most often seen in never smokers, was relatively more common under age 40 (p=0.02). Notably, of 15 patients under age 40 with mutations, 7 had *EGFR* exon 19 deletion, 4 *KRAS* G12D, 2 *KRAS* G12V, 1 *EGFR* L858R, and 1 *KRAS* G12S.
Conclusions: The distinctive age distribution and sex ratio associations of certain *EGFR* and *KRAS* mutations may signal differences in etiology and/or biologic potential. For instance, the lower age of patients with *EGFR* exon 19 deletion could reflect slightly faster progression when untreated compared to *EGFR* L858R, as suggested by some studies. Annotation of this unique dataset for stage, smoking history, and ethnic origin is ongoing and should provide further insights.

1792 High Prevalence of Atypical Mesothelial Proliferation in Extrapleural Pneumonectomy Specimens; Further Evidence of a Potential Precursor Lesion to Invasive Mesothelioma?

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Background: Atypical mesothelial proliferation (AMP) is thought to represent a potential precursor lesion to invasive pleural mesothelioma. To our knowledge there is no published literature describing the clinicopathologic characteristics of AMP. The aim of this study was to evaluate the prevalence of AMP in extrapleural pneumonectomy (EPP) specimens for invasive mesothelioma and to correlate AMP with clinicopathologic features.
Design: We studied 46 consecutive EPPs with available surgical material (mean 22 slides per case, range 12-30), performed at a single institution for invasive mesothelioma (IM) over a period of 16 months. Each case was assessed independently by two pathologists for the presence or absence of AMP according to currently established morphologic criteria. We evaluated architectural and cytologic features, the prevalence and extent of AMP and correlated clinicopathologic features between mesotheliomas with and without AMP.
Results: All 46 EPPs showed invasive mesothelioma (n=30 epithelioid, n=15 mixed and n=1 sarcomatoid). There were 40 men (87%) and 6 women (13%), with an average age of 62.9 years (range 38-79). AMP was identified in 10 (22%) EPP specimens, in a mean of 3.5 slides (range 1-6). Nine cases (90%) were associated with epithelioid mesothelioma and 1 case (10%) with mixed mesothelioma. Common architectural patterns of AMP were a single cell layer proliferation (n=8), stratified proliferations (n=5) and papillary proliferations (n=5). Six cases (60%) had mixed AMP growth patterns. In AMP with a single cell layer proliferation, prominent nucleoli were present in at least 50% of lesional cells. AMP was present in EPPs with lower weights (median 747g vs. 1110g, p=0.03) and in older patients (68 vs. 63 years, p=0.02, 95% CI -15.38 to -1.586).
Conclusions: In our study we show that the prevalence of AMP in EPPs is higher than anticipated at 22%, and is more frequent in older patients and in specimens with lower weights. Further studies are needed to investigate the clinical significance of AMP and the role of AMP in the pathogenesis of mesothelioma.

1793 Usefulness of EZH2 and IMP3 in Discriminating High Grade Neuroendocrine Carcinoma from Carcinoids in Small Biopsies and Fine Needle Aspiration Specimens of the Lung

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Background: Enhancer of zeste homolog 2 (EZH2) is the catalytic subunit of polycomb repressive complex 2 with histone methyltransferase activity. Insulin-like growth factor-II messenger RNA-binding protein-3 (IMP3) is a 580 amino acid oncofetal RNA-binding protein containing 2 RNA recognition motifs and 4 K homology domains. Our previous studies showed both EZH2 and IMP3 are highly expressed in large and small cell neuroendocrine carcinoma (LCNEC, SCLC) of the lung but not in carcinoids using mainly resected specimens. The aim of this study was to determine if EZH2 and IMP3 are diagnostically useful in their ability to discriminate different neuroendocrine tumors of the lung (NETL) on small biopsies and fine needle aspiration specimens.
Design: Fourteen cases of small biopsies (n=11) and fine needle aspiration specimens (n=3) of NETL, including 5 typical carcinoids (TCs), 1 atypical carcinoid (AC), 2 LCNECs and 5 SCLCs, were immunohistochemically studied using a monoclonal antibody against IMP3 (Dako) and a monoclonal antibody against EZH2 (Leica). The majority of these diagnoses were confirmed on resection. Cytoplasmic staining was considered positive for IMP3 and nuclear staining was considered positive for EZH2. The percentage of positively stained tumor cells was recorded and the staining intensity was graded as weak, moderate, or strong. A p value of <0.05, as determined by two-tailed Fisher exact test, was considered statistically significant.
Results: Immunohistochemical studies showed strong and diffuse EZH2 and IMP3 staining in all 2 LCNECs and 5 SCLCs. Among them, 4 cases had severe crush artifact. Conversely, there was no EZH2 staining in all 5 TCs and the 1 AC. There was weak IMP3 staining in 30% of tumor cells in 1 (20%) of 5 TCs. There was no IMP3 staining in the 1 AC case. EZH2 and IMP3 expression was significantly higher (p<0.01) in high grade neuroendocrine carcinoma than carcinoids (100%, n=7 and 100%, n=7 versus 0%, n=6 and 17%, n=6, respectively).
Conclusions: Our study demonstrates the usefulness of immunohistochemical detection of EZH2 and IMP3 in aiding in the discrimination of high grade neuroendocrine carcinoma from carcinoids in small biopsies and fine needle aspirations specimens of the lung which may have limited diagnostic material present or which may have a severe crush artifact.

1794 Napsin A Is Frequently Expressed in Pulmonary Adenocarcinoma but Rarely Detected in Neuroendocrine Tumors of the Lung

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Background: Napsin A is an aspartic proteinase that is involved in the maturation of surfactant B protein. Napsin A has been shown to be highly expressed in pulmonary adenocarcinoma and papillary renal cell carcinoma and occasionally detected in thyroid carcinoma. There are only a few reported cases of carcinoids and small cell lung

carcinomas (SCLCs) that were immunohistochemically studied with Napsin A. The aim of this study was to determine if neuroendocrine tumors of the lung (NETL), including typical carcinoid (TC), atypical carcinoid (AC), large cell neuroendocrine carcinoma (LCNEC) and SCLC, immunohistochemically express Napsin A.
Design: Forty-six surgically resected NETL including 14 TCs, 12 ACs, 11 LCNECs, and 9 SCLCs and a tissue microarray of 148 cases of adenocarcinoma of the lung were immunohistochemically studied using a monoclonal antibody against Napsin A (Leica). Cytoplasmic staining was considered positive. The percentage of positively stained tumor cells was recorded and the staining intensity was graded as weak, moderate, or strong. A case was considered positive if more than 5% of tumor cells had staining. Pathologic diagnosis was confirmed, with the use of prior immunohistochemical and mucicarmine stains. A p value of <0.05, as determined by two-tailed Fisher exact test, was considered statistically significant.
Results: Immunohistochemical studies showed all 12 cases of AC and all 9 cases of SCLC were negative for Napsin A staining. There was positive Napsin A staining in 1 (7.1%) of 14 TCs, with only weak staining in 30% of cells. There was positive Napsin A staining in 1 (9.1%) of 11 cases of LCNEC, with diffuse and strong positivity in greater than 90% of tumor cells. Napsin A positivity was detected in 126 (85.1%) of 148 cases of lung adenocarcinoma, including 23 (88.5%) of 26 well-differentiated, 68 (90.7%) of 75 moderately-differentiated and 35 (74.5%) of 47 poorly-differentiated adenocarcinomas. In non-neoplastic lung, Napsin A staining was present in type II pneumocytes.
Conclusions: Napsin A is rarely expressed in NETL. In contrast, it is expressed in a majority of pulmonary adenocarcinomas. These findings support the concept that the cell origins of adenocarcinoma and NETL are different. In addition, immunohistochemical detection of Napsin A expression may serve as a useful diagnostic tool in the distinction between NETL and poorly-differentiated pulmonary adenocarcinoma, in particular when the diagnostic tissue is limited in a small biopsy with a crush artifact.

1795 IMP3: An Immunohistochemical Marker for Invasive Squamous Cell Carcinoma and Its High-Grade Precursor Lesions of the Lung

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Background: Insulin-like growth factor-II messenger RNA-binding protein-3 (IMP3) has been reported to be expressed in multiple malignant neoplasms, but with only limited studies in squamous cell carcinoma (SCC) of the lung and none examining IMP3 expression in squamous precursor lesions. The aim of this study was to examine IMP3 expression in squamous precursor lesions including basal cell hyperplasia (BCH), squamous metaplasia (SM), low grade dysplasia (LGD), high grade dysplasia (HGD), and squamous cell carcinoma in situ (CIS).
Design: A total of 97 resected lung SCCs were examined for squamous precursor lesions including BCH, SM, LGD, HGD and/or CIS. Thirteen cases with such changes were identified on H&E-stained sections. Sections with these precursor lesions and tissue microarrays constructed from 97 invasive SCC cases were immunohistochemically studied using a monoclonal antibody against IMP3 (Dako). Cytoplasmic staining 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, as determined by two-tailed Fisher exact test was considered statistically significant.
Results: Eighty-six (88.7%) of 97 SCCs were IMP3 positive with predominantly strong and diffuse staining. In 13 cases with precursor lesions, there were 1 BCH, 2 SM, 3 LGD, 9 HGD and 10 CIS. All 9 cases with HGD had positive IMP3 staining in the HGD area, with moderate to strong staining in 7 (77.8%) of 9 cases. All 10 cases with CIS had positive IMP3 staining in the CIS area, with moderate to strong staining in 8 (80.0%) of 10 cases. In 1 (33.3%) of 3 cases with LGD there was weak staining in <5% of cells. There was no IMP3 staining in normal bronchial epithelium, BCH or SM. The difference in IMP3 staining between low risk precursor lesions (BCH, SM and LGD) and high risk precursor lesions (HGD and CIS) was statistically significant (p<0.01).
Conclusions: IMP3 is expressed in a majority of cases of HGD, CIS and invasive SCC of the lung. These findings indicate that IMP3 may play an important role in the initiation and progression of pulmonary SCC. Additionally, IMP3 may have utility in small biopsy or fine needle aspiration specimens to highlight HGD and CIS. The identification of these cells is critical as patients with such lesions are at a higher risk of developing invasive SCC.

1796 Malignant Mesothelioma of the Pleura with Pleomorphic Features: A Series of 44 Cases

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Background: Malignant mesothelioma [MM] is an heterogeneous cancer showing low grade cytologic atypia to extreme pleomorphism with bizarre cells that should not be dismissed as large cell carcinoma or metastatic sarcoma. We present a study of 44 MM with pleomorphic features, the largest series to date.
Design: All cases were retrieved from the MESONAT registry from 1998 to 2006. Clinical histories, radiological reports, follow up and demographic data were recorded using a structured questionnaire. All lesions were reviewed by the MESOPATH panel according to their standardized procedure of certification. Cases fulfilling the following criteria were included: diffuse proliferation of large bizarre/giant cells with pleomorphic nuclei, a sarcomatoid component of less than 10%. The immunohistochemical profile was a broad spectrum cytokeratins positivity, calretinin+ve/WT1+ve/CK5/6 +ve with a cut off >20% and negativity of p63, BerEP4, TTF-1, CEA, CD34, CD68, CD45. Statistical analysis were performed using Kaplan Meyer and cox model and survival was compared to a series of 1521 of non pleomorphic MM.
Results: There were 44 patients with an average age of 69 years old (range 50 to 69 years), with a M/F ratio 4.5/1, respectively 82% (n=36) for male and 18% (n=8) for female. A history of asbestos exposure was observed in 66 % of patients. Clinical data showed pain (42%), fatigue (54%), loss of weight (15%), dyspnea (27%). The initial presentation was: unilateral pleural effusion in 77% of cases, a diffuse pleural thickening in 54%, and a localized pleural based mass in 16%. Hyalin fibrous plaques were present in 4% of cases. The median survival of MM with pleomorphic features was 7 months with a survival at 2 years of 8% [0%;16%] compared respectively to 13 months and 18% for epithelioid type and 5 months with 9% survival at 2 years for sarcomatoid type. There was a significant difference at risk between MM with pleomorphic features compared to conventional epithelioid type (p= 0.001) and sarcomatoid type (p=0.097).
Conclusions: The findings suggest that MM with pleomorphic features is a distinct morphological variant of epithelioid (p=0.001) and sarcomatoid (p=0.097) MM.

1797 Immunohistochemical Study of mTOR Pathway Activation in Lymphangioleiomyomatosis

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Background: Lymphangioleiomyomatosis (LAM) is a rare and fatal lung disease affecting women in their reproductive age. LAM is characterized by proliferation of infiltrative smooth muscle (SM)-like cells that lead to cystic destruction of the lung and eventually respiratory insufficiency. Tuberous Sclerosis Complex 1 and 2 (TSC1/TSC2, also known as hamartin and tuberin) form a tumor suppressor dimer that negatively regulates the mammalian target of rapamycin (mTOR)/S6K1 signaling pathway. Since LAM occasionally develops in the background of tuberous sclerosis (TS) and since *tsc*2 functional mutations and loss of heterozigosity (LOH) have been found in the lesions of several patients with sporadic LAM, it is currently believed that the disease is caused by lack of functional tuberin. To better understand the role of TSC1/TSC2/mTOR/S6K1 pathway in LAM, we applied IHC to determine whether there is a correlation between presence and level of tuberin, hamartin, phosphorylated mTOR and phosphorylated S6K1 kinase.
Design: Eight sporadic and one TS-associated LAM cases were included in this study. FFPE sections from diagnostic wedge biopsies were immunostained with antibodies against SM actin and HMB45 (LAM markers), tuberin, hamartin, phosphorylated mTOR and S6K1 antibodies. Tissue array sections and normal lung were used as external controls. The immunostaining intensity of LAM lesions and normal lung tissue was classified from +++ (strongest) to - (negative).
Results: Normal lung parenchyma and all sporadic LAM lesions immunostained for tuberin and hamartin. The lung sample from the TS case was entirely negative for tuberin and positive for hamartin. In LAM lesions tuberin and hamartin immunoreaction was either focal or diffuse and of intermediate in intensity (+/++), but diffuse and strong in reactive epithelial and mesothelial cells (+++) and negative (-) in blood vessels. In five sporadic cases and the TS-associated case, the LAM lesions were focally and weakly positive for phosphorylated mTOR (+) and/or S6K1 (+). In the other three sporadic cases the LAM lesions were negative for both. Epithelial and mesothelial cells were strongly positive for both (+++) and blood vessels were negative (-).
Conclusions: Tuberin and hamartin were present in the lesions of sporadic LAM, but the TS-associated case lacked tuberin altogether. There was weak mTOR pathway activation in 5 cases and no activation at all in 3 cases. A weak but protracted activation of the mTOR pathway may contribute to the pathogenesis of LAM in some cases, while it does not seem to play a role in others.

1798 Mutations in the *tsc2* Gene Do Not Always Play a Role in the Pathogenesis of Sporadic Lymphangioleimyomatosis

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Background: Lymphangioleiomyomatosis (LAM) is a rare and fatal lung disease affecting women predominantly of child-bearing age. Mutation of one *tsc2* (tuberin) allele, preceded or followed by LOH (loss of herterozygosity) of the other allele, is currently considered to cause sporadic LAM. Therefore it is proposed that the lack of functional tuberin, leading to phosphorylation and activation of the mTOR (molecular target of rapamycin)/S6K1 signaling pathway, is essential to the pathogenesis of LAM disease. The propose of this study is to assess the roles of *tsc2* mutations or LOH and mTOR/S6K1 signaling pathway in the pathogenesis of sporadic LAM.
Design: DNA samples prepared from LAM lesions laser-microdissected from frozen lung tissue of 10 patients with sporadic LAM were studied for LOH by MLPA (multiplex ligation-dependent probe assay) and 7 of them for mutations in *tsc2*. These 7 samples were subject to whole genome amplification, and analyzed by deep sequencing of all *tsc2* exons using the Roche-454 platform. In addition, serial sections of FFPE lung tissue sampled from 3 of the sequenced cases were immunostained for LAM markers SM actin and HMB 45, tuberin C-terminus (missed in mutated/truncated tuberin), phosphorylated mTOR and S6K1.
Results: None of the 10 cases showed LOH. Five of the 7 samples sequenced showed one tsc2 mutation. One sample had two different mutations. The mutation frequency was below 10% in all but one case, in which it was 46%. One of the cases without tsc2 mutations (0M), a case with one tsc2 mutation (1M) and the case with two different tsc2 mutations (2M) were selected for IHC studies. Case 0M showed diffuse immunoreactivity for tuberin in LAM lesion, which is similar to that of normal alveoli, reactive epithelial and mesothelial cells (+++), but no immunoreactivity for phosphorylated mTOR and S6K1. Case 1M showed diffuse LAM lesion immunoreactivity for tuberin (+++), while in case 2M positivity for tuberin was focal (++). M1 and M2 cases showed focal immunoreactivity for phosphorylated mTOR and S6K1, which was (+) compared to (++++) in reactive epithelial and mesothelial cells and (-) in normal alveoli. In all cases vascular SM were negative (-) for tuberin, phospho-mTOR and phospho-S6K1.
Conclusions: Sporadic LAM does not require *tsc2* LOH to develop. Furthermore, some LAM cases have no abnormalities in either of the two *tsc2* alleles. Activation of the mTOR/S6K1 pathway does not always play a role in the pathogenesis of sporadic LAM.

1799 Cathepsin K Expression in Benign Clear Cell "Sugar" Tumor of the Lung

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Background: Cathepsin K is a papain-like cysteine protease that recently has been shown to be constantly, strongly and diffusely immunoexpressed in lymphangioleiomyomatosis. Lymphangioleiomyomatosis can be considered as part of the spectrum of proliferative lesions that have been previously defined under the name of "perivascular epithelioid cell tumours" (PEComas), including angiomyolipoma, clear-cell "sugar" tumour of the lung and extrapulmonary sites. The aim of this study is to evaluate Cathepsin K immunoexpression in clear-cell "sugar" tumour of the lung.

Design: We collected 3 clear-cell "sugar" tumours of the lung analysing the immunoexpression of cathepsin-k. We tested also Cathepsin K expression in 20 pulmonary adenocarcinomas, 20 squamous cell carcinomas of the lung and in 20 clear cell renal cell carcinomas.

Results: All the clear-cell "sugar" tumours were diffusely and strongly immunoreactive for cathepsin-k whereas none pulmonary adenocarcinoma, squamous cell carcinoma of the lung and clear cell renal cell carcinoma showed immunoreaction for this marker.

Conclusions: We demonstrated that: 1) Cathepsin K is constantly and strongly expressed in benign clear cell "sugar" tumor of the lung and can be useful in their differential diagnoisis with other primary and metastatic epithelial neoplasms of the lung. 2) The expression of Cathepsin K is an additional proof of the close relationship between benign clear cell "sugar" tumor and lymphangioleiomyomatosis of the lung.

1800 Immunohistochemical Detection of Monocarboxylate Transporters (MCT) and GLUT-1 in Pleural Malignant Mesothelioma and Reactive Mesothelial Hyperplasia: A Useful Tool for the Differential Diagnosis

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Background: Malignant tumor cells are highly proliferative and dependent on glycolysis for energy. Glucose transporter (GLUT)-1 enhances glucose influx in most human tumors. On the other hand, glucose metabolism via anaerobic glycolysis results in the production of large quantities of lactate, which cause intracellular acidosis and thus should be pumped out of cells by monocarboxylate transporters (MCT). Malignant mesothelioma (MM) is a highly aggressive neoplasm, and early diagnosis is critical for its treatment. However, differentiation of MM from reactive mesothelial hyperplasia (RMH) is sometimes difficult especially in small biopsy specimens. In this study, we examined whether immunohistochemical detection of MCT-1, MCT-4 and GLUT-1 could be a useful aid in differentiation between MM and RMH.

Design: We examined immunohistochemical expressions of MCT-1, MCT-4 and GLUT-1 in 34 cases of MM (22 epithelioid, 4 sarcomatoid, 7 biphasic, and 1 lymphohistiocytoid) and 20 cases of RMH.

Results: GLUT-1 expression was seen in 56% of MM and none of RMH. However, the expression rates (% of positive cells) were low: positive cells were less than 50% in 84% of the positive cases. MCT-1 was positive in all MM, and 79% of MM vs 15% of RMH showed high expression (≥50%) scores. MCT-4 expression was found in 88% of MM and none of RMH. Seventy-three percent of the positive cases showed high expression rates.

