Pulmonary

1959 Thymic Tumor Surgical Resection Margins Less Than 1mm Show Decreased Recurrence Rate Independent of Radiation Therapy (RT)

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Background: The best approach in the management of thymoma and thymic carcinoma is unclear. Patients referred to the British Columbia Cancer Agency (BCCA) with stage I disease are treated primarily with surgery while selected stage II patients receive adjuvant RT.

Design: This study presents a specific cohort of patients with stage II thymic epithelial neoplasms identified from a larger population-based cohort of cases referred to the BCCA from 1994 to 2009. Potential tumor-related prognostic factors (TRPF) including WHO subtype, tumor size, capsular presence and integrity, degree of invasion, distance to the closest margin and lymphoid hyperplasia were assessed by 2 pathologists following the 2011 published policies adopted by the International Thymic Malignancy Interest Group. Clinical data including age, sex, stage, treatment, recurrence and survival data were available for statistical analysis and correlation. Kaplan-Meier methodology was used to evaluate the relationship between margin status and overall survival (OS) and progression free survival (PFS).

Results: From a cohort of 170 patients, 54 stage II patients with known margin status were selected. Of these, 39 cases were Masaoka stage IIa and 15 were stage IIb. There were 28 females and 26 males, with a mean age of 64 years. There were 50 thymomas (5 type A, 9 type AB, 8 type B1, 18 type B2, 6 type B3, 3 mixed, 1 other) and 4 thymic carcinomas. 18 patients had surgery alone, 33 surgery and RT, 2 tri-modality treatment, and 1 no treatment. A total of 35 patients received RT. Of the surgically treated cases, 22 tumors had pathologically positive margins and 32 negative but close margins (20 at <0.5mm; 10 at 0.5-1mm; 2 at >1mm margin). Of the 22 patients with positive margins, 17 received RT and of those 4 recurred. Of the 30 patients with negative margins less than 1mm, 16 received RT and none recurred. Of the 14 patients with negative margins who were not irradiated, none recurred. Based on Kaplan-Meier curves, there were no statistically significant differences between margin status and OS (p=0.72), but there was a trend towards worse PFS in those with a positive margin (p=0.06).

Conclusions: Positive surgical margin status does not always trigger RT but may represent a negative prognostic factor for PFS in some stage II thymic epithelial malignancies. Patients with surgical resection margins within less than 1mm (but negative) show a decreased recurrence rate independent of RT and significantly better PFS than those with positive margins.

1960 Napsin A: Utility in Identifying Primary Mucinous Lung Adenocarcinomas Versus Mucinous Metastasis

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Background: Mucinous types of primary lung adenocarcinoma (including mucinous bronchioloalveolar) often present a challenge with lack of staining for traditional lung markers (e.g. TTF-1) and variable staining for CK7 and CK20. They may be difficult to distinguish from metastatic mucinous adenocarcinomas of the gastrointestinal tract and ovary. Napsin A is an aspartic proteinase that appears to be involved in the maturation of surfactant protein B and has been shown to be superior in sensitivity and comparable in specificity to TTF-1 for lung primary adenocarcinomas. The question remains, however, if Napsin A retains its usefulness for pulmonary adenocarcinomas with mucinous differentiation, the variants which are notorious for being morphologically and histochemically difficult to distinguish from metastatic adenocarcinoma (usually of colorectal origin). Inamura *et al.* found that immunoreactivity for Napsin A was lost in all 7 of the pulmonary adenocarcinomas with enteric differentiation that they examined. This study further examines Napsin A immunoreactivity in mucinous pulmonary adenocarcinoma in comparison to mucinous adenocarcinomas of the ovary, appendix and colon.

Design: A total of 40 cases of mucinous adenocarcinoma specimens (21 lung primary, 12 colorectal/appendiceal, 7 ovarian) were identified by computerized searches of pathology reports. Cases were selected regardless of age, gender, or ethnicity, spanning from 2000-2011. Napsin A, TTF-1, CK7, and CK20 immunohistochemical stains were performed on selected formalin-fixed paraffin embedded (FFPE) cell blocks. Slides were grouped by immunostain, randomly sorted, and blinded before review. Each slide was graded on stain intensity (0-3+) and percentage of tumor cells stained.