Conclusions: Combined immunohistochemical detection of MCT-1, MCT-4 and GLUT-1 is more sensitive and specific than that of GLUT-1 alone to descriminate between MM and RMH, and useful in early diagnosis of MM in small surgical specimens.

1801 Expression of KiSS-1 and MMP-9 in Non-Small Cell Lung Cancer and Their Relations to Metastasis and Prognosis

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Background: Non-small cell lung cancer (NSCLC) is a leading cause of death. Patients frequently present with advanced stage disease. KiSS-1 and MMP-9 have been reported to play important roles as metastasis suppressor and metastasis promoter genes, respectively, in a variety of malignancies. However, there is little information about their possible roles in NSCLC.

Design: The expressions of KiSS-1mRNA and protein, and MMP-9 protein were detected by in situ hybridization and immunohistochemistry respectively in 85 cases, including 56 cases of paired lymph node metastases. Integral optical density (IOD) was determined by image analysis system.

Results: The expressions of KiSS-1 mRNA and protein were significantly higher in low TNM stages of NSCLC compared more advanced stages. In advanced TNM stages, cases without metastasis have higher KiSS-1 gene expression compared to those with lymph node metastases. KiSS-1 expression is also higher in the primary tumors compared to the secondary tumors. In contrast, MMP-9 protein expression was significantly higher in tumors with a diameter > 3cm compared to tumors ≤ 3cm in diameter. In addition, MMP-9 expression was higher in stage III-IV cases compared to stage I-II tumors and higher in cases with metastasis than without metastasis. Furthermore, MMP-9 expression was significantly higher in the secondary tumors compared to the primary tumor. These findings indicate that the expression of KiSS-1 protein has a negative relation to MMP-9 protein in NSCLC. The 5-year survival rate in the cases with higher KiSS-1 protein expression were significantly higher than those with lower expression. However, the 5-year survival rates of cases with higher MMP-9 protein expression were lower than those with lower expression.

Conclusions: We conclude that KiSS-1 expression is increased in NSCLC, particularly in patients without metastasis and those with low-stage disease. Our results also suggest that the invasion and metastasis of NSCLC may be related to the levels of KiSS-1 and MMP-9. Metastatic progression of NSCLC appears to be promoted by down-regulation of KiSS-1 and up regulation of MMP-1, whereas the opposite expression patterns inhibit metastatic progression. Hence, these markers could serve as a potential targets for the development of lung cancer therapies in the future.

1802 Gene Expression Profiling of the Tumor Microenvironment during Non Small Cell Lung Carcinoma (NSCLC) Progression

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Background: Genes involved in tumor-microenvironment interactions may provide novel targets for diagnostic development and therapeutic strategy. Our understanding of the interactions between epithelial and stromal components of NSCLC, however, remains limited at the molecular level. We conducted in this study a comparative analysis of global gene expression in the stromal compartiments of NSCLC and healthy lung tissues.

Design: We combined laser capture microdissection (LCM) and gene expression microarrays to analyze 18 patient-matched normal stroma and tumor-associated stroma frozen specimens. Briefly, highly enriched populations of normal stroma (from peribronchial areas) and tumor-associated stroma were procured by LCM using a PixCell II system (Molecular Devices, USA). Total RNA was isolated from captured cells, amplified, labeled and hybridized to whole genome arrays containing 25.484 distinct oligonucleotide probes covering most of the known human transcripts (list of probes available at http://www.microarray.fr). Data were processed using the Bioconductor package with default parameters for back ground correction, quantile normalization and signal summation. Differential gene expression analyses were performed using linear regression models in the limma package. TaqMan real time PCR was performed on amplified RNA used for microarray analysis.

Results: Highly upregulated genes in the tumor-associated stroma include constituents of the matrix metalloproteases and extracellular matrix, cell-cycle-related genes, and different receptors. Among the top100 genes differentially expressed in the tumor associated stroma, *MMP11*, *MMP7*, *SPARC*, *STAT3*, *semaphorin 3B*, *decorin*, *a2 smooth muscle actin*, *VEGF, CXCL9, fibronectin 1* were particularly upregulated in comparison to the level in normal matched stromal tissues.

Conclusions: The present study provides the first comparative analysis of the *in situ* gene expression profiles of patient-matched normal stroma and stromal compartments of invasive stages of NSCLC. This study supports the view that the tumor microenvironment is an important co-conspirator rather than a passive bystander during tumorigenesis. Moreover, molecular alterations within the stroma offer novel avenues for therapeutic strategies and disease prognosis

1803 Detection of Circulating Tumor Cells (CTCs) in Patients Undergoing Surgery for Non-Small Cell Lung Cancer (NSCLC). A Pilot Study Comparing an Indirect Technique, the CellSearch System, and a Cytomorphological Approach, the Isolation by Size of Epithelial Tumor Cells Method

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Background: Despite recent advances in the management of patients developing NSCLC, the cure rate of these patients remains low. The prognosis is largely determined by the occurrence of distant metastases. This relapse can be mainly caused by clinically occult micrometastasis present at primary diagnosis. In this regard, early detection of circulating tumor cells (CTCs) before surgery might help to improve therapeutic strategy. Currently, there is a lack of data comparing direct and indirect methods for CTCs detection performed from the same cohort of patients undergoing surgery for NSCLC. We have conducted this study to look for the presence of CTCs in peripheral blood of patients with NSCLC before surgery by using an indirect method of detection, the CellSearch (CS) system (Veridex), and a direct method of detection, the Isolation by Size of Epithelial Tumor (ISET) cells technology (Metagenex).

Design: A total of 114 consecutive patients undergoing surgery for NSCLC and 30 healthy individuals were included in this study. Presence of CTCs was evaluated at the same time by the CS (using the CellSearch Epithelial Cell Kit) and by the ISET [using a double immunolabeling with anti-pan-cytokeratin (KL1) and anti-vimentin antibodies] technologies.

Results: CTCs were detected in 73/114 (64%) patients using CS and/or ISET. CTCs were detected in 30/114 (26%) and in 62 /114 (54%) patients using ISET and CS, respectively. 19/114 patients (17%) showed CTCs detected both by CS and ISET. However, 18/114 patients (16%) had vimentin positive cells of uncertain origin detected by ISET method only. The presence and the number of CTCs detected by these 2 methods was independent of the pTNM staging and the histological subtypes. Subjects of the control group had no positive individual detected by ISET and by CS.

Conclusions: When using CS and ISET methods, CTCs can be detected before radical surgery in NSCLC patients in a high number of cases. However, only 17% of NSCLC patients had simultaneous detection of CTCs by ISET and CS, underlying that these two methods could be complementaries to detect CTCs preoperatively in NSCLC.

1804 Is Low-Grade Lymphomatoid Granulomatosis an IgG4-Related Interstitial Lung Disease?

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Background: Lymphomatoid granulomatosis (LYG) of the lung is a rare B-cell lymphoproliferative disorder, that is usually Epstein-Barr virus (EBV)-positive, often

accompanied by an exuberant reactive perivascular infiltrate with a predominance of T-cells, and a variable number of plasma cells. Recently Yamashita et al (Am J Surg Pathol 2008;32(11):1620-6) described 3 cases of lung involvement by IgG4-related disease showing a histology reportedly indistinguishable from grade 1 lymphomatoid granulomatosis (LYG-G1). These authors suggest that given the absence or paucity of EBV-positive cells, atypia, and monoclonality, what has been described as LYG-G1 may not be part of the spectrum of LYG-G2/G3 and may instead correspond to IgG4-related lung disease. The aim of this study was to establish if cases of low grade LYG diagnosed at our institution would fulfill the criteria for IgG4-related lung disease.

Design: All cases diagnosed as pulmonary LYG-G1 or LYG-G2 or IgG4-related lung disease in our institution between 1996-2009 were retrieved through an electronic database search, and the slides reviewed. Diagnosis and grading of LYG were performed according to the 2008 WHO criteria. CD138, IgG4, CD20, and CD3 immunostains as well as EBER ISH were performed on all cases. The density of plasma cells was estimated by counting the number of CD138+ plasma cells in four 40x field "hot-spots". The number of IgG4-staining cells was counted in the same spots and the ratio of IgG4/CD138-staining plasma cells was calculated.

Results: 6 cases of pulmonary LYG (3 grade 1, 3 grade 2) from patients aged 48-80, M/F=2/4 and one case of IgG4-related lung disease were identified. The IgG4-related lung disease had a histologic pattern similar to LYG-G1. No IgG4-positive plasma cells were identified in any of the LYG cases but over 30% of plasma cells were IgG4+ in the single case diagnosed as IgG4-related lung disease.

Age/Sex	Entity	EBER/HPF	Clusters of large B-cells	CD138 count	IgG4/CD138
65 M	IgG4-related	0	no	174	32%
65 F	LYG-G1	<3	no	1	0
53 M	LYG-G1	<3	no	42.5	0
80 F	LYG-G1	1	no	67.5	0
48 F	LYG-G2	19	no	72.5	0
64 F	LYG-G2	10	yes	13	0
65 M	LYG-G2	15	yes	45	0

Conclusions: Our results support the existence of LYG-G1 as an entity separate from IgG4-related lung disease. However, in cases of LYG-G1 pattern that are EBV negative, a IgG4 immunostain should be performed to evaluate for the possibility of IgG4-related lung disease.

1805 Increased Length of Survival after Lung Transplantation Is Not Dependent on Patient Age at Transplantation – Analysis of 625 Cases from a Single Center

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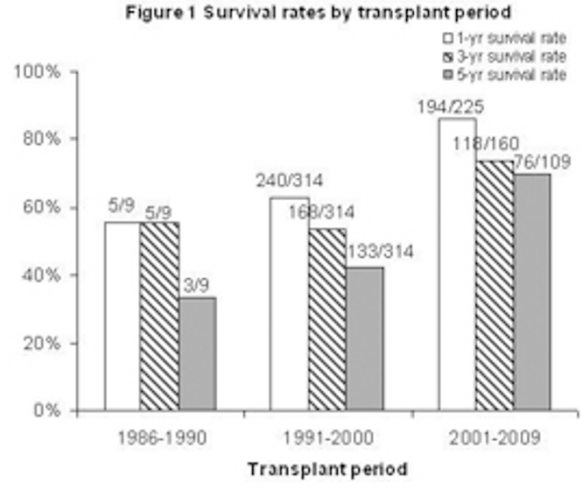
Background: Long term survival post lung transplantation (tx) has been increasing with improvments in surgical techniques and immunosuppressive therapies but there is a paucity of data regarding other factors that may determine the outcome.

Design: We analyzed retrospectively 625 patients who underwent lung tx at our institution between 1986 and 2009. To evaluate the trend in survival rate, we grouped the patients chronologically into 3 cohorts: 1986-1990, 1991-2000, and 2001-2009. In order to determine if the recipient's age influenced the outcome of lung tx, the patients were categorized into two groups, Group A (<40 years) and Group B (>=40years). Chi-square test was used to compare the difference in survival rates in different time intervals. Survival estimates were calculated according to the Kaplan-Meier method and curves were compared by the Wilcoxon and log-rank tests.

Results: There were 303 single-, 296 bilateral- and 26 heart-lung tx. The mean age of the recipients was 47.46 ± 13.66 years. Over the three periods studied, there had been a significant improvement in the 1-, 3- and 5-year survival rates for lung tx ($p<0.05$).



However, there was no signficant difference in survival between younger (< 40 years old) versus older patients (<40 years old) in all three time intervals. Kaplan-Meier survival curves showed no significant difference in post-tx survival time in patients who were < 40 years old versus recipients who were >=40 years (log rank, p =0.19; Wilcoxon, p=0.36).

This suggests that the age of lung recipients per se does not affect the outcome of lung tx.

Conclusions: Survival post lung tx has improved over the last decade. However, younger age of lung tx recipients is not associated with a better outcome of lung tx. Conversely, older patient age, per se, does not appear to affect survival outcome post lung tx.

1806 Dual Effect of Carbonic Anhydrase (CA) Isoforms IX and XII on Non Small Cell Lung Carcinoma (NSCLC) Progression. A Clinico-Pathological Study from 555 Patients from a Single Institution (CHU of Nice, France)

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Background: Adaptation of tumor cells to hypoxia is a critical driving force in tumor progression and metastasis. The expression of the membrane associated CAIX and CAXII is tightly controlled by oxygen levels in multiple epithelial tumor types, including lung cancer. Whereas tumor expression of CAIX in many tissues correlates with poor patient survival, the significance of CAXII has been poorly investigated.

Design: TMA-immunohistochemistry analysis was used to study CAIX and CAXII expression in 555 NSCLC, including 281 adenocarcinomas (AC), 184 squamous cell carcinomas (SCC), 43 large cell carcinomas (LCC), and 47 other tumor subtypes (NOS). Membrane immunoreactivity of each protein was assessed with a semi-automated tissue arrays image-analysis workstation (Spot Browser V7, Alphelys) in all tissue cores. An ELISA assay (R&D Systems) was performed to measure CAIX serum level in 208 of these patients and in 57 healthy individuals. Results were compared with clinicopathologic variables and clinical outcome.Statistical correlations were made with R software Windows version 2.8.1.

Results: CAIX overexpression was noted in 133/555 (24%) cases and correlated with tumor type [37% SCC vs 36% LCC vs 32% NOS vs 13% AC, p= 0.016] and shortened overall survival (p=0.022). The mean serum CAIX level in NSCLC patients was higher (45.4±4.81 pg/ml) than in healthy individuals (2.47±0.63 pg/ml). High CAIX serum level was related to tumor size (p=0.048), high rate of local recurrence (p=0.03), poor overall (p=0.017) and disease-free survival (p=0.047). CAXII overexpression was observed in 104/555 (18%) cases and correlated with tumor type [34% SCC vs 13% NOS vs 11% LCC vs 11% AC, p<0.001], pTNM staging [30% II stage vs 16% I stage vs 16% III stage vs 14% IV stage, p=0.011], and longer disease-free survival (p=0.0001). On multivariate analysis, CAIX and CAXII expression independently predicted patient survival.

Conclusions: These data indicate that CAIX is a strong predictor of recurrence, progression and poor overall survival of patients with NSCLC. Conversely, overexpression of CAXII is a favorable prognostic factor in NSCLC. Altogether, these results call for the development of new specific anti-CA drugs, taking into consideration the CA isoform profile of a given tumor subtype.

1807 Alveolar Trapping in Non Small Cell Carcinoma of Lung

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Background: Histologic features have been studied as prognostic factors in non small cell lung carcinomas (NSCC). Alveolar trapping (AT) inside the tumor is a known phenomenon but its relationship with other features and survival has been very little studied. We study it in a series of NSCC.

Design: We evaluated AT in 552 NSCC from two hospitals. It was graded as scanty (1), moderate (2) or abundant (3); extension of the trapping as peripheral (1), between the periphery and 2 mm inside the neoplasm (2) and more than 2 mm inside. Stroma (1 scanty, 2 moderate and 3 abundant), necrosis (0 absent, 1 scanty, 2 moderate and 3 abundant) and growing into adjacent alveoli (1 present, 2 absent) were evaluated. We have follow-up of 473 of the 552 cases (median 51 months). We compare these histological features between them, with histologic type and 5 year global survival.

Results: 260 of the 552 cases were squamous cell carcinomas (SC), 220 adenocarcinomas (AC), 32 large cell (LCC), 18 bronchioloalveolar (BAC), 18 adenosquamous (ASC), 1 mixed AC and small cell and 2 unclassified (UCC). AT was found in 141 cases (27%)

[118 SC, 11 AC, 6 LCC, 5 ASC and 1 UCC]. In 35 (24.82%) AT was scanty (1), in 54 (38.29%) grade 2 and in 52 (36.87%) grade 3; the extension in 11 (7.8%) peripheral (1), in 22 (15.6%) grade 2 and in 108 (76.59%) grade 3. The stroma was scanty (1) in 24 cases (17.02%), (2) in 91 (64.53%) and (3) in 26 (18.43%). Necrosis was absent (0) in 10 (7.09%), (1) in 30 (21.27%), (2) in 55 (39%) and (3) in 45 (31.91%). AT is more common in SC ($p<0.0001$). Grade and the extension were related between them ($p<0.0001$), necrosis was more abundant in cases with AT ($p<0.0001$) and cases with AT showed more often growing into adjacent alveoli ($p<0.0001$). We have data of survival in 473 cases (106 with AT). Patients with scanty AT had worse 5 year survival ($p=0.046$). Likewise patients with only peripheral AT ($p=0.02$). At the multivariate study cases with AT grade 1 versus grade 2 and 3 show HR (increase of mortality) 1.97 (CI95% 1.020-3.834) ($p=0.044$).

Conclusions: Alveolar trapping is common in NSCC, more frequent in SC and in cases with necrosis and alveolus to alveolus spreading. The grade and extension of AT were significantly related. Patients with abundant and extensive AT have better survival.

1808 Elastic Tissue Stain: A Valuable Diagnostic Tool for the Assessment of Visceral Pleura Invasion in Patients with Non-Small Cell Lung Cancer

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Background: Breaching of the visceral pleura by tumor cells has long been known to negatively impact the outcome of patients with lung cancer. A recent proposal for the classification of visceral pleural invasion (VPI) defines VPI as invasion beyond pleural elastic lamina (PL1), with PL2 signifying tumor at the pleural surface and PL3 denoting tumor in chest wall tissue. This study was undertaken to test the above cited classification proposal and to determine the potential staging value of elastic tissue staining in a cohort of 96 surgically resected patients with non-small cell tumors 3 cm or less in diameter, initially staged as $T_1N_0M_0$ on the basis of H&E stain alone.

Design: All of the 96 evaluated tumors were subpleurally located but actual distances of tumors to the pleural surface varied considerably requiring segregation of the tumors into 2 groups: less than 0.2 cm and 0.2 cm or more. Breaching of elastic lamina was independently evaluated by 3 observers. Unanimity was defined as uniformity of result by 3 observers. Resolution of discrepancies was achieved by subsequent joint review.

Results: Consensus diagnoses were achieved in all 96 cases by initial unanimity in 57 cases or subsequent joint review in 39 cases. Among the 96 cases, 72 (75%) were classified as PL0 requiring no TNM upstaging. However, 18 (18.75%) were classified as PL1 requiring upstaging to $T_2N_0M_0$.

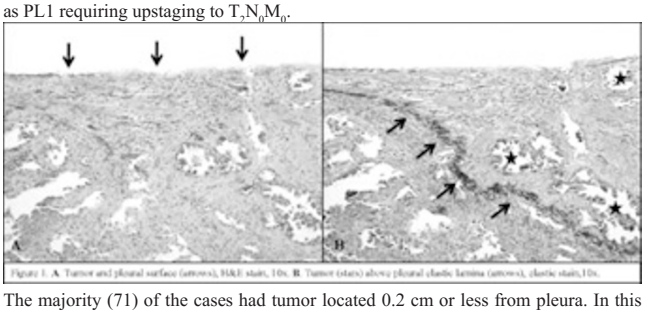

Figure 1. **A**. Tumor and pleural surface (arrows), H&E stain, 10x. **B**. Tumor (stars) above pleural elastic lamina (arrows), elastic stain,10x.

The majority (71) of the cases had tumor located 0.2 cm or less from pleura. In this subset of patients the proportion of understaged tumors was greater (25.35%). Only one patient was classified as PL2, and five difficult to categorize cases were classified as PLX (5.2%).

Conclusions: Our study suggests that the use of elastic tissue stain allows recognition of a sizable proportion (18.75%) of understaged lung cancer patient who may otherwise be denied potential benefits of adjuvant chemotherapy. Our study further suggests that elastic tissue staining is particularly valuable in the subset of patients in whom the lung cancers were closest (within 0.2cm) to the pleura. In this subset, the proportion of understaged patients was 25.35%.

1809 DNA Mismatch Repair Deficiency in Malignant Pleural Mesotheliomas

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Background: DNA mismatch repair (MMR) abnormality is the pathway of carcinogenesis in hereditary non-polyposis colorectal cancer (HNPCC) syndrome and in a minority of sporadic neoplasms. Sporadic MMR abnormalities may be caused by methylation of the hMLH1 gene promoter or loss of heterozygosity (LOH). We recently encountered a sentinel case of sarcomatous pleural malignant mesothelioma (MM) in an HNPCC patient with germline complete hMLH1 deletion; MM has not previously been described in HNPCC. As hMLH1 is located on 3p21.3, and 3p21.3 deletion has been described in 69% of MM, we hypothesized that MLH1 LOH and MSI may occur in MM.