Results: See Table 1

Results

TTF-1	**Positive**	**Negative**
Lung	7	14
Appendix/colon	0	12
Ovary	0	7
Napsin-A	**Positive**	**Negative**
Lung	11	10
Appendix/colon	1	10
Ovary	0	7
CK7	**Positive**	**Negative**
Lung	20	1
Appendix/colon	4	8
Ovary	7	0
CK20	**Positive**	**Negative**
Lung	4	17
Appendix/colon	11	0
Ovary	6	1

Conclusions: All 7 cases of lung mucinous adenocarcinoma that were TTF-1 positive were also positive for Napsin-A; however, an additional 4 cases were positive for Napsin-A and not TTF-1. Based on these results, Napsin-A demonstrates greater sensitivity (0.52 versus 0.33) and nearly equivalent specificity (0.94 versus 1.0) compared to TTF-1 when applied to primary pulmonary adenocarcinomas with mucinous differentiation in comparison to mucinous tumors of the appendix/colon and ovary.

1961 Detection of EGFR Mutations in Lung Adenocarcinoma by Immunohistochemistry Using Mutant Specific Antibodies: Are We There Yet?

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Background: Somatic mutations of the EGFR tyrosine kinase are the most prevalent and well studied in lung carcinoma. E746-A750 deletion in exon 19 and L858 point mutation in exon 21 account for ~90% of these mutations. Immunohistochemistry (IHC) for EGFR mutation using mutant specific antibodies was studied recently in lung carcinoma and found to be ~90% sensitive, thus allowing use as a screening tool.

Design: We evaluated the IHC expression of the two mutant specific EGFR antibodies in 38 cases of lung adenocarcinoma on a tissue microarray obtained from Japan. Ten cases had exon 19 mutation, 8 cases had exon 21 mutation, 1 case had both exon 19 and exon 21 mutation and 1 case had exon 18 mutation by PCR. In 18 cases no mutation was detected. Antibodies used included L858R specific for the exon 21 and E746-750 specific deletion for exon 19 EGFR mutation (Cell Signaling). The method followed for performing IHC was exactly same as the one previously published (antigen retrieval using EDTA pH9(Dako) for 30 minutes, dilution 1:100 for primary antibodies, and the EnVision+kit (Dako) for detection). In addition, various dilutions of primary antibody at 1:50 and 1:25 were used. Depending upon the intensity of cytoplasmic staining, cases were scored as 0, 1+, 2+ and 3+. Staining intensity of 0 to 1+ was counted negative and 2+ to 3+ was counted positive.

Results: Results are shown in Table 1. Using 1:100 dilution, the detection rate was 0% with both the antibodies. Using 1:50 dilutions the detection rate was 9% for exon 19 and 0% for exon 21 mutation. Using 1:25 dilution the detection rate was 73% for exon 19 with 22% false positivity and 11% for exon 21 with no false positivity.

Table1

Primary antibody	Dilution	No Staining	Positive staining (2+ and 3+)	
		(0 -1+)	True Positive	False Positive
Exon 19	1:100	11/11	0/11(0%)	0/27 (0%)
	1:50	10/11	1/11 (9%)	0/27 (0%)
	1:25	3/11	8/11(73%)	6/27(22%)
Exon 21	1:100	9/9	0/9(0%)	0/29(0%)
	1:50	9/9	0/9(0%)	0/29(0%)
	1:25	8/9	1/9(11%)	0/29(0%)

Conclusions: We conclude that IHC for EGFR mutation detection cannot be used as a reliable screening tool in lung carcinoma cases yet.