Design: We retrieved 25 cases of MM diagnosed at Long Beach Memorial Medical Center between 1993 and 2008. The sentinel case is not included in this study. IRB approval was obtained. Formalin fixed paraffin embedded tissue (FFPE) was used for 4 mm tissue microarray after reviewing the H&E sections for selection of tumorous areas. Immunohistochemistry (IHC) using standard techniques, for hMLH1, hMSH2, hMSH6, and hPMS2 was performed on the microarray. Cases with abnormal staining on microarray were confirmed on whole FFPE blocks. In addition, MSI was evaluated by PCR using the 5 National Cancer Institute recommended microsatellite primers. LOH of hMLH1 was evaluated employing primers targeting an hMLH1 intronic region using PCR.

Results: H&E examination showed 8 sarcomatous, 13 epithelioid, and 4 biphasic tumors. 2 of 8 sarcomatous MM were desmoplastic type. DNA was obtained for molecular analysis in 21 cases. 2 of 25 (8%), both of desmoplastic variant, showed a marked loss of nuclear expression of hMLH1 and hPMS2 by IHC on both the tissue microarray and whole FFPE blocks. These 2 cases also showed MSI (2 and 3 of 5 microsatellites unstable respectively), and hMLH1 LOH. 2 additional cases, both epithelioid type, showed MSI only. 4 of 21 (19%) cases showed hMLH1 LOH, including the 2 cases with MSI and abnormal IHC. The other 2 cases with hMLH1 LOH were microsatellite stable and showed normal IHC results. In summary, IHC abnormality was found in 8% of cases, MSI in 19% and LOH in 19%.

Conclusions: MMR deficiency is a feature of a subset of MM. Our small series suggests a relationship between the desmoplastic subtype of MM and MMR deficiency. MM may be a rare neoplasm in the spectrum of tumors seen in patients with HNPCC syndrome, or MM may occur through sporadic abnormalities of MMR.

1810 Pathological Features of Interstitial Pneumonia Associated with Undifferentiated Connective Tissue Disease

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Background: NSIP pattern is the commonest pathological pattern in cases with Connective Tissue Disease (CTD). Meanwhile, cases which show clinical features of CTD but do not meet the criteria of American College of Rheumatology are now recognized as Undifferentiated CTD (UCTD). Recent report raises the question if majority of idiopathic NSIP may be the lung manifestation of UCTD. Further analysis is necessary to clarify the histological features of UCTD. Herein, we report the histological features of UCTD cases in our consultation case profile.

Design: 145 VATS in archives of Toyama University Hospital obtained from 2007-2009 were reviewed. We excluded the cases clinically diagnosed as CTD and picked up cases that meet the criteria of UCTD (both positive for serum antibody/ESR and symptoms of CTD). Eventually, 8 cases were selected. We investigated the pathological diagnoses according to ATS-ERS 2002 classification and histological findings; distribution of fibrosis (D), dense fibrosis (F), honeycombing (HC), fibroblastic focus (FF), organizing pneumonia (OP), lymphoid follicle (L), pleural thickening (P), peribronchiolar metaplasia (PBM), reactive type2 cell (RT) vasculitis (V), wall thickening of pulmonary artery (PA), hyaline membrane(HM), fibrin (FB) and normal lung (N).

Results: Eight cases consisted of 2 males and 6 females with age from 49 to 74. Smoking states were available to 7; 1 current, 1 ex-smoker and 5 never smoker. Radiological findings were available for 4 cases that revealed 3 NSIP and 1 unclassifiable pattern. Pathological diagnoses were 4 NSIP, 2 discordant UIP, 1 probable UIP and 1 LIP. As findings, diffuse distribution was found in 6/8 cases. F in 8/8, HC in 3/8, OP in 4/8, FF in 2/8, L in 8/8, P in 8/8, PBM in 7/8, RT in 7/8, V in 0/8, PA in 5/8, HM in 0/8, FB in 2/8, N in 3/8 were detected, respectively.

Conclusions: We investigated the pathological features of interstitial pneumonia associated with UCTD. We found that NSIP was the commonest pattern, although there were some cases with UIP or comorbid UIP. Common pathological findings are lymphoid follicle, pleural thickening, PBM and reactive type2. No vasculitis or hyaline membrane was found. Presence of fibroblastic foci, honeycombing or fibrin was rare.

1811 Immune Infiltration and Melanoma Target Antigen Expression in Lymphangioleiomyomatosis (LAM)

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Background: LAM belongs to PECOMAs, tumors showing perivascular epithelioid cell differentiation, which co-express myoid and melanocytic markers and show marked female predominance. Although PECOMAs elsewhere in the body are mostly benign, LAM relentlessly progresses to pulmonary failure. Several melanocytic markers may be expressed by LAM including: HMB-45, which is reactive with melanoma-associated antigen gp100 and MART-1. In melanoma and vitilligo, infitrating T cells have been shown to target both gp100 and MART-1 and this may be associated with depigmentation and tumor regression. Lungs affected by LAM are also frequently infiltrated by immune cells; however, there is a paucity of data regarding their role in LAM and levels of expression of various melanocytic markers.

Design: We performed immunohistochemical (IHC) and FACS analysis of lung tissue from 5 LAM patients, 3 controls and 3 melanoma tumors. Melanoma markers (HMB-45, MART-1, tyrosinase, TRP-1 and TRP-2) were tested by IHC and their expression was measured semi-quantitatively. Infiltrating immune cells were counted by cell sorting using FACScanto and tested with T cell (CD4 and CD8), dendritic and macrophage markers and were semi-quantified. LAM was compared to normal lung and melanoma.

Results: Expression of gp100, MART-1, TRP-1 and TRP-2, but not tyrosinase, was detected in LAM. Expression of TRP-1 was more prominent than gp100 in LAM and more prominent than in melanoma, while TRP-2 was comparable in both. In LAM, partially overlapping subsets of cells expressed gp100 and MART-1. T cell density in LAM and normal lung (4% of sorted cells) was similar but it was reduced compared to melanoma. $Cd11c^+$ dendritic cells in LAM were 50% more abundant than in normal lung but 50% less than in melanoma. Macrophages were much more abundant in LAM than in control lung and comparable to melanoma tumors. The differences in T cell density between LAM and melanoma were primarily due to more abundant $CD8^+$ T cell infiltration in melanoma.

Conclusions: These data suggest that infiltrating lymphocytes may be involved in the immune targeting of LAM cells, and that this targeting extends beyond antigens detectable by HMB-45 stain. The infiltrating immune cells in LAM may specifically target gp100 and MART-1, the same antigens that are targeted in melanoma. Further

testing of the cytotoxic potential of the infiltrating immune cells in LAM is needed in order to explore the possibility of therapeutic immune modulation in LAM and the feasibility of using anti-melanoma vaccines for the treatment of LAM.

1812 Genotype-Phenotype Correlation in Lung Adenocarcinoma

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Background: With the advancement of personalized treatment for non-small cell lung cancer, especially adenocarcinoma (ADA), identification of molecular targets in an efficient fashion is of the utmost importance in therapeutic decision making. However, there have been controversial reports on using histologic features to predict the presence of some mutations.

Design: Seventy-four ADAs, surgically resected or excised and submitted for molecular testing, were classified based on WHO criteria as ADA with bronchioloalveolar (mucinous or non-mucinous), papillary, acinar and/or solid patterns. Of those, 110 described mutations of 13 genes were studied using mutiplex PCR in 58 tumors (33 positive for mutation, 25 negative for known mutation). Point mutations of EGFR or KRAS were found by direct sequencing in the remaining 16 cases. The association between dominant histologic features and mutations were evaluated.

Results: While the majority of cases studied displayed a mixed histologic pattern, a dominant pattern was always evident.

Correlation between Histologic Pattern and Mutation Type

	All patterns	BAC, non-mucinous	BAC,mucinous	Papillary/ Micropapillary	Acinar	Solid	Others
EGFR	26	11	0	10	4	1	0
EGFR+CTNNB1 +/-TP53	4	0	0	0	1	0	3
KRAS	17	4	4	3	3	3	0
PIK3CA	2	1	0	0	0	1	0
No mutations	25	6	1	8	5	4	1
Total cases:	74	22	5	21	13	9	4

EGFR mutations were associated with the 2 most common histologic patterns, non-mucinous BAC (42%) and papillary (38%). In contrast, K-ras mutations were most commonly associated with BAC, either mucinous (4/17) or non-mucinous (4/17), and some extent of mucinous differentiation was present in a total of 10 K-ras mutants (59% vs. 12 % of non-Kras mutants, $p < 0.001$). While EGFR and K-ras mutations were mutually exclusive, 4 EGFR cases demonstrated an additional mutation in ß-catenin (CTNNB1), which was usually associated with a unique phenotype, such as a fetal lung pattern (2/4) or prominent cytoplasmic clearing (1/4). Finally, 43% of cases (25/58) lacked a detectable mutation using our panel and exhibited various dominant histologic patterns.

Conclusions: The results confirm and expand previously described genotype-phenotype correlations in lung ADAs. However, many tumors do not express a known mutation, despite displaying the various histologic patterns. Therefore, expansion of molecular testing and comprehensive histologic sub-typing is required to define new potential molecular targets for therapy and to discover unanticipated histologic-genetic correlations.

1813 Mutational Profiling of Non-Small Cell Lung Cancer (NSCLC) by Sequenom's OncoCarta™ Panel

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Background: Large scale genomic sequencing of lung adenocarcinomas have recently revealed the frequent occurrence of mutations within a large number of genes known to play an important role in carcinogenesis. These include oncogenes, growth factor receptors, signal transduction and tumor suppressor genes. The presence of mutation in some of these genes (e.g. KRAS and EGFR) has been correlated with sensitivity or resistance to chemotherapy and/or novel targeted therapies. In the near future, the management of lung cancer patients will require knowledge of the full mutation spectrum of the tumor; as this will play an important role in guiding treatment decisions.

Design: The goal of this study was to explore the feasibility of high throughput profiling of potential mutations in formalin-fixed and paraffin embedded primary NSCLC samples. Using the HE slide as a guide, high tumor cellularity areas of the tumor paraffin blocks were sampled using the 1 mm coring needle of tissue microarrayer. The isolated DNA was analysed by the Oncocarta™ panel (Sequenom®, San Diego, CA), which has the potential to identify 238 mutations across 19 oncogenes using PCR with detection by mass spectrometry.

Results: Altogether, 100 cases of resected NSCLC were studied. Mutations were detected in 38 (38%) cases, including 32 (59%) of adenocarcinoma and 6 (30%) of squamous cell carcinoma. The mutations detected included the those on *EGFR* tyrosine kinase domain, *KRAS*, and *PI3KCA*. Consistent with previous reports, *KRAS* and *EGFR* mutations were found mainly in adenocarcinoma (30% and 20%, respectively), while *PI3KCA* mutations were found mainly in squamous cell carcinomas.

Conclusions: Mutational profiling of routine clinical lung cancer samples is feasible for identification of critical genetic aberrations in NSCLC.

1814 Utility of Glucose Transporter 1 in the Differentiation of Peritoneal and Pleural Mesothelioma from Non-Malignant Mesothelium

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Background: Mesothelioma, of either peritoneum or pleura, is a rare malignancy. The diagnosis is often difficult to reach, in part because of the overlap between the morphology of reactive and malignant mesothelial cells. Glucose Transporter 1 (GLUT-1) is a glucose transporter typically found on erythrocytes. Though it stains various carcinomas, it has recently been reported as specific and fairly sensitive in discriminating between malignant pleural mesothelioma and reactive hyperplasia. The application of GLUT-1 staining in peritoneal mesothelioma has not been previously reported.

Design: Tissue microarrays containing 104 malignant peritoneal mesotheliomas and 31 malignant pleural mesotheliomas were studied and slides of 18 benign or reactive mesothelial specimens were stained with a GLUT-1 monoclonal antibody. Two observers scored each for percentage of mesothelial or tumor cells demonstrating positive staining. A cut-off of 5% was set for positivity.

Results: There were no false positives (100% sensitivity) in either the pleura or the peritoneum. Of the total 135 malignancies, 67 demonstrated positive staining (overall 49.6% sensitivity). Malignant versus benign determination had a Chi Square statistic of 16.78, p=.0001. In the peritoneum 52 of 104 malignancies demonstrated positive staining (50% sensitivity), In the pleura, 15 of 31 were positive, showing similar sensitivity in both sites.

Conclusions: As established in pleural disease, GLUT-1 staining is a useful adjunct in the differentiation of peritoneal mesothelioma from benign or reactive mesothelium. Due to modest sensitivity only positive staining is particularly informative. Our experience with pleural mesotheliomas supports what has been previously reported, but showed lower sensitivity. In both instances the utility of the stain was limited by non-specific staining (e.g. in necrotic areas) as well as brightly staining erythrocytes and lymphoid elements. Nonetheless, GLUT-1 can help differentiate malignant mesothelioma from reactive or otherwise benign mesothelium.

1815 CD24, a Novel Cancer Biomarker Predicting Disease-Free Survival of Nonsmall Cell Lung Carcinomas: From the Viewpoint of Forthcoming (Seventh) New TNM Classification

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Background: Metastasis-associated protein CD24 has been identified as a new prognostic factor and stem cell marker in the human neoplasm. However, the importance of the CD24 in nonsmall cell lung carcinomas (NSCLC) has not been elucidated well.

Design: We evaluated CD24 expression in 267 consecutive cases of NSCLC by immunohistochemistry (IHC) using a tissue microarray (TMA) technique and correlated with clinicopathological parameters including forthcoming (Seventh) new TNM classification.

Results: CD24-high expression was demonstrated in 87/267 (33%) and was associated with advanced new pathologic stage (35/81(43%) vs. 52/186(28%); $P = 0.016$) and higher in adenocarcinoma (ADC) histology than in squamous cell carcinoma (SCC) histology (64/165(39%) vs. 20/88(23%); $P = 0.023$). Patients with CD24-high tumors tended to have a higher risk of disease progression ($P < 0.001$) and cancer-related death ($P = 0.002$). Multivariate analysis proved CD24-high expression as independent prognostic factors of disease progression and cancer-related death ($P = 0.011$, hazard ratio (HR) = 1.64, 95% confidence interval (CI) =1.12~2.41 and $P = 0.030$, HR = 1.82, 95% CI =1.06~3.11). CD24-high expression was correlated with new pathological stage ($P = 0.016$) rather than old pathological stage ($P = 0.069$). On multivariate analyses, weak correlations between new pathological stage and progression-free survival ($P = 0.051$) were also found without reaching formal statistical significance.

Conclusions: CD24 expression in NSCLC is associated with advanced newly proposed TNM stage and ADC histology as well as disease progression and cancer-related death, indicative of aggressive tumor behavior.

1816 DNA Methylation Profile of Multistage Progression of Pulmonary Adenocarcinomas: Atypical Adenomatous Hyperplasia, Bronchioloalveolar Carcinoma and Adenocarcinoma

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Background: Atypical adenomatous hyperplasia (AAH) is considered as the precursor lesion of pulmonary adenocarcinomas (ADC). However, the epigenetic alterations of the AAH- bronchioloalveolar carcinomas (BAC)-ADC sequence are not clearly established.

Design: We first analyzed the methylaton status at 62 CpG island loci of 10 nonsmall cell lung cancer (NSCLC) tissues and 10 paired normal tissue using MethyLight assay and then chose 18 genes of cancer specific hypermethylation. Consequently, we analyzed the methylaton status at 18 CpG island loci of 20 normal lung tissues, 21 AAHs, 30 BAC, and 60 ADCs using MethyLight assay.

Results: Methylation of more than one CpG island loci was observed in 15 (71.4%) of the 21 AAH, 27 (90%) of the 30 BAC, 59 (98.3%) of the 60 ADC. The mean number of genes methylated was significantly higher in ADC than in AAH and BAC (6 and 2, 3, respectively; $p = 0.003$). The data shows that a higher prevalence of *GATA3* (16%), *HOXA1* (37%), *RARB* (38%), *TMEFF* (54%) promoter methylation was observed in AAH and BAC showing early carcinogenesis. The genes frequently methylated in ADC were *BCL2* (42%), *CCND2* (20%), *CDH13* (25%), *DLEC1* (30%), *GRIN2B* (27%), *HOXA1* (85%), *HOXA10* (15%), *MT1G* (28%), *PENK* (43%) and *RUNX3* (40%). Four different classes of methylation behaviors were found: (a) genes methylated in ADC only (*HOXA10*), (b) genes with low and similar methylation frequency (0-25%) in three-step lesions (*CRABP1, GATA3, ITGA4, RARRES1, SEZ6L* and *SFRP5*), (c) genes with high and similar methylation frequency (35-72%) in three-step lesions (*TMEFF2* and *RARB*), (d) genes showing an increasing tendency with or without fluctuation of the methylation frequency along the progression (*BCL2, CCND2, HOXA1, MT1G* and *RUNX3*).

Conclusions: Our results indicate the involvement of epigenetic alterations in the progression of ADC and that aberrant CpG island methylation tend to accumulate along the multistep carcinogenesis. High levels of CpG island hypermethylation of *BCL2,*

CCND2, HOXA1, MT1G or *RUNX3* might serve as a potential biological marker for the progression of invasive ADC.

1817 C-MET Subcellular Localization in Patients with Malignant Mesothelioma in a Series of 157 Cases from the MESOPATH Center

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Background: C-Mesenchymal-Epithelial Transition factor (C-MET) receptor tyrosine kinase a critical pathway for lung carcinoma and malignant mesothelioma (MM) is activated by binding of Hepatocyte Growth Factor, inducing autophosphorylation. The resulting phospho-C-MET is rapidly translocated to the nucleus and concomitantly initiates intracellular signaling transduction pathways, that result in alteration of cell functions such as spreading and migration, leading to metastasis. It is therefore anticipated that c-met targeted therapy could result in efficient antiproliferative and anti-metastasis drugs. We aim to analyse the expression of C-MET and its activated form on our series.

Design: We investigated the correlation between immunostaining of both C-MET and its activated form, phospho-C-MET and overall survival (OS) in 174 MM specimens referred in our pathological center for expert pathological diagnosis certification. A semi-quantitative score was attributed taking account the number (0 to 100%) of stained cells and the intensity (0 to 3) of staining. Semi-quantitative scores (0 to 300) were studied as continuous variables, without any pre-determined cut-off.

Results: Among the 157 slides giving reliable immunostaining results, positive C-MET expression was found in 119 MM (75.8%), mostly in epithelioïd subtype (87%, p<0.0001). Among those 119 +ve C-MET specimens, 77 (64.7%) were also +ve for Phospho-C-MET. Both C-MET and Phospho-C-MET scoring were independent of patient gender (p= 0.98 and p=0.36 respectively) or age (p= 0.296 and p=0.62 respectively). Neither Phospho-C-MET scoring nor Phospho-C-MET localization discriminate patients subgroups with different median OS. Conversely, in patients with a C-MET scoring either higher than 100 or intensity higher than 1 when exclusively confined to plasma membrane, median OS was 25 months *versus* 13 months for other patients. Compared with patients having C-MET scoring lower than 100 (p=0.0012) or patients with C-MET cytoplasmic or nucleus localizations, the survival differences were significant either in univariate (p=0.02) or multivariate analysis (Cox model, p=0.043), adjusted for age.

Conclusions: Our results suggest that immunodetection of C-met receptor-tyrosine kinase specifically at plasma membrane, could be relevant to stratify a subgpoup of patients with worse prognosis. Whether those patients could benefit from c-met targeted therapies, remains to be established in future prospective clinical trials.

1818 Immunohistochemistry with Mutation Specific Antibodies Detecting the Status of EGFR Mutations in Non-Small-Cell Lung Cancer

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Background: The association between EGFR mutations and response to EGFR tyrosine kinase inhibitors (TKIs) in non-small-cell lung cancer(NSCLC) has been consistently confirmed in a number of studies. Deletions in exon 19 and the L858R substitution in exon 21, accounting for approximately 90% of all EGFR mutations, are the best characterized sensitizing mutations in adenocarcinoma (AC). This study was designed to evaluate immunohistochemistry (IHC) with mutation specific antibodies to detect mutant EGFR proteins in NSCLC for patient selection to TKI therapy.

Design: We have screened 140 cases of NSCLC patient tumor samples, which included 90 cases of AC and 50 cases of SCC, by IHC using total EGFR antibody and exon 19 delE746_A750 and L858R mutation specific antibodies. The result was confirmed by direct DNA sequencing. In addition, we compared patient response to TKIs with the IHC staining result in 32 patients who were treated by TKI prior the screening.

Results: 10 cases were positively stained by EGFR and exon 19 delE746_A750 specific antibodies. 9 cases were positively stained by EGFR and L858R specific antibodies. Squencing found 24 cases with EGFR mutations. 14 cases were exon 19 deletions (10 type I and 4 other subtypes). 10 were L858R. All positive cases were from AC. All IHC positive cases were confirmed by DNA sequencing, but the rest 5 DNA sequencing positive cases were negatively stained by IHC (1 exon 19 delE746_A750, 3 other subtypes deletion and 1 L858R mutation). The specificity of IHC is 100% and sensitivity is 79.2%. 32 advanced disease cases were treated by Iressa until disease progression. In three month following up: 1 complete response (CR), 8 partial response (PR), 2 stable disease (SD), 21 progression of disease (PD). The total effective rate (CR+PR) was 28.1%, disease control rate (CR+PR+SD) was 34.4%. 9 of the 32 cases (28.1%) were IHC positive. 1 CR, 7 PR, 1 SD. Total effective rate (CR+PR) was 88.9% and disease control rate (CR+PR+SD) was 100.0%.

Conclusions: IHC with mutation specific antibodies to detect mutant EGFR proteins is a rapid, specific and cost efficient methodology. The positive staining is not just highly correlative to the sequencing result, but also correlative to tumor response to TKIs.

1819 Minute Nests of Neuroendocrine Cells in the Mediastinal Lymph Nodes: Real Metastasis?

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Background: It is continuously debatable whether lymph nodes can harbor a primary neuroendocrine tumor. The presence of neuroendocrine cells in rare peri-pancreatic lymph nodes (LNs) suggests that neuroendocrine cell proliferation can arise in situ from neuroendocrine cells native to any LN. It is difficult to determine the clinical outcome just based on the finding of these cells by immunohistochemistry in patients with broncho-pulmonary carcinoids.

Design: Two cases of mediastinal LNs harboring minute nests of neuroendocrine cells in patients with no history of neuroendocrine tumor were reviewed. For comparison, 71 carcinoids including 62 typical carcinoids (TCs) and 9 atypical carcinoids (ACs) with 315 LN biopsies were retrieved. All slides were examined and the sizes of LN metastasis were recorded.

Results: Case 1. The patient was 67 year old man who had a 7.0 cm, hypermetabolic tumor in the left lower lobe of lung detected by CT and PET scans. Lobectomy with LN biopsies was performed. Well-differentiated adenocarcinoma was diagnosed and <1 mm size of nested neuroendocrine cells were identified in level 10 LN. One level 9 and 2 hilar LNs were unremarkable. Case 2. The patient was 74 year old man who had 3.8 cm and 2.0 cm, hypermetabolic tumors in the left hilum and thyroid by PET, respectively. Multiple left neck LNs were hypermetabolic as well. Endobronchial biopsy was performed to show poorly differentiated adenocarcinoma. Total thyroidectomy and left neck lymph node dissection were performed and papillary thyroid carcinoma with multiple LN involvement was diagnosed. Subsequently, LNs including level 5, 6 and 10 were biopsied and <1 mm of nested neuroendocrine cells was identified in level 5 LN. In both case 1 and 2, the neuroendocrine cells were strongly positive for chromogranin, synaptophysin and TTF-1, but negative for calcitonin and thyroglobulin. D2-40 stain failed to detect any lymphatic channels surrounding these cells. Slides of TCs, ACs and LNs were reviewed to show >4 mm in size of individual metastases in 7 involved LNs (62 TCs with 5 positive LNs and 9 ACs with 2 positive LNs) and the morphology of metastatic carcinoids was similar to that of the primary tumors. No <1 mm in size of nested neuroendocrine cells was identified in LNs.

Conclusions: Minute nests of neuroendocrine cell proliferation similar to the histology and protein expression observed in tumorlet of the lung can be incidentally identified in mediastinal LNs. Close clinical followup is warranted although these cells are unlikely metastasized from the lung.

1820 Aldehyde Dehydrogenase 1 (ALDH1) Expression Is Associated with Clinical Outcome in Non Small Cell Lung Cancer (NSCLC)

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Background: ALDH1 is an enzyme involved in the regulation of intracellular retinoic acid metabolism. ALDH1 expression has been associated with solid tumor stem cells and progenitor cells and linked to resistance to chemotherapy. ALDH1 expression has not been widely studied in NSCLC.

Design: Formalin-fixed, paraffin embedded sections from 114 NSCLC, including 31 squamous cell carcinomas (SCC), 45 adenocarcinomas (AC), and 38 bronchioloalveolar carcinomas (BAC) were immunostained by automated methods (Ventana Medical Systems, Inc, Tucson, AZ) with mouse monoclonal ALDH1 (clone 44/ALDH; BD Biosciences). Cytoplasmic immunoreactivity was semiquantitatively assessed in the tumor for all cases. Scoring was based on staining intensity and percentage of tumor cells. Results were correlated with clinicopathologic variables.

Results: ALDH1 immunoreactivity was predominantly cytoplasmic. ALDH1 overexpression was noted in 54/114 (47%) tumors and correlated overall with tumor type [68% SCC vs 29% AC vs 45% BAC, p<0.0001], tumor grade [26% well differentiated vs 65% moderately differentiated vs 41% poorly differentiated, p=0.007], and gender [60% male vs 33% female, p=0.004]. Within the SCC subgroup, ALDH1 overexpression correlated with tumor grade [0% well differentiated vs 95% moderately differentiated vs 55% poorly differentiated, p=0.007], gender [88% male vs 43% female, p=0.013] and shortened overall survival [88% expired vs 43% expired, p=0.013]. On multivariate analysis, only advanced tumor stage and poor tumor differentiation independently predicted overall survival.

Conclusions: ALDH1 expression in NSCLC is associated with SCC tumor type, higher tumor grade and male patients. With in SCC subgroup, ALDH1 overexpression is positively correlated with higher tumor grade, male patients and poor survival. The potential role of ALDH1 in NSCLC as a prognostic factor and a predictor of chemoresistance warrants further study.

1821 Retrospective Analysis of Lung Transplant Patients between 1991-2009: Improved Survival Is Related to Native Lung Disease

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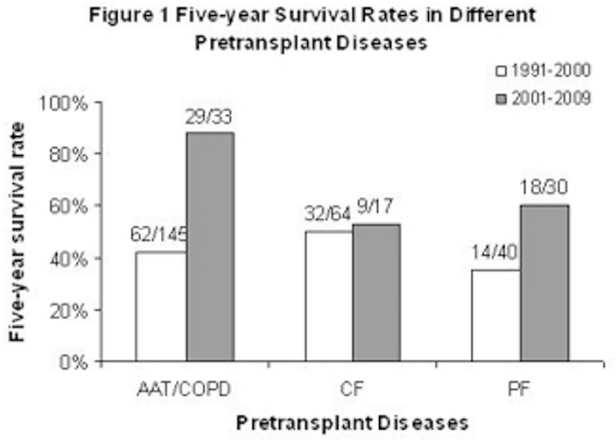
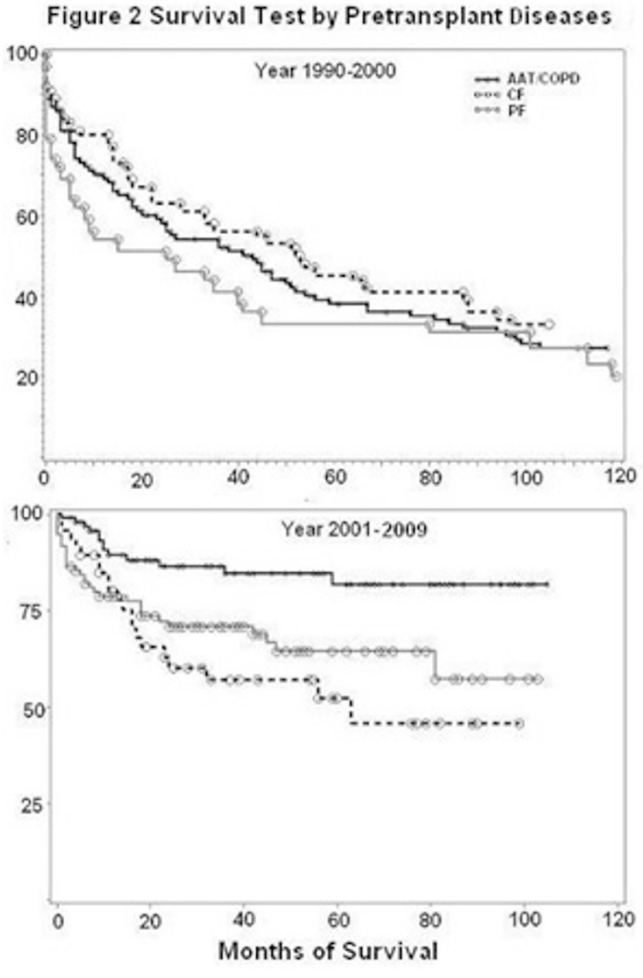
Background: Survival of post lung transplantation (tx) patients has significantly improved consequent to modified therapies but there is a paucity of data regarding overall survival in different pre-tx diseases.

Design: We retrospectively reviewed 616 patients who underwent lung tx at a single lung tx center between 1991-2009. We evaluated survival rates chronologically (1991-00 and 2001-09) according to the primary pre-tx disease: cystic fibrosis (CF), pulmonary fibrosis (PF), α 1 antitrypsin deficiency/chronic obstructive pulmonary disease (AAT/COPD). Survival rates were compared in these three groups. Predicative survival curves were computed by life table analysis and differences between the three pre-tx diseases were analyzed by the generalized Wilcoxon test.

Results: Of the 616 lung tx patients, 111 had CF (18%), 246 had AAT/COPD(39%), and 135 had PF (22%). Recipients' mean age was 47.46 ± 13.66 years. The 1-, 3-, and 5-year survival rates in the AAT/COPD and PF groups were significantly increased (p<0.05) in the 2000s. However, there was no significant improvement in the 1-, 3- and 5-year survival rates for lung tx recipients with CF, even though these patients were younger. Mean age of patients with CF was 28.1±8.6 yrs, versus 55.6±6.8 in AAT/COPD and 53.3±10.3yrs in PF. This indicates that survival post lung tx is not dependent on age alone.The Wilcoxon test showed that before 2001, there was no significant difference in survival in the three groups, but, since 2000, recipients with AAT/COPD or PF had a higher survival rate than those with CF(p=0.001).





Conclusions: There was a significant increase in survival in AAT/COPD and PF; however, there has not been a significant improvement in the survival of patients with CF in last two decades. More studies are needed to ascertain the cause of this and to determine the optimal management regime for lung tx recipients.

1822 CD138 (Syndecan-1) in Thymic Neoplasms: Correlation with Various World Health Organization Types and Clinical Outcome

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Background: The purpose of this study is to investigate the expression of CD138 (Syndecan-1) in thymic neoplasms, and analyze its interrelationship with clinicopathologic variables.

Design: A series of 64 thymic neoplasms were reviewed and classified according to the World Health Organization (WHO) classification system. Key clinical information including Masaoka stage, recurrence free and overall survival was obtained. Staining pattern (cytoplasmic versus membranous) as well as the percentage of positive tumor cells were recorded. A percentage of ≥10% was used as a cutoff for tumor positivity with CD138. Correlation of CD138 expression with WHO type and clinicopathologic variables was statistically analyzed using Fisher's exact test and log-rank test.

Results: 29 of 64 cases (45.3%) stained positive with CD138. Positive staining was seen in 6 of 7 (85.7%) type A, 7 of 15 (46.7%) type AB, 1 of 8 (12.5%) type B1, 1 of 5 (20%) type B2, 11 of 19 (58%) type B3 and 3 of 10 (30%) type C (p=0.04). While 10 of 11 (91%) CD138 positive type B3 had membranous expression; cytoplasmic expression was identified in 6 of 6 (100%) type A, and 6 of 7 (86%) type AB ($p < 0.0001$). Positive CD138 expression was noted in 19 of 30 (% 63.3) cases with Masaoka stage I ($p= 0.01$). While negative expression of CD138 was seen in 24 of 34 (71%) cases with advanced Masaoka stages (II, III or IV). Tumor recurred in 4 cases (7%), all of which had negative CD138 expression (p=0.008, log rank test) (Figure 1).

Conclusions: CD138 immunoexpression in thymic neoplasms could be used as an ancillary study to differentiate between WHO histologic types, particularly types A, AB and B3. CD138 negativity can also be used as a predictive factor for worse clinical outcome.

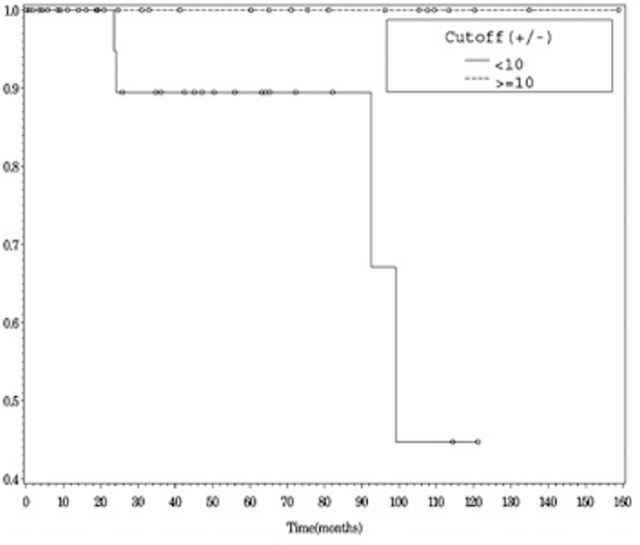

Figure 1: Kaplan-Meier curve for RFS in patients with thymomas excluding type C. Patients with CD138-negative tumors showed tumor recurrence more often than patients with CD138-positive tumors.

1823 Squamous Cell Carcinoma Antigen (SCCA)-2 Is Overexpressed in End-Stage Idiopathic Pulmonary Fibrosis and Correlates with Epithelial Proliferation

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Background: Idiopathic pulmonary fibrosis (IPF) is a progressive interstitial lung disease of unknown etiology. A high incidence of lung cancer in patients with IPF is the main reason for the poor prognosis of the disease. Abnormal re-epithelization with aberrant proliferation of metaplastic cells often observed in highly remodeled fibrotic areas are believed to be a part of the precancerous process leading to lung cancer. Squamous cell carcinoma antigens (SCCA)-1/2 (also known as SERPIN B3/B4), are members of the serpin superfamily and fundamental for the control of proteolysis through an inhibitory function of different proteases. Recently several studies have documented an important role of SCCA-1/2 in the modulation of fibrosis, including lung fibrosis. The aim of this study was to confirm the role of SCCA in lung fibrosis and investigate its influence on epithelial proliferation. The most prevalent isoform, SCCA-1 or SCCA-2, was also studied.

Design: Native lungs of 47 patients consecutively transplanted for IPF (Group A: 33 males and 14 females; mean age±standard deviation: 55±7 years; DLCO, mean±SD: 23±10% of predicted values, three of them with histological foci of neoplastic transformation) were studied. Ten native lungs of non-IPF subjects (Group B) were used as a control group. In consecutive serial sections from all cases immunohistochemistry for SCCA-1/2, transforming growth factor (TGF)β and Ki-67 was performed and the quantification restricted to strongly stained metaplastic epithelial cells distinguishing cuboidal, bronchiolar and squamous cells. Values were expressed as percentages of positive cells and Ki-67+ cells represented the proliferative index (PI). Quantitative real-time reverse transcriptase polymerase chain reaction (RT-PCR) was used to characterize the most prevalent SCCA isoform in all cases.

Results: SCCA was overexpressed in Group A (median values, range: 32% of cuboidal, 9-65%; 36% of bronchiolar, 14-61%; and 88% of squamous cells, 24-96%), and only sporadically detected in Group B (median, range: 2%, 0-6%). SCCA expression was directly correlated to both PI ($p<0.05$) and TGFβ ($p<0.0001$). Although SCCA-1 was present in all patients, SCCA-2 was overexpressed in IPF patients.

Conclusions: These results confirm the strict relation between SCCA-1/2 and the pro-fibrogenetic cytokine TGFβ. The isoform SCCA-2 plays a key role in aberrant regeneration occurring in IPF.

1824 Placental Alkaline Phosphatase (PLAP) and Podoplanin (D2-40) Define Two Subtypes of Cells in Lymphangioleimyomatosis

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Background: Lymphangioleiomyomatosis (LAM) is a poorly understood rare fatal lung disease of premenopausal women. LAM lesions show infiltrating smooth muscle (SM)-like cells causing cystic destruction of lungs, eventually mandating lung transplant. LAM cells often metastasize to regional lymph nodes, circulate in blood, and occasionally produce distal metastases, including, to transplanted lungs. Occasionally LAM occurs in association with tuberous sclerosis disease and *tsc2* mutations were found in a few sporadic LAM cases. Since we noticed that vascular SM immunoreact for PLAP (Placental Alkaline Phosphatase) we evaluated LAM lesions for its expression. Moreover, since PLAP circulates patients with PLAP-positive tumors, we sought to evaluate the integrity of the vasculo-lympatic channels in/around LAM lesions.

Design: Serial sections of FFPE lung tissue sampled from 21 patients with LAM were immunostained for LAM markers SM α actin (SMA) and HMB45, PLAP, vascular endothelial marker CD31 and lymphatic endothelial marker D2-40 (podoplanin).

Results: SMA was detected in most LAM cells followed in frequency by D2-40. PLAP and HMB45 were detected in a lower number of cells. Predominantly D2-40-positive lesions also had abundant HMB45-positive cells, but only rare PLAP-positive cells. Predominantly PLAP-positive lesions were negative or occasionally weakly positive for D2-40 and had a few HMB45-positive cells. Furthermore, cells that expressed PLAP did not express D2-40 or HMB45. Blood vessels stained weakly for PLAP but were negative for D2-40 or HMB45. D2-40 stained only lymphatic channels in normal or other disease lung controls. CD31 stained most of the epitheloid cells lining the LAM cysts.

Conclusions: - PLAP is a novel marker for a subpopulation of LAM cells, immunoreacting significantly stronger than vascular or airway SM cells. - Since PLAP, a GPI-anchored membrane protein is extruded on exosomes and circulating LAM cells can also carry PLAP, it should be cleaved from both by serum Phosphatidyl Inositol Phospholipase D (PIPLD). In such a case serum PLAP level could potentially be a surveillance marker for LAM patients. - Podoplanin (D2-40) is a novel marker for a large subpopulation of LAM cells. Since a larger number of LAM cells stain for D2-40 than HMB45, the former may be a better diagnostic marker for LAM. - Since podoplanin is involved in cell invasion, its presence in LAM lesions may have functional implications in progression of the disease. - There are either PLAP-predominant or podoplanin-predominant LAM lesions/cases.

1825 Radiation Therapy Does Not Improve the Prognosis of Patients with Stage II Thymoma, Supporting Previous Evidence Suggesting That the Presence of Transcapsular Invasion Is Not a Significant Prognostic Feature

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Background: Recent metaanalysis of best available evidence showed that patients with stages I and II thymoma do not have significantly different prognosis. This suggested that detection of transcapsular invasion during the pathologic evaluation of these lesions does not provide significant prognostic information, a conclusion that was tentative because only some patients with stage II disease had been treated with radiation therapy and the possibility of treatment bias could not be excluded.

Design: A systematic review of the English literature from 1962-present was performed to identify best evidence regarding the prognostic value of radiation therapy in patients with stage II thymomas. Only studies that evaluated more than 10 patients treated with radical thymectomy, post-operative radiation therapy and provided a minimum of 5-year follow-up information were included. The level of evidence of each study was assessed using the criteria published by the Cochrane collaboration. Patients were divided into 2 groups, those that were treated with post-operative radiation therapy and those that received only thymectomy. The data was analyzed with Comprehensive Metaanalysis software (Biostat Inc Englewood, N.J.).

Results: Twenty-five level III and IV studies evaluating 1183 number of patients with stage II thymoma reported the use of post-operative radiation therapy but only 9 of these studies provided information consistent with our inclusion criteria. They reported 197 number of patients (median=24 and range 1-41) treated with radiation therapy with doses ranging from 25-72 Gy and 193 patients (median=18 and range 1-73) that were treated only with radical thymectomy. Survival proportions for patients in these two groups were 84% and 68%, respectively. Metaanalysis showed odds ratios >0.05 for the comparison of survival proportions of these two groups of patients and demonstrated the presence of significant data heterogeneity as shown by funnel plot and Egger's regression test p>0.05.

Conclusions: There is a lack of randomized clinical trials evaluating the prognosis of patients with stage II thymoma undergoing radiation therapy after complete resection. Thetreatment protocol for thymomas needs revision.

1826 Metastatic Endometrial Stromal Sarcoma in the Lung: Importance of Immunohistochemical Staining, Clinical History and Imaging Studies

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Background: Discrimination between metastatic pulmonary endometrial stromal sarcoma (ESS) and other spindle cell neoplasms including solitary fibrous tumor, and sarcomatoid carcinoma and mesothelioma can be challenging when diagnostic material is a small and crushed core biopsy and when clinical history is lacking. Immunohistochemical staining can be a powerful tool to segregate ESS from others; however, it may be a pitfall because a portion of ESS cases were reported to be positive for cytokeratin. Here we assessed the importance of a panel of immunostaining, clinical history and imaging studies in rendering this diagnosis.

Design: Eleven cases of metastatic pulmonary ESS surgically resected (n=10) or biopsied (n=1) were retrieved. Clinical and imaging data were available for analysis. Lung histologic slides were reviewed and the immunohistochemical staining panel including AE1/AE3, CK7, CK19, Cam5.2, CD10, bcl-2, CD34, TTF-1, ER, PR, and caldesmon were performed. Percentage and intensity of stained tumor cells were recorded.

Results: Seven cases of metastatic lung ESS were low grade (LGESS) and 4 were high grade (HGESS). Patients ranged in age from 44 to 70 years for LGESS and from 50 to 73 years for HGESS, respectively. All the cases presented with one to multiple unilateral or bilateral lung nodules detected by CT. Primary ESS was diagnosed from hysterectomy specimens except one by endometrial biopsy, 0.5 to 23 years prior to metastasis. Immunohistochemical studies showed that all ESS cases were moderately to strongly positive for bcl-2 and CD10 with >50% of tumor cells stained except one HGESS being negative for CD10. ER and PR neagtive were detected in 7 cases with diffuse and moderate to strong positivity. Three ER and PR negative cases were HGESS. TTF-1, CK7 and CD34 were neative in all cases. One LGESS and one HGESS were positive for caldesmon with patchy and strong positivity. Interestingly, two cases of LGESS showed moderate to strong AE1/AE3 positivity in >50% of tumor cells with one case having moderate CK19 and Cam 5.2 staining in >30% of tumor cells.

Conclusions: Caution should be taken when assessing spindle cell neoplasms in female patients with a history of hysterectomy. A panel of immunohistochemical staining and imaging studies are useful in nailing down the diagnosis.

1827 The Presence of P311 in Lymphangioleiomyomatosis Lesions May Link TGFß1 Expression with the mTOR Pathway

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Background: Lymphangioleimyomatosis (LAM) is a rare fatal lung disease effecting women predominantly of child-bearing age. LAM is characterized by the infiltration of the lung by smooth muscle (SM)-like cells leading to cystic destruction and pulmonary insufficiency. The finding of circulating LAM cells in blood suppports the notion that extra-pulmonary involvement is due to metastasis rather than multifocal disease. LAM had been associated with mutations in the tuberous sclerosis complex gene 2 (TSC2 or tuberin) leading to activation of the mTOR (molecular target of rapamycin)/S6K1 signaling pathway. Active mTOR binds to eIF3 (eukaryotic initiation factor 3), displacing and activating S6K1, which promotes translation of cell growth-regulation genes. P311 is an 8-kDa protein found in SM cells and neurons. We recently found that *p311* null mice have a severe decrease in SM TGFß1 translation. Since LAM cells exhibit SM-like differentiation, we evaluated LAM lesions for expression of P311 and TGFß1.

Design: Serial sections of FFPE lung tissue sampled from 21 patients with sporatic LAM were immunostained for LAM markers SM actin and HMB 45, P311 and TGFß1. Additional LAM lesions were laser-microdissected for P311 co-immunoprecipitation studies using a specific anti-P311 antibody, followed by mass spectrometry to identify P311 binding partners.

Results: P311 was focally positive in LAM lesions with intensity similar to or higher than that of neighboring vascular SM. LAM cells expressing P311 also showed strong staining for TGFß1, while neighboring blood vessel SM did not express TGFß1. Co-immunoprecipitation followed by mass spectrometry demonstrated that P311 interacts with eIF3.

Conclusions: - LAM lesions are focally and concomitantly positive for P311 and TGFß1. Since the lack of P311 in *p311* null mice results in a decrease in TGFß1 level, we postulate that the expression of TGFß1 in LAM cells is related to the production of P311 by these cells. - Since TGFß1 promotes tumor tolerance and stimulates the production of extracellular matrix degrading proteases, the expression of TGFß1 by LAM cells is likely to contribute to the invasive nature of the disease. - Based on the fact that P311 interacts with eIF3, we propose that by such interaction P311 facilitates the binding of active mTOR to eIF3 in the tuberin-deficient LAM cells. However, since vascular SM has normal tuberin function, the mTOR pathway is inactive and is not recruited to promote translation of TGFß1.

1828 Smoking Related Interstitial Fibrosis (SRIF): A Common Form of Interstitial Fibrosis in Smokers To Be Distinguished from the Fibrosing Idiopathic Interstitial Pneumonias

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Background: In examining non-tumorous lung parenchyma in lobectomy specimens from smokers, we were surprised by the frequent presence of severe interstitial fibrosis in the apparent absence of clinical and radiographic evidence of interstitial lung disease. This observation prompted us to undertake a systematic analysis of non-neoplastic lung parenchyma in smokers.

Design: Twenty lobectomy specimens excised for neoplasms from smokers were extensively sampled by dividing them into 27 evenly spaced segments and taking one section per segment. Each case was examined semi-quantitatively for interstitial fibrosis, fibroblast foci, peribronchiolar metaplasia, honey-comb change, emphysema, and respiratory bronchiolitis (RB).

Results: Interstitial fibrosis involving more than 25% of slides was identified in 12 of 20 cases. One case each was diagnosed as usual interstitial pneumonia (UIP), scarred Langerhans cell histiocytosis, and asbestosis, while 9 were considered to represent examples of smoking related interstitial fibrosis (SRIF). This lesion was characterized by varying degrees of alveolar septal widening by hyalinized collagen deposition with minimal inflammation. The fibrosis predominated in subpleural parenchyma but was also found in deeper areas away from the pleura. All cases were associated with emphysema, which was often severe. The fibrosis usually surrounded enlarged

airspaces of emphysema, and it also involved non-emphysematous parenchyma. Respiratory bronchiolitis accompanied the changes in all cases. No patient had clinical or radiographic evidence of interstitial lung disease, and clinical progression was not documented in any case, although follow-up was short.
Conclusions: SRIF is a distinct form of interstitial fibrosis that is commonly encountered in cigarette smokers. Additional investigation will be required to determine its clinical significance and its relationship, if any, to other smoking related diseases. It is important, however, that SRIF be distinguished from specific forms of fibrosing lung disease that may be associated with poor prognoses, especially UIP.

1829 Biopsy Findings in Acute Pulmonary Histoplasmosis: Unusual Histologic Features of a Potential Mimic of Lymphomatoid Granulomatosis

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Background: Most examples of pulmonary histoplasmosis are characterized by necrotizing granulomatous inflammation. Only disseminated histoplasmosis is recognized as causing a different reaction which consists of ingestion of organisms by macrophages without granuloma formation. The histologic features of acute pulmonary histoplasmosis are not well described since this form of the disease is rarely biopsied.
Design: Four surgical lung biopsies of acute pulmonary histoplasmosis were retrieved from consultation files (3) and our surgical pathology archives (1). Each was stained with H&E, Ziehl-Neelsen and Grocott methenamine silver (GMS). Clinical and radiographic findings were documented for all cases, and various histologic features were recorded.
Results: There were 3 men and 1 woman who ranged in age from 40 to 68 years. All presented with fever and other flu-like symptoms for a duration ranging from 3 days to less than 30 days. Radiographically, 3 cases showed a solitary nodular infiltrate while one showed bilateral reticulonodular infiltrates. Histologically, all 4 biopsies showed a parenchymal inflammatory infiltrate composed of lymphocytes and histiocytes filling alveolar spaces and expanding the adjacent interstitium. Large areas of parenchymal necrosis were additionally present in 3 cases. Vasculitis composed of lymphocytes and histiocytes was present in all, and was striking in 3, resulting in a resemblance to grade 1 lymphomatoid granulomatosis (LYG). The tip-off to the correct diagnosis, present in 3 cases, was a few small necrotizing granulomas scattered within the lymphohistiocytic infiltrate. The diagnosis was confirmed by the presence of Histoplasma yeasts in the GMS stain.
Conclusions: Acute pulmonary histoplasmosis may cause a lymphohistiocytic infiltrate with necrosis and vasculitis that is suggestive of LYG. The correct diagnosis is easily established by examination of silver stains. This observation emphasizes the importance of examining special stains for organisms before diagnosing Grade 1 LYG.

1830 Subclassification of Poorly Differentiated Non Small Cell Carcinoma on Small Biopsy Specimens: Utility of a Panel of Immunohistochemical Stains

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Background: The availability of targeted therapies has created a need for precise subtyping of non small cell lung carcinomas. The most difficult cases to classify are poorly differentiated or extensively necrotic carcinomas in small biopsy specimens. The aim of this study was to assess the utility of immunohistochemical markers in subtyping such tumors. The study is unique in that it includes only those non-small cell carcinomas that could not be precisely classified on biopsy and in which subsequent resection specimens were available for determination of the final gold standard diagnosis.
Design: Cases of poorly differentiated non small cell lung carcinomas diagnosed on small lung biopsies (bronchial biopsies and core biopsies) were included in the study if: (1) they could not be further subtyped on the biopsy specimen on the basis of H&E morphology alone, and (2) a subsequent resection specimen was available. The resected tumor was classified on the basis of H&E morphology alone according to WHO criteria by two pulmonary pathologists blinded to immunohistochemical findings. Each biopsy was stained with CK7, TTF-1, napsin, p63, CK5/6 and 34βE12. For all markers, any expression of the marker within tumor cells was considered positive. The utility of the various stains in classifying the tumors was assessed.
Results: On resection, there were 15 squamous cell carcinomas (SQCCA), 7 adenocarcinomas (ADCA), 3 adenosquamous carcinomas, 1 large cell carcinoma, 1 sarcomatoid carcinoma and 1 small cell carcinoma. Positivity for 2 squamous markers occurred in 8/15 SQCCAs (34βE12 and p63 in 5, 34βE12 and CK5/6 in 2, CK5/6 and p63 in one) but in none of the 7 ADCAs. Positivity for both TTF-1 and napsin was seen in 3/7 ADCAs but none of the 15 SQCCAs. p63 staining was seen in 2 ADCAs that were also TTF-1-positive, while TTF-1 staining occurred in 2 SQCCAs that were also 34βE12-positive. Staining for CK7 was not helpful, since it was present in all 7 ADCAs and 11/15 SQCCAs.
Conclusions: Poorly differentiated non small cell lung carcinomas can be accurately subtyped on small biopsy specimens in about half of all cases using a panel of immunohistochemical stains. The combination of TTF-1, napsin, p63, CK5/6 and 34βE12 is most informative.

1831 Occurrence and Frequency of Heterogeneity in EGFR Immunohistochemical Expression in Lung Adenocarcinomas of Mixed Type – Possible Consequences for EGFR Predictive Testing

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Background: In industrialized countries adenocarcinomas are now the most frequent type of lung cancer. Adenocarcinomas are often morphologically heterogeneous, with a mixed sub-type representing 80%. The evaluation of the epidermal growth factor receptor (EGFR) status is now commonplace, due to targeted therapy. The purpose of this study was to investigate if variation in immunohistochemical (IHC) staining intensity for EGFR correlated with the histomorphological heterogeneity in adenocarcinomas.
Design: 26 surgical specimens from lung neoplasms, diagnosed as primary adenocarcinomas of mixed type, were sub-typed according to their growth pattern (WHO-classification 2003) and evaluated by IHC for EGFR (clone 31G7, Zymed™, 1:25). The IHC expression of EGFR was scored 0-3 as follows: 0, no staining or faint membrane staining in <10% of the tumour cells; 1+, faint/barely perceptible staining in ≥10%; 2+, weak to moderate staining in ≥10%; 3+, strong membrane staining in ≥10%. Heterogeneity in IHC expression was considered significant when growth patterns displayed a difference in staining intensity score of 2 or more.
Results: All tumours, in varying intensity, stained positively for EGFR. The staining pattern was generally diffuse with a gradual variation in intensity. Abrupt and significant differences in staining intensity were only noticed in areas of transition from one growth pattern to another with an accompanying change in cellular atypia. 5 out of 26 (19 %) adenocarcinomas displayed a significant variation in staining intensity between different growth patterns. In two of these cases an increased expression was shown in the acinar compared to the bronchoalveolar component. In one of these cases the result was confirmed with FISH-analysis, showing a high-grade amplification in the acinar component. Furthermore, an inverse expression pattern was displayed in one case, with decreased staining intensity in the acinar component compared to the bronchoalveolar. In another two cases a decrease in the expression of the solid component was shown compared to the acinar.
Conclusions: This study showed a significant heterogeneity in EGFR IHC expression in 19% of the cases and staining intensity was correlated with variations in growth pattern. This could be of importance when selecting material for EGFR mutation detection, in order to avoid false negative results. Our suggestion is therefore to pre-screen resection material with either IHC or FISH/CISH before mutation analysis.

1832 Cilliated Epithelial Differentiation in Lung Adenocarcinoma

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Background: Lung carcinomas are a heterogeneous group of tumors at the cytologic, histologic, immunophenotypic and molecular genetic levels, and this heterogeneity is particularly prominent in the adenocarcinoma category. Histopathologically adenocarcinoma often contains a variety of morphologic patterns and cell types, the latter of which often show features of Clara cells, type II pneumocytes, and mucinous cells. Tumor cells in well-differentiated adenocarcinoma generally show differentiation to Clara cells or type II pneumocytes, hence, they are often referred to as adenocarcinoma of terminal respiratory unit. Ciliated cells are a major cell type in the proximal airway, but although their presence has been documented in adenocarcinomas at the ultrastructural level, it is believed that they are not microscopically seen in pulmonary adenocarcinomas. FoxJ1 is a transcription factor indispensable for ciliogenesis.
Design: A total of 177 cases of non-small cell carcinomas including 149 adenocarcinomas and 28 squamous cell carcinomas were studied. There were 6 bronchioloalveolar carcinomas (BAC), 19 acinar, 4 papillary, 39 solid, 7 mucinous, and 74 mixed adenocarcinomas, 50 cases of which contained BAC component. Immunostain for FoxJ1 was performed and the extent of staining was graded as 1+, 5-25%; 2+, 25-50%; 3+, 50<%.
Results: FoxJ1 reaction was seen in 40/149 adenocarcinomas (27%), among which 29 cases contained only a few FoxJ1-positive neoplastic cells whereas 10 tumors showed 1+ and one showed 2+. The tumor with 2+ was a solid adenocarcinoma, and the 10 tumors with 1+ constituted 6 acinar, 2 solid, 1 papillary, and 1 BAC. 29 tumors with only a few FoxJ1-reactive cells consisted 4 acinar, 5 solid, 1 BAC, and 19 mixed type (2 of which contained BAC element). No cilia, however, was identified on H&E sections. FoxJ1 was completely negative in all squamous cell carcinomas.
Conclusions: The presence of FoxJ1-positive cells in lung adenocarcinomas indicates that ciliogenesis is in progress in some tumor cells, suggesting lung adenocarcinoma can differentiate toward the epithelium of the conducting airway (bronchi). This finding is interesting given that the current hypothesis favors that lung stem cells reside in the terminal airway and can differentiation into pneumocytes and Clara cells but probably not cells of conducting airway, and that these stem cells are involved in tumorigenesis of lung adenocarcinoma. This finding is in keeping with the current view of lung organogenesis, i.e., the proximal and distal airways are derived from the same progenitor cells of the anterior foregut endoderm.

1833 Interstitial Lung Disease Is Applicable to Tissue Microarray Analysis with Spiral Tissue Microarray Technique

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Background: Interstitial lung disease (ILD) is a histologically heterogenous disease and composed of multiple combinations of histopathology. Unlike neoplastic disease, key findings of ILD is often a mixture of multiple histological events such as lymphocytic infiltration, epithelial reaction, fibrosis and vascular injury. Due to its uneven histological distribution, application to TMA study has been considered to be difficult. We have recently developed a novel tissue microarray (TMA) technique named Spiral TMA (S-TMA). S-TMA block can be constructed by embedding multiple reeled thick-cut specimens vertically to a designed recipient block. Its two biggest advantages are to cover tissue heterogeneity and skip damaging donor blocks. We created both conventional TMA (c-TMA) and S-TMA of ILD cases, and investigated if S-TMA can reasonably cover histological variations of ILD.
Design: Twenty five cases with ILD in the lobectomy specimen performed against primary lung cancer at Toyama University were collected. S-TMA was constructed with 100 micron thick sections of the blocks. c-TMA with 2mm cores was also constructed. Areas including best histology were carefully selected by a trained pathologist. Presence

of bronchiolar epithelia, large pulmonary arteries (PA), lymphocytic infiltration, type II epithelia, airspace filling macrophage and fibrosis was examined in S-TMA, c-TMA, and whole HE slides of original blocks. Large PA was defined with presence of both internal and outer elastic layers which were confirmed by EVG staining. The degree of fibrosis was also confirmed by EVG staining.
Results: Areas seen in S-TMA ranged from 1.4 to 2.8mm^2 (mean 2.04mm^2), while those in c-TMA was always 3.14mm^2. Each finding seen in S-TMA/c-TMA/whole original slides was as follows. Bronchiolar epithelia, 16/14/25; Large PA, 20/22/25; lymphocytic infiltration, 13/4/24; type II epithelia, 21/8/25; Airspace filling macrophages, 20/20/24 and fibrosis, 24/24/25.
Conclusions: S-TMA covered more of histological variations in ILD than c-TMA. Application of S-TMA on ILD cases may contribute accelerating upcoming molecular-expression studies in the area of ILD.

1834 Prognostic microRNA Signature in Early Stage Lung Adenorcarcinomas (ADK): Preliminary Results

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Background: 20% of stage I Non Small Cell Lung Carcinomas (NSCLC) relapse within 2 years after surgery. Therefore, the identification of biologic prognostic factors of early stage NSCLC is relevant. The clinical significance of miRNAs signature in early stage NSCLC remains unclear. The purpose of this study was to evaluate the prognostic value of microRNAs in patients with stage I ADK.
Design: Crieteria of eligibility were T^1-T_2N^0 primary lung ADK treated with surgery between 2005 and 2007. Nine patients presented local or metastatic recurrence (= group 1). Each of these patients were matched with 2 patients without any recurrence (= group 2), according to the following criteria : tumor size, pleural involvment, adjuvant chemotherapy, % of tumor cell within the tumor sample, TTF-1 status. MicroRNA microarray expression profiling of tumors and paired nontumorous tissues was performed on these 27specimen.Total RNAs were extracted with TRIzol solution from frozen sample. MicroRNAs were purified on column with the Mirvana kit and chemically labelled with Alexa Fluors. Labelled-miRNA hybridized on microChip containing 2054 probes, including the whole human microRNome. The expression of each microRNA was quantified in tumor and corresponding healthy tissue. Disease-free survival (DSF) was defined with the Kaplan-Meier method.
Results: Tumor samples had a specific microRNA signature compared with healthy tissue(such as down regulation of mir-126, mir-30, let 7, mir-145, up regulation of mir-21 and mir –35). A hierarchical clustering was performed in 35 selected microRNA.When comparing levels of expression of microRNA between group 1 and 2, 4 microRNAs were significantly associated with cancer recurrence : up regulation of mir-21, mir-297, and downregulation of mir-30a and mir-99a, consisting in a specific microRNA signature. Two-years DFS was 33% in patients with the specific Micro-RNA S signature (MRS) vs 89% for the others ($p<0.0001$). In this set of patients, sensitivity and specificity of the specific MRS for disease rcurrence was 78% and 89%, respectively.
Conclusions: This study is the first to demonstrate that a specific MRS of early stage lung ADK could predict the risk of cancer recurrence. These results are under validation in a multicentric independant cohort of stage I lung adenocarcinomas in order to transfert these findings in clinical practice.

1835 Array CGH on Archival Fixed Paraffin Embedded Tissues on a Series of Malignant Mesothelioma

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Background: Array based comparative genomic hybridization (aCGH) is a promising tool for molecular subclassification in malignant mesothelioma (MM). Because of the interest to perform retrospective studies applied to a specific focus on the slide, we aim to evaluate the reliability of archival FFPE tissues on a series of MM cases.
Design: Total DNA was extracted from 15 frozen and 9 FFPE tissue samples retrieved from the MESOPATH center. FFPE were selected up to 10 years, with time of fixation up to 7 days. aCGH was performed on a 180 K pangenomic Agilent array yelding a 13kb overall median probe spacing. The sensitivity of aCGH was investigated using serially diluted tumor DNA specimens. The minimum percentage of cells and amount of DNA was also evaluated.
Results: Focusing on FFPE samples we tested ULS and Klenow method for aCGH. Noticeably the Klenow one was the best. We also obtain better quality increasing the amount of recommended DNA. One of the crucial points was to co-hybridize same quality DNA. Therefore we extracted tumoral and non tumoral DNA from FFPE. Highly sensitive detection required a previous dissection from the H&E to obtain at least 70% of tumor cells. Among the FFPE tumor samples 88% yielded a high quality aCGH showing deleted and amplified fragments ranging from 3.5 Kb up to a full chromosome deletion. Among the many interesting detected aberration, we found a gain of JUN (3.5kb) and an amplification of FOS(37.5kb amplified fragment). The homozygous deletion of 9p21 was observed in 66% of the cases correlated with loss of immunoexpression of p16 in 100%.
Conclusions: Long term archival FFPE is suitable for aCGH analysis if the DNA quantity is adequate with at least 70% of tumor cells.

1836 Protein Expression and Gene Rearrangement of ALK in Non-Small Cell Lung Carcinomas

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Background: A subset of non-small cell lung carcinoma (NSCLC) is associated with anaplastic lymphoma kinase (ALK) gene translocation, mainly forming echinoderm microtubule associated protein like 4 (EML4)-ALK fusion gene. The frequencies of ALK gene translocation have been variably reported depending on detection methods and geographic areas. We investigated the frequencies of (1) ALK protein expression, (2) fusion gene EML4-ALK and (3) the correlation between protein expression and transforming fusion gene EML4-ALK in 470 NSCLC specimens of Korean patients.
Design: From May 2003 to December 2008, 470 consecutive surgically resected cases of NSCLC at Seoul National University Bundang Hospital were enrolled in this study. ALK protein expression was detected by immunohistochemistry and EML4-ALK gene translocation by fluorescent in situ hybridization (FISH) using LSI ALK dual color break-apart rearrangement probe.
Results: ALK protein immunoreactivity was detected in 30 out of 470 NSCLC cases (6.4%), consisting of 28 adenocarcinomas, one squamous cell carcinoma and one atypical carcinoid tumor. On FISH analysis, 14 out of 470 NSCLC cases (3.0%) showed ALK locus rearrangement, all of which showed ALK protein expression by immunohistochemistry. Specifically, ALK gene break-apart was observed in nine adenocarcinoma cases, while another five cases containing four adenocarcinomas and one squamous cell carcinoma showed isolated orange signal.
Conclusions: ALK immunoreactivity in NSCLC was correlated with ALK locus translocation by FISH despite immunohistochemistry and FISH analysis showed a discrepancy in detection rate. These results suggest that ALK immunohistochemistry may be a useful detection method to screen the presence of EML4-ALK fusion transcript. The two methods might be mutually complementary if the mechanism of the discrepancy is well clarified, which would provide more information for new therapeutic options.

1837 Sarcomatoid Peritoneal Mesothelioma: Clinicopathologic Correlation of 13 Cases

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Background: Peritoneal mesothelioma is rare and the sarcomatoid variant is more infrequent. Several case series have described the morphologic features of sarcomatoid peritoneal mesothelioma (SPe); however, the clinicocopathologic features are not well characterized.
Design: We reviewed our database of 2859 mesothelioma cases, of which 2524 (88%) were pleural and 335 (12%) were peritoneal. Of the peritoneal mesotheliomas, 252 (75%) were epithelioid, 70 (21%) were biphasic, and 13 (4%) were sarcomatoid variant.
Results:

Table 1: Demographics, Pathologic Findings, and Fiber Analysis

Case	Age	Sex	Occupation/Exposure	Parietal Pleural Plaques	Asbestosis	Tumor Type	Asbestos Content
1	70	M	Johns-Manville, many yrs.	Y	Y	DSPe	ND
2	74	M	Shipyard electrician, 16 yrs; Power plant worker, 21 yrs.	Y	ND	SPe LHC	ND
3	66	M	Johns-Manville, 39 yrs.	Y	Y	DSPe	ND
4	70	M	Merchant marine seaman, 23 yrs.	ND	ND	SPe	ND
5	65	F	HHC; Husband, shipyard worker	N	N	SPe	Elevated
6	69	M	Shipyard insulator, 49 yrs.	Y	ND	SPe	ND
7	70	M	ND	N	Y	SPe	Elevated
8	59	F	HHC; Husband, construction worker, 42 yrs.	ND	ND	SPe	WNL
9	59	M	Construction, 31 yrs.	ND	Y	SPe	ND
10	49	F	HHC; Husband, power plant	ND	ND	SPe H	WNL
11	54	M	Janitor	ND	ND	SPe	ND
12	53	M	ND	N	ND	SPe H	ND
13	72	M	Electrician, 24 yrs	N	ND	SPe	ND

HHC, household contact; SPe, sarcomatoid peritoneal mesothelioma; DSPe, desmoplastic SPe; LHC, lymphohistiocytic; H, heterologous elements; ND, no data; WNL, within normal limits

Conclusions: To our knowledge, this is the first large series reporting the clinicopathologic features of SPe. The average age at diagnosis was 65.5 years and there was a male predominance (M:F = 3:1). All cases stained positive for cytokeratins, and two contained heterologous elements. Seven cases had objective markers of asbestos exposure (plaques, asbestosis, and/or elevated lung asbestos content), and two additional cases (4, 13) had occupations that are strongly associated with mesothelioma. Two cases with alleged household contact exposures (8, 10) could not be confirmed by lung fiber analysis. SPe is a rare variant of mesothelioma that is frequently related to asbestos exposure (9/13 cases).

1838 Different Prevalence of Transactivating (TA) p63 and Non-TAp63 Isoforms in Pulmonary Adenocarcinomas: A Useful Diagnostic Tool

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Background: p63 protein, a member of the p53 family of nuclear transcription factors, is a fundamental player in the development of pulmonary squamous cell carcinomas (PSSCs), but little is known about the prevalence of different p63 isoforms in pulmonary adenocarcinomas (PACs).
Design: We assessed twenty PACs for p63 by using immunohistochemistry on paraffin sections with 1A4 clone, recognizing all p63 isoforms, and p40 polyclonal antibody recognizing all non-TA p63 isoforms. Moreover, paired frozen samples of the same

tumors were analyzed by real-time PCR assay for the 10 different p63 isoforms thus far recognized (α, β, γ, δ, ε), of either TA or non-TA type. A few PSCCs were used as controls for assays.
Results: p63 immunoreactivity with clone 1A4 was found in 15% of PACs (range 10-70% tumor cells), independent of growth patterns, whereas none exhibited p40 immunostain, revealing a strong prevalence of TA isoforms. PSCCs were always diffusely positive for both 1A4 and p40 antibodies. Real-time PCR analysis confirmed that TA isoforms prevailed by far on non-TA isoforms in PACs (with a prevalence of α over β), whereas the reverse held true for PSCCs (with a prevalence of α over β, γ and δ, in that order). In turn, ε isoform was never found in either PACs or PSCCs.
Conclusions: The absence of p40 immunoreactivity in PACs, paralleling a strong prevalence of TA isoforms, may be a useful diagnostic clue when the distinction from PSCCs is crucial for neoadjuvant therapy, for example in the setting of poorly differentiated tumors and/or small biopsies.

1839 PAX-2 and Napsin A in the Differential Diagnosis of Lung Versus Kidney Primary Site in Patients with Renal Cell Carcinoma and a New Lung Mass: A Comparison with TTF-1 and RCC Antigen

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Background: In the work-up of metastatic renal cell carcinoma (RCCA) versus primary lung carcinoma, some of the immunohistochemical (IHC) markers commonly used for a renal primary, such as CD 10, EMA, and vimentin, are not helpful because of lung reactivity. More useful markers include TTF-1, a lung marker, and RCC antigen, a renal marker. Since these IHC markers are not 100% sensitive nor specific, the availability of an expanded panel can be of value in indeterminate cases in the work-up of metastases of unknown primary or when the differential diagnosis is metastasis versus lung primary. We compare two newer markers, Napsin A for lung and PAX-2 for kidney with these more established markers.
Design: Using a tissue microarray, we compared 88 non-small cell lung carcinomas (34 squamous, 42 adenocarcinomas, and 12 large cell carcinomas, including 4 large cell neuroendocrine carcinomas) with 52 RCCA (25 clear cell, 10 chromophobe, and 17 papillary). We interpreted the stain as positive if 5% or more of the malignant cells stained, regardless of intensity, as follows: TTF-1 and PAX-2 nuclear staining, RCC and CK 7 cytoplasmic staining, and Napsin A granular cytoplasmic staining were evaluated.
Results:

IHC Results:

	RCC clear	RCC papillary	RCC chromophobe	Lung squamous	Lung adeno	Lung large cell
PAX-2	16/25 (64%)	13/17 (76%)	5/10 (50%)	0/31 (0%)	0/38 (0%)	0/12 (0%)
RCC antigen	12/25 (48%)	16/17 (94%)	1/10 (10%)	0/32 (0%)	2/40 (5%)	0/12 (0%)
TTF-1	0/25 (0%)	0/17 (0%)	0/10 (0%)	2/31 (6%)	36/39 (92%)	8/12 (67%)
Napsin A	11/25 (44%)	17/17 (100%)	7/10 (70%)	9/31 (29%)	37/40 (93%)	7/12 (91%)
CK7	7/25 (28%)	13/16 (81%)	6/10 (60%)	12/30 (40%)	39/39 (100%)	10/11 (91%)

Number of positive cases/Total number (Percentage of positive cases)

Note: A few cases were excluded if the corresponding "dot" of the case had no tumor present or had been lost in processing.
Conclusions: For the distinction of lung non-small cell carcinoma versus RCCA in the work-up of metastases of unknown primary, or separation of metastatic RCCA in patients with a history of RCCA and a new lung mass, we found that TTF-1 is very specific for lung, and RCC and PAX-2 are very specific for kidney. CK 7 and Napsin A were not helpful in the work-up.

1840 Evaluation of miRNAs Levels of p53 Pathways in Non Small Cell Lung Cancer

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Background: The tumor suppressor gene p53 has been broadly studied in Non Small Cell Lung Cancer (NSCLC). It is found mutated in 55% of cases in NSCLC. MicroRNAs (miRNAs) are small RNA molecules that regulate mRNA translation to protein. Some miRNAs have been shown linked to p53 network. Protein p53 through his DNA binding domain activates the expression of miR-34 family in response to DNA damage or oncogenic stress, to induce apoptosis or cell cycle arrest. Recently, it has been described a novel p53 interaction through Drosha complex proteins, which allows p53 to regulate the maturation process of miR-16 and miR-143 to suppress cell proliferation.
Design: To explore the relationship between p53 mutation and miRNAs we have studied the clinical implications of miR-16 and miR-143 expression levels in 70 NSCLC patients and their relation to p53 mutations and miR-34a levels. It was possible to assess by sequencing p53 mutations in just 60 samples. We performed the same evaluation in normal tissue and in the tumor of all patients.We studied the expression of miR-16, 143 and 34a by real time PCR and analyzed data with SPSS 15.0.
Results: The data showed that miR-16 and miR-143 were downregulated in tumor tissues compared to normal tissues ($p<0.001$ and $p=0.001$ respectively). Concerning miR-16 expression, it emerged as an independent factor only for overall survival (OS) (RR=2.17; p=0.004). In the analysis that included miR-16 and p53 mutational status, both emerged as independent factors for disease free survival (DFS) (RR=1.9; p=0.024), but only mR-16 confirmed itself as an independent factor for overall survival. The analysis of miR-16 and miR-34 allow us to stratified three groups with different prognosis for DFS (p=0.002) and for OS ($p<0.001$) inside the patients that showed high miR-34a expression (good prognosis patients).
Conclusions: MicroRNA-16 appeared as a good marker for overall survival and seems to play a synergic role with miR-34a inside the p53 pathway. However, there is no relationship of their expression levels and p53 mutations in our set of patients. These miRNAs may be good targets for new therapeutics strategies in NSCLC. Supported by: FIS-PI060087 and FIS- PI040123; SEPAR; CIBERES.

1841 Diagnostic Significance of Cell-Kinetic Parameters in WHO Type A and B3 Thymoma and Thymic Carcinoma

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Background: Recent studies have shown only moderate interobserver agreements for the WHO classification of thymoma and the distinction between thymoma and thymic carcinoma (TCa). Our data showed common disagreements for WHO B3 thymoma vs TCa, followed by WHO A vs B3, and A vs TCa, disagreements of 26%, 7.4% and 6.7%, respectively. However, the prognosis of TCa is much worse than thymomas with reported 5-yr survival of 28%. Furthermore, several studies suggest that WHO B3 thymoma have worse prognosis than other thymoma with reported 5-yr survival as low as 51%, in contrast to WHO A thymoma with usual 5-yr survival of 100%. Therefore, adjunctive studies are sought to facilitate the distinction between these histological subtypes.
Design: Medical records from 64 patients with thymic neoplasm (1946-2008) were reviewed. Two pathologists independently classified all cases according to WHO and agreed upon type A (n=31), B3 (n=22) or TCa (n=11). Ki-67 LI (n=56) was expressed as %pos/100 epithelial cell (EC) nuclei (mean of 1000 EC counted over 3 areas) and mitotic activity (MA) (n=64) as mitoses/10Hpf (mean of 50Hpf counted). Bcl-2 (n=56) expression was graded as 0 (neg) through 3 (>90% EC+). Statistical analyses were performed. Data are presented as median (Q1,Q3) for each group, and were compared with Kryskal-Wallis tests.
Results: 40 men & 24 women had a median age of 59.4 yrs.

Results of Cell-kinetic Parameters by WHO Type

	KI67 LI	MA	Bcl-2
A[1]	3.0 (2.4,5.3)	1.6 (0.8,4.4)	1.0 (1.0,3.0)
B3[1]	7.5 (5.7,13.7)	3.3 (1.2,5.6)	1.0 (1.0,1.0)
TCa[1]	23.2 (17.9, 39.9)	21.0 (6.4,23.4)	2.0 (1.0,3.0)
A vs B3[2]	**<0.0001**	0.17	0.019
B3 vs TCa[2]	**0.0066**	**0.0003**	0.97
A vs TCa[2]	**<0.0001**	**0.0001**	0.11

[1]median (Q1,Q3); [2]p-values, **only p≤0.017 are considered significant**

Significant Bcl-2 expression (>90% EC) was observed in types A (n=12/29), B3 (n=2/16) and TCa (n=5/11). However, there was no significant difference in Bcl-2 expression between types A, B3 and TCa. Ki67 LI significantly differed between WHO A and B3 thymomas and TCa. MA was not significant in distinguishing WHO A vs B3 thymomas.
Conclusions: Ki67 and MA differ significantly between WHO types A, B3 and TCa and might represent a useful tool to distinguish between these subtypes. Although Bcl-2 might be important in the pathogenesis of thymic neoplasms, its expression by EC was not of diagnostic significance.

1842 Usual Interstitial Pneumonia in Lung Resections for Carcinoma

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Background: Usual interstitial pneumonia (UIP) is the defining histologic feature of idiopathic pulmonary fibrosis, a form of progressive lung fibrosis. Studies have observed an increased incidence of peripheral squamous cell carcinomas (SCC) in patients with UIP. We determined the incidence of UIP in lungs resected for SCC and non-SCC lung carcinomas.
Design: This is a retrospective observational case-based study in which hematoxylin and eosin stained slides from all lung resections (lobectomy, bilobectomy, or pneumonectomy) for primary lung SCC from 1999-2009 (n=164) were reviewed. A cohort of resected non-SCC carcinomas (n=183) was also reviewed. Slides were evaluated for tumor type and tumor location. Tumors were classified as peripheral if they were located <3 cm from visceral pleura without involving a cartilaginous airway. Sections taken from non-neoplastic lung were assessed for degree of fibrosis, honeycomb change, respiratory bronchiolitis, emphysema, and osseous metaplasia and classified as UIP, probable UIP (>25% fibrosis and honeycomb change but without characteristic patchwork distribution), or not UIP. Clinical data was abstracted from medical records.
Results: UIP/probable UIP was diagnosed in 9.1% of SCC resections and 5.5% of non-SCC resections (p=0.02; see Table). UIP/probable UIP was present in 7.2% of resections for peripheral tumors and 7.1% of resections for central tumors regardless of tumor type; there was no statistically significant increase in UIP/probable UIP between peripheral and central SCC or between peripheral and central non-SCC. UIP was originally diagnosed in only 6.3% and 30% of affected patients with SCC and non-SCC, respectively.

Table: Summary of Clinical and Histologic Findings

	SCC without UIP (n=148)	SCC with UIP/probable UIP (n=15)	Non-SCC without UIP (n=173)	Non-SCC with UIP/probably UIP (n=10)
Peripheral tumor	71	8	121	7
Central tumor	77	7	52	3
Mean age , yrs (range)	68 (44-85)	68 (60-78)	66 (26-85)	70 (54-87)
M:F	1.7:1	3:1	0.7:1	9:1
Mean size, cm (range)	3.7 (0.6-12)	3.3 (0.8-9)	3.0 (0.5-13)	4.1 (1-6.7)

Conclusions: UIP is present in 5-10% of patients who undergo resection for lung carcinoma and is more common in men and patients with SCC. Careful sampling and review of non-neoplastic lung is important in resected lung cancers.

1843 Expression of Estrogen Receptor Beta 1, but Not Estrogen Receptor Beta 2 or Alpha Is Linked to Worse Prognosis in Stage I Adenocarcinoma, in Women, in a Large Epidemiological Cohort but Not in a Smaller, Single-Hospital-Based Series

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Background: Data support the existence of gender differences in the incidence and mortality of lung cancer. However, the extent of such differences and its biological basis remain incompletely characterized. Particularly, the role that sex hormone receptors have on survival and their relation to the clinical and pathological variables of Non Small Cell Lung Cancer (NSCLC) remain poorly defined.

Design: We studied estrogen receptor alpha, beta 1 (EstRB-1) and beta 2 by immunohistochemistry in a series of 504 NSCLC, accrued through a local cancer registry, as a part of an ongoing epidemiological study of lung cancer (series 1). These included 397 women, 107 men; 349 adenocarcinoma, 75 squamous cell carcinoma, 50 poorly differentiated NSCLC, 30 of other histology, with a mean follow-up of 64.7 months. We also studied (series 2) 72 adenocarcinoma consecutively resected at our institution, composed of 28 women, and 44 men, with a mean follow-up of 130 months. All markers were scored separately for intensity (1 lowest, to 3 highest) and extent of positivity (1≤10%;2=11-50%;3≥50%). The two scores were multiplied to generate a final score (1-9): cases where this was ≥ 3 were considered expressors.

Results: Among the hormone receptors studied in series 1, only a positive EstRB-1 constitutes a predictor of reduced prognosis and this is seen only in women with stage I adenocarcinoma (mean follow-up 63.9 months, p value=0.02); a positive EstRb-1 has no relation to age, gender or race. In series 2, EstRB1 does not constitute a significant predictor of survival, in either local or advanced disease, men or women.

Conclusions: 1) Expression of Estrogen Receptor Beta-1, but not estrogen receptor beta 2 or alpha, affects survival in adenocarcinoma but not other histiotypes of NSCLC. 2) EstRB-1 only affects survival in women and stage I disease.3) The effect of EstRB-1 on prognosis is revealed by analysis of a large cohort, while goes unnoticed in a small single-hospital based series.4) EstRB-1 levels may contribute, in conjunction with other factors, to the reported gender differences existing in the biology of lung cancer.

1844 Cyclooxygenase-2 Overexpression Is Linked to Increased VEGFR-3 Levels in Lung Adenocarcinoma but Its Association with Survival Is of Borderline Significance

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Background: Cyclooxygenase-2 (Cox-2) regulates important pathways in carcinogenesis, including angiogenesis, and has been a target of molecular therapy in clinical trials, in Non Small Lung Cancer (NSCLC).Yet, it remains unclear whether its expression has a prognostic role in NSCLC and what its relation to the other molecules involved in angiogenesis is.

Design: In order to define the prognostic role of Cox-2 in NSCLC we studied whether its expression predicts prognosis, in resected adenocarcinoma (Aca) and Squamous Cell carcinoma (SqCC) and whether its expression is linked with that of Vascular Endothelial Growth Factor Receptor 3 (VEGFR3), in Aca. 59 Aca (34 stage I, 9 stage II and 16 stages III-IV; mean follow-up 131 months) and 27 SqCC (16 stage I, 6 stage II, 5 stages III-IV, mean follow-up 67 months)were evaluated for Cox-2 expression, using immunohistochemical analysis. Expression of Cox-2 and VEGFR3 were scored based on staining intensity (0;1=low to 3=highest) and percentage of positive cells (1≤10%; 2=11-50%; 3=>50%). A final score was obtained multiplying the two scores (0 to 9) and cases were classified as low (0-4) or high (6-9) expressors. Survival data were obtained through the charts and the Detroit Metropolitan Cancer Surveillance System.

Results: Kaplan-Meier survival analysis showed that high Cox-2 expression has a trend in predicting better survival in Aca, when all stages were analyzed (p=0.056), whereas it showed no statistical association with survival when only stage I were studied. In SqCC, Cox-2 levels were not predictive of survival, at any stage. In Aca, Cox-2 high expression significantly correlated with high VEGFR3 expression (p=0.037).

Conclusions: 1- The trend we found for an association with survival of Cox-2 levels, in Aca confirms most published data, supporting the hypothesis that Cox-2 does not have a major influence on the prognosis of NSCLC. 2-Cox-2 may have a different impact on the biology of Aca and SqCC, including its prognosis. This may be due to the inherently different pathobiology of these cancers. 3-The correlation of high Cox-2 expression with high VEGFR3 expression we found confirms the reported role of Cox-2 in tumor angiogenesis. However, further studies on the interaction of Cox-2 with the other growth factors, inhibitors and receptors of this pathway are needed to understand the molecular details of this interaction and its ultimate impact on vessel formation in NSCLC.

1845 Usefulness of *Ki-67* for Predicting Metastatic Potential of Carcinoid Tumour of the Lung: A Study of 48 Cases

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Background: *Ki-67* is an emerging proliferation marker for the evaluation of high risk subsets of Lung Carcinoid Tumors (LCTs). The objectives of this study are 1) to evaluate the usefulness of *Ki-67* index as well as mitoses and tumor size in predicting metastasis 2) to compare the Manual Conventional Method (MCM) and the Computer Assisted Image Analysis Method (CAIAM) for its calculation.

Design: Forty-eight patients with LCTs were studied from 1982-2007. For *Ki-67* immunostudy two sections of tumor were stained (Vector laboratories; clone MM1). Digital images of 5000 cells were counted using a image processing software (Northern Eclipse version 7.0) and 2000 cells by MCM (Zeiss microscope, 40 x objective). Mitoses/10HPF were counted.

Results: The age of the patients ranged from 17-81 (mean 52, 18 M, 30 F). Of the 48 patients, 7 developed metastasis in lymph node (6), liver (1) or both (2). Median follow up for metastatic (MG) and non-metastatic groups (NMG) were 45 and 35 months respectively. The mean tumor size was 2.7 cm (range 0.5-9.5). 37 were typical carcinoids (TCs) and 11 atypical carcinoids (ACs) (Travis criteria). There was a strong correlation between *Ki-67* index by MCM (1.5) and CIAM (0.75) (r = 0.929, *P*= .001). There were positive relationships between metastasis and carcinoid type (*P*=.039) and mitoses (≥2) (*P*=.017). Although not statistically significant, the mean *Ki-67* index for ACs was higher than for TCs by both counting methods (0.95% vs. 0.72% by CIAM, *P*=.299; 2.32% vs.1.37% by MCM, *P*=.71). Similarly although not statistically significant, the mean *Ki-67* index for MG was higher than for NMG (1.01% vs. 0.71% by CIAM, *P*=.281; 2.10% vs. 1.39% by MCM, *P*=.239). However when *Ki-67* index data was categorized at various levels, there is suggestion of a useful cutoff (≥0.50%) to predict metastasis (*P*= .106 by CIAM, .166 by MCM). A significantly higher proportion of patients with mitosis ≥2 and *Ki-67* index ≥0.50% had metastasis (*P*=.033) than other patients. Similarly patients with tumor size ≥3cm and *Ki-67*≥0.50% had a greater percentage of metastases than others (*P*=.039).

Conclusions: This study confirms that mitoses ≥2 is a powerful predictor of metastasis in LCTs. Analysis of *Ki-67* index along with mitoses may be a useful adjunct for predicting metastasis in LCTs for which adjuvant therapy may be considered.

1846 Sox2 Protein Expression Is an Independent Poor Prognostic Indicator in Stage I Lung Adenocarcinomas

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Background: Despite surgical excision, 40% of patients with stage I non-small cell lung carcinoma will recur. Histologic parameters such as tumor subtype, grade, and the presence of lymphovascular invasion are inconsistent predictors of outcome. Prognostic immunohistochemical markers may help to identify patients at high risk of recurrence. Sox2 is a marker of embryonic stem cell pluripotency that has been associated with aggressive tumor behavior and is expressed in some germ cell tumors, lung and esophageal squamous cell carcinomas, and a subset of lung adenocarcinomas. We hypothesized that Sox2 expression may provide prognostic information in early stage lung adenocarcinomas.

Design: Formalin-fixed paraffin embedded lung adenocarcinomas were retrieved from the surgical pathology files. We evaluated a test cohort of 62 lung adenocarcinomas resected between 1997 and 1999, including 37 stage I tumors, and a validation cohort of 67 stage I lung adenocarcinomas resected in 2000. Sox2 expression was analyzed by immunohistochemistry and compared to clinicopathologic features and time to progression (TTP), defined as the interval between date of surgery and clinical or radiographic progression.

Results: In the test and validation cohorts, respectively, males comprised 35 and 44%, median age was 70 and 65 years, smokers comprised 78 and 81%, and lobectomy (versus wedge resection) was performed in 68 and 60%. Overall, 75% of adenocarcinomas were mixed subtype; acinar was the most common predominant pattern. Sox2 expression was detected in 40% of the test cohort and 52% of the validation cohort. Sox2 expression was not associated with age, gender, smoking status, tumor stage (Ia vs Ib), grade, or histologic subtype. Survival analysis showed that patients with stage I adenocarcinoma with Sox2 expression had a shorter TTP than those without expression (n=17, HR=7.3, P=0.005 in the test cohort; n=35; HR=2.7, P=0.02 in the validation cohort). By multivariate analysis, Sox2 independently predicted a shorter TTP in both the test and validation cohorts (P=0.02 and P=0.003, respectively).

Conclusions: In this study we show that Sox2 is expressed in approximately half of stage I lung adenocarcinomas and is an independent predictor of poor outcome, and we confirm the findings in an independent validation dataset. Sox2 expression can help stratify patients with stage I adenocarcinoma who have an increased risk of recurrence.

1847 Identification of Early Pathologic Markers of Interstitial Lung Diseases

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Background: The absence of longitudinal pathologic data in patients with developing interstitial lung disease (ILD) limits our understanding of the pathogenesis of these diseases. We retrospectively examined surgically resected "uninvolved lung" taken from patients with stage I non small cell lung carcinoma (NSCLC) in an attempt to discover early features of ILD, particularly usual interstitial pneumonitis, in this susceptible population. We correlated the pathologic findings at the time of surgical resection with the subsequent clinical and radiologic outcomes.

Design: Microscopic slides of uninvolved lung for 66 sequential patients who underwent surgery for stage I NSCLC in 2000 were evaluated by two pulmonary pathologists. None of the patients had clinical or radiologic evidence of ILD at the time of surgical treatment. Follow-up chest radiology and clinical features were extracted from the medical record.

Results: Mean patient age at time of surgery was 70, with 38 (58%) women and 54 (82%) smokers. Mean follow-up was 5 years and median survival was 7.8 years. 57 (86%) had at least mild emphysema, 22 (33%) had respiratory bronchiolitis, 19 (29%) had at least focal interstitial fibrosis, and 20 (30%) had at least moderate vascular disease. Two patients (3%) were subsequently radiologically diagnosed with ILD during follow-up; neither patient had received adjuvant therapy. One patient who developed bilateral lower-lobe predominant honeycomb change after 3 years had patchy subpleural interstitial fibrosis with small scattered fibroblast foci and minimal architectural distortion on the original biopsy. The other patient who developed bilateral subpleural reticular opacification and honeycombing after 8 years showed NSIP/DIP-like changes on the original biopsy.

Conclusions: Retrospective pathologic review of surgical lung cancer resections is a feasible approach to identifying the early lesions of ILD before they become clinically or radiologically manifest. Further study of these early lesions by molecular genetic techniques may provide insight into the early stages of development of these diseases. In our initial study, smoking-related changes were common findings in the lungs of patients with early stage NSCLC. These findings warrant a larger study to identify specific histologic features that predict which patients may go on to develop bona fide ILD.

1848 Subtyping of Non-Small Cell Lung Carcinoma (NSCLC): Comparison of Cytology and Small Biopsy Specimens

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Background: The accurate classification of NSCLC has gained importance due to the emergence of histology-based treatment options. The majority of NSCLCs are unresectable at presentation and treatment is usually determined by small biopsy (Bx) or cytology (Cyto) specimens. While Cyto has been established to be superior to Bx for the diagnosis (Dx) of small cell lung carcinoma (SCLC), the relative efficacy of these modalities in subtyping of NSCLC has not been established.

Design: A review of the departmental database was conducted to identify all primary lung carcinomas with concurrent Cyto and Bx specimens including cases with subsequent resection during a two-year period (9/1/2006-9/1/2008). Metastases, SCLCs and carcinoids were excluded. In our clinical practice, Cyto and Bx specimens are reviewed independently. 102 paired specimens were identified (M:F ratio 1:1.9, average age 68, age range 37-91) including fine needle aspirates with core Bx (n=66), and bronchial wash/brush/lavage specimens with transbronchial Bx (n=36). 20 cases had subsequent resection.

Results: Of 102 cases, Cyto Dx was definitive (adenocarcinoma or squamous cell carcinoma) vs favored vs unclassified in 70% vs 19% vs 11%, whereas the distribution for Bx was 72% vs 22% vs 6%, respectively. The unclassified rate was reduced to 4% when the two modalities were considered together. Overall, Cyto was more definitive in 13% of cases, and Bx in 17%. All Cyto Dx except 2 were rendered purely on morphology, whereas immunohistochemistry (IHC) (typical panel TTF-1, p63, 34βE12) aided in the classification of 22% of Bx. Tumor type was concordant in Cyto and Bx for 93%, whereas discordant Dx were reached for 7% of cases (n=7). For these cases, resection and/or additional IHC revealed the correct Dx was rendered by Cyto in 3 cases and Bx in 4 cases. Tumor type in all concordant Cyto/Bx specimens was supported by subsequent resection.

Conclusions: We find that in our routine clinical practice both Cyto and Bx achieve high rate of NSCLC subtyping with comparable accuracy. Combining the modalities reduces the rate of unclassified NSCLC to 4%. Although the use of IHC may be withheld in Cyto with a known paired Bx, morphological features alone are sufficient to subtype the majority of cases. Furthermore, similar to SCLC, Cyto is superior to Bx in classification of NSCLC in a subset of cases. Our data confirms the suitability of both small Bx and Cyto specimens, particularly when combined, for histology-based treatment paradigms.

1849 Prognostic Significance of Cytologic Features of Lung Adenocarcinoma, Mixed Subtype

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Background: Adenocarcinoma (AD) mixed subtype, the most common histologic type of lung cancer, is characterized by a variety of different architectural patterns. Recently, a three-tiered histologic pattern-based grading system was developed for stage I lung AD which stratified patients into low, intermediate, and high risk categories for disease recurrence. However, many lung cancer patients present with inoperable disease and cytology may serve as the primary method for diagnosis. Attempts to correlate architectural arrangements between parallel cytologic and histologic preparations have not been successful. Therefore, we evaluated the cytomorphologic features of AD in patients previously scored by a histologic grading scheme to identify features of potential prognostic significance.

Design: Retrospectively, we reviewed the FNA specimens from 79 patients with mixed subtype ADs while blinded to a risk-stratification score previously assigned using a histologic pattern-based grading system. Specimens consisted of Diff Quik, H&E, Papanicolau, and ThinPrep stained slides. The following cytomorphologic features were evaluated: cell groups (flat sheets vs. 3D clusters vs. single cells), nuclei (size variability, shape, and contour), nucleoli (single vs. multiple and/or macronucleoli), presence of nuclear inclusions, chromatin (fine, coarse, or clumped), and the quality of the smear background. Then, we grouped the specimens according to the three grades of histologic tumor differentiation and analyzed the distribution of these cytologic characteristics.

Results: Histologically well-differentiated tumors correlated with the following cytomorphological characteristics: a clean background (100%), small nuclear size (<5x area of a resting lymphocyte) (p=0.0372), a predominance of flat sheets (p=0.0001), single nucleoli, and nuclear size uniformity. Features correlating with poor histologic differentiation included a wide variation in nuclear size, giant tumor nuclei, and a predominance of 3D clusters (p=0.0001). There was no difference among the grades of tumor with regard to the presence of nuclear inclusions, the presence of single tumor cells, or the relative prominence of nucleoli (p>0.05).

Conclusions: We have identified several distinctive cytologic features of AD, mixed subtype, that correlate with levels of histologic differentiation shown to have prognostic significance.

1850 ERCC1,VEGFR, NF-kB and Gender in Chemotherapy-Treated Advanced Stage NSCLC: An Ongoing Study Report

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Background: Excision repair cross-complementation group 1 (ERCC1) expression/resistance to cisplatin therapy has been corroborated by studies in patients with advanced non-small cell lung cancer (NSCLC). VEGFR has been studied as prognostic indicator. NF-kB or p65 transcription factor expression is seen in cancers with advanced local growth. Gender may be a prognostic factor in advanced lung cancer. Our aim was to evaluate ERCC1, VEGFR, NF-kB expression, and gender as prognostic indicators in advanced stage NSCLC patients given cisplatin-based and non-cisplatin based therapy.

Design: Advanced stage NSCLC patients from the VAMC and University of Cincinnati were retrospectively reviewed from 1998-2007. We reviewed the tissue sections for adequacy and selected 40 patients treated with cisplatin-based chemotherapy and 10 treated with non-cisplatin based chemotherapy. IHC for ERCC1 (Mouse monoclonal ab-8F1, Thermo Scientific, 1:200), VEGF-KDR, (rabbit monoclonal 1:200, Cell signaling) and NF-kB (rabbit monoclonal C-20 1:100; SCBT) were independently graded (0 to 2+ for ERCC1; 0 to 3+ for NF-kB and 0 to 2 for VEGFR). ERCC1, VEGFR, NF-kB reactivity were correlated with overall survival (defined as date of diagnosis to time of death from any cause or last follow up (Cox regression model; Kaplan Meier).

Results: Of 40 patients (mean age=64; range: 42-84); treated with cisplatin, ERCC1 negativity was strongly associated with longer survival (p=0.007); high expression of VEGF-KDR receptor showed better overall survival of 14 vs 9.7 months with lower expression, (not reaching statistical significance). NF-kB (p65) showed expression in 4/46 patients (9.9 months survival vs 13 months with no expression). Women (n=8/all cisplatin based therapy) compared to men (n=42) had a statistically significant better median survival of 31.7 compared to 12.5 months (p=0.04).

Conclusions: In advanced stage NSCLC with chemotherapy: 1. ERCC1 decreased expression may predict longer survival following cisplatin-based therapy. 2. Higher expression of VEGFR may have longer overall survival. 3. NF-kB (p65) expression may have shorter survival. 4. Female gender is associated with longer survival. 5. Studies on a larger sample would be warranted to evaluate these trends.

1851 KRAS Amplification in Non-Small Cell Lung Carcinoma

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Background: Amplification of 12p12.1, containing the KRAS gene, is one of the commonest amplification events in lung adenocarcinoma. Although activating KRAS mutations are well characterized, KRAS amplification as an oncogenic mechanism is relatively unexplored. We have previously demonstrated that KRAS amplification is associated with increased p21 expression in NSCLCs, and that amplification is often associated with an activating KRAS mutation. In the current study, we sought to determine the frequency of KRAS amplification in NSCLC and to identify associated clinicopathologic features.

Design: Fluorescence in situ hybridization, utilizing a probe for the KRAS gene, was applied to a series of 385 NSCLSs, including 300 tumors consecutively resected with curative intent. KRAS amplification was compared with clinicopathologic features derived from a prospectively collected patient database. Statistics: categorical variables, Fisher's exact test; continuous variables, student's t test; overall survival, Kaplan-Meier method and log-rank test; $p<0.05$.

Results: Among 385 NSCLCs, 58 (15%) exhibited KRAS amplification. Amplification was significantly associated with larger mean tumor size (p=0.003), poor differentiation (p=0.004) and pleural invasion (p=0.046). KRAS amplification was not associated with patient age, gender, race, or smoking history; angiolymphatic invasion; or node status. Although the rate of KRAS amplification did not differ between squamous cell and adenocarcinomas, among adenocarcinomas KRAS amplification was far less common in tumors consisting of pure bronchioloalveolar subtype versus those of mixed or invasive subtypes (p=0.004). At a median followup of 2.3 years, no difference in overall survival based on KRAS amplification status was noted.

Conclusions: KRAS amplification is seen in a substantial minority of NSCLCs, and is often but not always associated with an activating KRAS mutation. KRAS amplification is associated with increased p21 protein levels, as well as poor prognostic indicators including larger tumor size, poor differentiation and pleural invasion. Further studies will be necessary to characterize the ocogenic mechanisms of KRAS amplification, its relationship with activating KRAS mutation, and its prognostic significance.

1852 Distinguishing Adenocarcinoma from Squamous Cell Carcinoma in the Lung Using Double Stains: Napsin A and p63 and TTF-1 and CD141

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Background: To distinguish primary lung tumors from metastatic tumors to the lung, a panel of antibodies is used. The current FDA-approved standard treatment for non-small cell lung cancer is Carboplastin/Taxol/Avastin. However, based upon survival benefit; patients with squamous cell carcinoma (SCC) should not receive Avastin due to a 30% mortality rate by fatal hemoptysis. The use of TTF1, CK7 and p63 has been used to differentiate primary lung cancer from metastases. Thrombomodulin (CD141) has been shown to be sensitive for SSC and Napsin A has been shown to be a specific marker of lung adenocarcinoma (LADC); however, information on specificity of Napsin A from other cancer sites is desirable. We investigated the specificity and sensitivity of Napsin A on a wide spectrum of normal and neoplastic tissues including lung cancers and double stain protocols with Napsin A, CD141, TTF1 and p63 were developed.

Design: Tissue microarrays (TMA) were constructed from archival normal and neoplastic tissues. TMAs were deparaffinized in the usual manner and antigen

retrieval was performed. Napsin A was evaluated for specificity and sensitivity. Lung cancer TMAs were evaluated with double-stain procedures for TTF1, CD141, p63 and Napsin A.

Results: Napsin A was positive in 50% of renal cell carcinomas; 21% of thyroid cancers; 2% of ovarian cancers; 0% of cervical cancer (squamous cell) and 16% of cervical adenocarcinomas. Breast, colon, prostate, bladder, seminoma, liver, lymphoma, leiomyosarcoma and pancreatic cancers were all negative. In normal tissues, only lung and kidney were positive. In LADC, Napsin A demonstrated equal sensitivity to TTF1 (77.4%) but was slightly more specific (Table 1). Napsin A was negative in 93.4% of SSC, while CD141 and p63 were positive (89.8% & 75%) in SCC respectively. There were 2 cases of SCC that did not express p63 or CD141, but expressed Napsin A and TTF1.

Table 1

Lung Cancers	Antibody	Cases	Positive
Adenocarcinoma	p63	31	2
	TTF1	31	24
	Napsin A	31	24
	TTF1 + Napsin A	31	26
	CD141	31	5
SCC	p63	49	43
	TTF1	49	4
	Napsin A	49	3
	CD141	49	33
	p63 + CD141	49	44
Adenosquamous Cell Carcinoma	p63	7	4
	TTF1	7	5
	Napsin A	7	5
	CD141	7	4

Conclusions: Double stains provide invaluable information when target antigens are visualized on a single slide. We demonstrated that the combination of Napsin A and TTF1 are a highly-specific in a large proportion of primary LADC, and that p63 and CD141 are sensitive for SCC. These results indicate that Napsin A is a promising marker for differential diagnosis of adenocarcinoma in the lung.

1853 Reproducibility of Histopathological Subtypes in Pulmonary Adenocarcinoma. An International Interobserver Study

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Background: Histological subtyping of pulmonary adenocarcinoma has been associated with predictive and/or prognostic features, but inconsistencies may originate from difficulties in the proper subclassification. This study aimed to establish the reproducibility of these subtypes amongst pulmonary pathologists worldwide.

Design: In a ring study 26 pathologists involved in the IASLC reviewed 115 cases submitted by 20 pathologists, of POWERPoint images consisting of 5 typical histologic patterns: acinar (n=20), non-mucinous BAC (lepidic, n=19), micropapillary (n=16), papillary (n=19) and solid (n=20), in addition to a 6th problem case (n=21). All cases were randomized (JK) and reviewed in 3 subsequent rounds. For each case the reviewer provided a diagnosis of one dominant pattern and also additional pattern(s), if any. Kappa score was separately calculated for the 5 typical patterns and the difficult cases.

Results: In total number of diagnoses for the typical patterns combined and the difficult cases were 2444 and 546, respectively. The mean kappa score (±SD) for the 5 typical patterns combined and for difficult cases were 0.77 ± 0.07 and 0.38 ± 0.14, respectively. More than one pattern was reported for the typical and difficult cases in 35% and 65% of the cases, here the overlap was between papillary and micropapillary (22%); acinar and solid (15%); acinar and papillary (14%); BAC and papillar (10%); acinar and BAC (9%); acinar and micropapillary (6%); BAC and micropapillary (6%) and among several other combinations(19%).

Conclusions: Substantial reproducibility was found for typical patterns of pulmonary adenocarcinoma subtypes. When multiple patterns are present, reproducibility is fair.

1854 CD8 T Cells Promote the Resolution of Prolonged Airway Inflammation in a Murine Model of Asthma

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Background: Human asthma is characterized by persistent airway inflammation, mucus production, and airway hyperreactivity (AHR). The role of $CD8^+$ T cells in the chronic airway inflammation of asthma is controversial. Previous reports have suggested that CD8 effector ($CD62L^{low}CD44^{hi}$), and not CD8 central memory ($CD62L^{hi}CD44^{hi}$) T cells mediate airway inflammation and AHR. Our previous studies revealed that in a murine model of asthma, mice that received Fas-deficient T cells developed a persistent phase of airway inflammation, mucus production, and AHR. Interestingly, although the acute phase of inflammation was dominated by CD4 effector cells, during the persistent phase we found an increase in the number of $CD8^+$ T cells in the BAL, lungs and spleens. These $CD8^+$ cells expressed activation markers that are characteristic of effector T cells, and therefore may actually be driving the active persistent airway inflammation.

Design: 1) To determine whether there are more $CD8^+$ T cells in airways of asthma patients, endobronchial biopsies were collected from 5 severe persistent asthma patients and immunohistochemistry was performed to indirectly determine the presence of $CD8^+$ and $CD4^+$ T cells. 2) To investigate the role of $CD8^+$ T cells in our murine model of prolonged airway inflammation, T cells were adoptively transferred into $Rag^{-/-}$ mice intravenously at day -15. Reconstituted mice were sensitized and challenged with allergen at days -14, -7 and 0, respectively. At the end of the acute phase, $CD8^+$ T cells were depleted *in vivo* by *I.P.* injection of anti-CD8 depletion antibody or control Ig for 4 consecutive days after the last challenge. Mice were sacrificed for study two weeks later to investigate inflammation resolution.

Results: Severe persistent asthma patients appear to have more airway $CD8^+$ T cells than $CD4^+$ T cells. In addition, in the absence of $CD8^+$ T cells, inflamed mice had a prolonged eosinophilia, mucus production, and peribronchial and perivascular inflammation. Furthermore, cytokine analysis showed that $CD8^+$ T cells produced IFN-γ which promoted the resolution of the airway inflammation.

Conclusions: Our study suggests that endogenous effector $CD8^+$ T cells in the airways have a promoting rather than inhibitory effect on the resolution of persistent airway inflammation

1855 SOX2 Amplification Is a Frequent Event in Squamous Cell Lung Cancer

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Background: Transcription factor SOX2 (3q26.3-q27) is a key regulator of pluripotency in embryonic stem cells and cooperates in the generation of induced pluripotent stem cells. In foregut development, SOX2 plays a critical role by maintaining cells in a pluripotent state. Recently, we found that SOX2 is amplified in about 30% of squamous cell lung and esophageal cancers (Bass et al. Nat Genetics. 2009). These findings suggest that SOX2 is activated by amplification as a lineage-survival oncogene. Activated SOX2 might return adult cells into a stemness state and thus participate in the carcinogenesis and progression of squamous cell lung carcinomas.

Design: Aim of our study was to verify SOX2 amplification and protein overexpression in non-small cell lung cancers (NSCLC). A total of 902 NSCLCs from two independent population-based cohorts (New York, NY: adenocarcinomas of the lung (ACL) n=298 ; squamous cell lung carcinomas (SCLC) n=48, and Zurich, Switzerland ACL: n= 243; SCLC: n=273) were assessed by fluorescence in-situ hybridization and immunohistochemistry. Within the SCLC cohort from Zurich, we assessed for association between SOX2 amplification and clinicopathologic features.

Results: In the New York cohort, 5.9 % of ACL and 60.4 % of SCLC showed a low level amplification of SOX2. High level amplification was found in 8.3 % of the SCLC samples. In the Zurich cohort, low level amplification was detected in 6.5 % of ACL and in 63 % of SCLC samples. 9.6 % of SCLC and 0.5 % of ACL exhibited a high level amplification of SOX2. SOX2 amplified cases had a significantly higher SOX2 expression compared to non-amplified samples. Within the Zurich cohort we found that high level SOX2 amplification was significantly associated with higher pN stage and an average gain of 20 cigarette packyears.

Conclusions: We could confirm frequent SOX2 amplification and overexpression in a large subset of NSCLCs. According to our findings, SOX2 amplification is highly specific for squamous differentiation. The clinical relevance of SOX2 amplification status needs to be further analyzed on independent cohorts.

1856 Wnt Signal Pathway and EMT Gene Expressions Are Possible Mechanisms of Acquired Resistance of EGFR-TKI

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Background: Epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKI) such as gefitinib and erlotinib have been used for the treatment of non-small cell lung cancer, but drug resistance eventually develops in most patients despite the initial good response. The acquired resistance of EGFR-TKI was found in the HCC827 lung cancer cell line, which harbored L858R point mutation without T790M mutation or MET gene amplification. The molecular mechanism of EGFR-TKI resistance is still questionable.

Design: To address the possible genetic mechanisms on EGFR-TKI resistance, we compared gene expression profiles with the use of DNA microarrays between HCC827 cell line and HCC 827 sublines showing EGFR-TKI resistance (HCC827/CLR). We assessed the level of Wnt signaling activity and EMT markers by western blotting in the cell lines.

Results: Two significant gene expressions- Wnt signal pathway and Epithelial Mesenchymal Transition (EMT)-were identified. Wnt 1, 3, 5A, 6, 9A, GSK 3β, Axin2, LRP 6, 10, 12 and FZD2, 4, 7 genes were increased in HCC827/CLR sublines compared to HCC827 cell lines. Adhesion molecule genes including Integrin, E-cadherin, N-cadherein, B-catenin were decreased. On the contrary, EMT markers, such as Snail, Slug, SIP-1, Twist, Vimentin were increased in HCC827 CLR. HCC827/CLR showed morphologic features of Epithelial-to-mesenchymal transition (EMT), such as loss of polarity and spindle shaped morphology.

Conclusions: Wnt signal pathway gene and EMT related gene expressions are possible genetic mechanisms of acquired resistance of EGFR-TKI.

1857 Usefulness of Napsin A and TTF-1 in Discriminating Metastatic Carcinoma from Primary Adenocarcinoma of the Lung

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Background: The differential diagnosis between metastatic and primary lung carcinoma is a frequent perplexity. TTF-1 has been considered as a reliable marker to distinguish between primary adenocarcinoma (AC) and metastasis of the lung. However, recent studies have shown that TTF-1 can be detected in extrapulmonary ACs, which makes the interpretation diffcult in certain cases. Fortunately, napsin A has been reported

to be highly expressed in the lung ACs. The aim of this study was to determine if immunohistochemical detection of napsin A and TTF-1 can be powerful in discriminating primary lung AC from metastasis.

Design: Twenty-nine resected metastatic carcinomas of the lung metastatic from 7 colonic ACs, 10 conventional renal cell carcinomas (RCCs), 3 papillary or Hürthle cell carcinomas of thyroid, 1 endocervical AC, 1 ovarian endometrioid carcinoma, 1 prostatic ACA, 2 hepatocellular carcinomas, and 2 adrenocortical carcinomas and 2 breast carcinomas along with tissue microarrays (TMA) of 121 the lung ACs, and 92 clear cell, 15 papillary and 4 chromophobe RCCs and 4 oncocytomas were immunohistochemically studied using antibodies against TTF- and napsin A. Nuclear and cytoplasmic staining for TTF-1 and napsin A were considered positive, respectively, and the percentage of positively stained cells was recorded along with intensity (graded as weak, moderate, or strong). A p value of <0.05, as determined by Fisher's exact test, was considered statistically significant.

Results: Three of 7 metastatic colonic AC showed weak to moderate and patchy nuclear staining for TTF-1 in 5% to 20% of the tumor cells; one of 10 clear RCC, 1 ovarian carcinoma and 1 prostatic AC also exhibited 5% to 30% of tumor cells weakly to moderately positive for TTF-1. Reactive type II pneumocytes were strongly positive for TTF-1. All cases were negative for napsin A. In the lung AC, napsin A and TTF-1 were detected in 85.9% (104/121) and 81.0% (98/121), respectively and the sensitivity between the two was not statically different. Papillary RCCs were positive for napsin A (>80% tumor cells, moderate to strong) in 12 of 15 cases (80%) and all other renal epithelial neoplasms were negative for napsin A. TTF-1 was not detected in all cases in the RCC TMA, including oncocytomas.

Conclusions: This is the first time to report that TTF-1 is detected in both clear cell RCC and prostatic AC metastatic to the lung. Combined TTF-1 and napsin A immunostains are more powerful in separating the primary lung AC from metastasis.

1858 EGFR Mutation and p53 Overexpression in the AAH-BAC-Small Adenocarcinoma Sequence of the Lung

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Background: A progression model of atypical adenomatous hyperplasia (AAH) to bronchioloalveolar carcinoma (BAC) to invasive adenocarcinoma(ADC) has been proposed. However, the genetic alterations of the AAH-BAC-ADC sequence are not clearly established. We examined the mutation of the epidermal growth factor receptor (EGFR) gene and p53 protein overexpression in the AAH, BAC and small ADC to understand their role in the pulmonary adenocarcinoma pathogenesis.

Design: Twenty AAH, 39 BAC (19 Noguchi type A and 20 type B) and 32 ADC (Noguchi type C) were enrolled in this study. EGFR mutations at exons 18-21 and p53 protein expression were examined by PCR-direct sequencing and immunohistochemistry, respectively.

Results: Mutations of the EGFR gene were noted in 32 (35.2%) lesions, which included 7 (35.0%) of AAH, 13 (33.3%) of BAC and 12 (37.5%) of small ADC. Eighteen (19.8%) of the mutations were detected as exon 19 deletion, 13 (14.3%) as exon 21 point mutation and 1 (1.1%) as exon 18 point mutation. Overexpression of p53 protein was found in 16 (17.6%) lesions, none of AAH, 4 (10.3%) of BAC and 12 (37.5%) of ADC.

Table 1. Comparison of EGFR gene mutation and expression of p53 protein

Histology (no.)	EGFR gene mutation negative	EGFR gene mutation positive	p value	p53 protein expression negative	p53 protein expression positive	p value
AAH (20)	13 (65.0%)	7 (35.0%)		20 (100.0%)	0 (0.0%)	
BAC (39)	26 (66.7%)	13 (33.3%)		35 (89.7%)	4 (10.3%)	
ADC (32)	20 (62.5%)	12 (37.5%)		20 (62.5%)	12 (37.5%)	
Total (91)	59 (64.8%)	32 (35.2%)	0.935	75 (82.4%)	16 (17.6%)	0.001

Conclusions: High frequency and similar incidence of EGFR mutation in AAH, BAC, and ADC supports that EGFR gene mutation seemed to be associated with early stages of pulmonary ADC, and BAC with EGFR mutation might progress into invasive adenocarcinoma easier than those without mutation. On the contrary, p53 overexpression was identified in the late step of the AAH-BAC-ADC sequence model. The genetic alterations of the EGFR and p53 might play a role in the different stages of the peripheral pulmonary adenocarcinoma development.

1859 Prognostic Significance of the Proposed IASLC/ATS/ERS Revised Classification of Lung Adenocarcinoma in 514 Stage 1 Lung Adenocarcinomas (ADC)

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Background: The International Association for the Study of Lung Cancer (IASLC), the American Thoracic Society (ATS) and European Respiratory Society (ERS), have proposed a revised classification of lung adenocarcinoma. There are no studies investigating the prognostic significance using the proposed criteria. We sought to investigate the usefulness of this classification to identify prognostically significant ADC subtypes.

Design: A total of 514 patients were classified as Adenocarcinoma in situ (AIS), minimally invasive adenocarcinoma (MIA), lepidic predominant non-mucinous (LPNM), acinar predominant (AP), papillary predominant (PP), micropapillary predominant (MPP), solid predominant (SP), colloid predominant (CP) and mucinous adenocarcinoma (MA). Statistical analysis was performed using SPSS version 17 with crosstable using Chi-square statistics. Survival analysis was performed using Kaplan Meier analysis for disease free survival (DFS) and Cox regression.

Results: We found 323F (63%) and 191M (37%) with 376 1A (73%) and 138 1B (27%); mean age 68 yrs (33-89 yrs). 5-yr DFS for males was significantly worse (77%) than that for females (88%, p=0.011); it was also worse for IB (75%) than for 1A (86%, p=0.001). Three overall prognostic groups for 5 yr DFS were identified: 1) 100% for AIS: n=1, MIA: n=7 and LPNM: n=28; 2) 85% for PP: n=143, 86% for AP: n=232 and 86% for MA: n=15 and 3) 69% for SP: n=66, 62% for MP: n=12 and 69% for CP: n=9 (p<0.001). In addition, survival was significantly worse for MA (76%) compared to LPNM (100%, p=0.014). In multivariate analysis stratified for stage, proposed IASLC classification, lymphatic invasion and sex were independent prognostic predictors of survival.

Conclusions: The proposed IASLC/ATS/ERS classification identifies prognostically significant categories of Stage I lung ADC. AIS and MIA are rare tumors at MSKCC, comprising less than 2% of all cases and LPNM accounted for only 5.4% of all tumors. These data support the proposal to use the predominant subtype for classifying the remaining 93% of our lung adenocarcinomas which were invasive.

1860 Mucinous Carcinoma (Colloid Carcinoma) of the Lung, an Immunohistochemical and Molecular Analysis

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Background: Mucinous (Colloid) Carcinoma of lung is an uncommon subtype of pulmonary adenocarcinomas. Differentiating between primary pulmonary and metastatic mucinous adenocarcinoma of extrapulmonary origin, namely lower GI tract origin, can be challenging; as there is a considerable histological and immunophenotypic overlap between the two. Current study was performed to evaluate immunohistochemical profile and EGFR and KRAS gene mutation status in 12 cases of mucinous carcinoma of the lung.

Design: H&E stained sections from surgical resection of 17 cases of pulmonary mucinous carcinoma were obtained from MD-Anderson. Selection was subsequent to exclusion of five patients, found to have mucinous adenocarcinoma of extrapulmonary origin. Immunohistochemical probes were utilized for detection of: CK7, CK20, TTF-1, SP-A, and CDX2; extent of expression was assessed by light microscopy in scale of 0-4+, 0: none and 4+: more than 75% staining. Molecular analysis for EGFR, exons 18-21, was carried using dye terminator PCR sequencing method. KRAS codons 12, 13, and 61 were analyzed by pyrosequencing. All EGFR and KRAS sequence variants were confirmed by independent PCR from at least two micro-dissections, sequenced in both directions.

Results: The immunohistochemical results are listed in the table 1. Molecular analysis detected wild type EGFR sequence in all cases studied. 3 cases had KRAS mutation of codons 12 or 13.

Table 1

Case #	CK7	CK20	TTF-1	SP-A	CDX2
1	4+	2+	0	0	2+
2	4+	4+	0	0	3+
3	4+	4+	1+	0	4+
4	4+	2+	0	0	2+
5	4+	4+	1+	0	4+
6	4+	4+	0	0	4+
7	4+	3+	0	0	4+
8	4+	0	0	0	3+
9	2+	4+	N/A	0	N/A
10	4+	3+	1+	0	2+
11	4+	2+	1+	0	2+
12	3+	1+	0	1+	4+

N/A: not available

Conclusions: Our results indicate that the use of immunohistochemistry and clinical/radiological correlation remains the gold standard for the site of origin of mucinous carcinomas occurring in lung. Strong and diffuse expression of CK7 in colloid carcinoma of the lung can help in differentiation from metastatic mucinous adenocarcinoma of lower GI tract origin. Occurrence of mutation in EGFR tyrosine kinase domain (exon 18-21) is less frequent in Colloid Carcinoma than other lung adenocarcinoma subtypes. However, the frequency of KRAS mutation is similar to that of other lung adenocarcinoma subtypes.

Quality Assurance

1861 Quality of Reporting Gallbladder Carcinoma – An Audit of a Consultation Practice at a Tertiary Care Hepatopancreaticobiliary (HPB) Centre

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Background: Over the past decade, much attention has been given to improving the quality of surgical pathology reports for cancer cases. In our jurisdiction, a successful pathology reporting project has focused on completeness through a Province-wide adoption of the College of American Pathologists (CAP) checklists. In five common disease sites, colorectal, breast, lung, prostate, and endometrial carcinoma specimens, the CAP synoptic reporting was mandated. Less common cancers, however, received less attention. The purpose of our study was to assess the completeness of reporting of gallbladder resection specimens in our referred-in cases as an example of reporting on a less common site.

Design: Consultation reports on gallbladder carcinomas were searched from the surgical pathology database at Sunnybrook Health Sciences Centre. Surgical pathology reports from the original hospitals were obtained, and evaluated for the presence of the following parameters as per the CAP Gallbladder Cancer protocol: Specimen type, Histologic type, Tumor site, Tumour Grade, Cystic duct margin, Liver bed margin, lymphovascular invasion, perineural invasion, pTNM staging. Our referral pool was community hospital-based laboratories who subscribed to the Province-wide pathology reporting project.

Results: Thirty-two cases of gallbladder carcinoma were received for consultation/review during the study period. Out of these cases, only one fulfilled CAP requirements for completeness. Missing required elements included: Tumor site (28/32, 87%); Tumor size (25/32, 77%); Histologic grade (6/32, 20%); Liver bed margin (23/32, 73%); Cystic