1962 Utility of PAX-8, CD117 and CD5 in Distinguishing Thymic Carcinoma from Poorly Differentiated Lung Carcinoma

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Background: Distinguishing thymic carcinoma from poorly differentiated lung carcinoma in a mediastinal biopsy can be challenging. PAX-8 is a nuclear transcription factor expressed during organogenesis, specific to kidney, ovary and other organs. During neoplastic transformation of the epithelium, the gene is re-expressed. PAX-8 expression has not been documented during thymic organogenesis. However, recent studies comparing PAX-8 expression in malignant and benign tissues, which included only small numbers of thymic carcinomas, reported positivity in 0-80% of thymic carcinomas. Lung carcinomas were largely negative, with positivity in few squamous carcinomas. The aim of this study was to determine if PAX-8 immunostaining can distinguish between thymic carcinoma and poorly differentiated lung carcinoma.

Design: Archived cases of proven thymic carcinoma (n=13) and poorly differentiated lung carcinoma (n=15) were analyzed for the intensity and proportion of expression of PAX-8 (nuclear), CD117 (membranous) and CD5 (membranous) with the interpreters kept blind to the diagnoses. Staining in less than 10% of cells was interpreted as negative.

Results: PAX-8 was positive in 69.2% (9/13) of thymic carcinomas and 6.6% (1/15) of lung carcinomas. The intensity varied from weak to strong granular nuclear staining in 30-100% of cells. A single lung carcinoma positive for PAX-8 had focal squamous differentiation. While CD117 was positive in 84.6% (11/13) of thymic carcinomas, a significant proportion (26.6%) of lung carcinomas was also positive. 53.8% of thymic carcinomas and none of the lung carcinomas were positive for CD5. 46.1%, 53.8% and 69.2% of thymic carcinomas were dual positive for combinations of CD5/PAX-8, CD117/CD5 and CD117/PAX-8, respectively. None of the lung carcinomas were dual positive for any of these combinations. Positivity for any two of the three markers (CD117, CD5 and PAX-8), was seen in 76.9% (10/13) of thymic carcinomas and in none of the lung carcinomas. 53.8% of thymic carcinomas were triple positive, while 15.3% of thymic carcinomas and 66.3% of lung carcinomas were triple negative.

Conclusions: PAX-8 is a sensitive marker for thymic carcinoma and is an important addition to the diagnostic panel for differentiating thymic carcinoma from poorly differentiated lung carcinoma. Adding PAX-8 to CD117 and CD5 increases the diagnostic yield for thymic carcinoma, especially when at least two of three markers are positive. However, triple negativity does not exclude the diagnosis of thymic carcinoma.

1963 Mixed Small Cell Undifferentiated Squamous Cell Carcinoma of the Thymus – A Clinicopathologic Study of 10 Cases

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Background: Mixed small cell undifferentiated squamous cell carcinoma of the thymus was described as an entity by Snover et al in 1978. They reported three cases where the tumor was composed of sheets of undifferentiated cells within which were present nests of squamous cells exhibiting only a mild degree of atypia. The transition was relatively abrupt with one to two layers of cells with mixed morphology. More recently, an additional case of a tumor with this morphology has been reported. However, the entity remains poorly described.

Design: To better understand this rare disease, following IRB approval, we reviewed our database of over 600 cases of thymic tumors, including 120 thymic carcinomas, to identify cases with mixed undifferentiated and squamous carcinoma features. The presenting symptoms, morphological spectrum, and outcomes data of these patients are presented. All but two patients were referred from outside institutions and had limited number of slides available for review.

Results: Ten patients were identified, six male and four female; age at diagnosis ranged from 22 to 79. One patient had a history of prostate cancer. The patients predominantly presented with respiratory related symptoms or chest pain, although three patients presented only with systemic symptoms such as fatigue and weight loss. Three patients also had signs or symptoms of paraneoplastic syndromes. Most patients had advanced disease at presentation with four patients presenting with unresectable disease. The diagnosis was based on review of H&E slides (range 3–20 slides/patient). The squamous component in all cases was well differentiated with slight to minimal degree of atypia. The undifferentiated component varied in cell size and often contained large/intermediate cells. The stroma was fibroblastic in all but one case. All but one patients developed metastases or died within 3 years of diagnosis. The tumors had been previously diagnosed as poorly differentiated carcinoma (four), well differentiated carcinoma (two) and in one case each high-grade non–small cell carcinoma, large cell nonkeratinizing with focal squamous differentiation and squamous cell carcinoma. Neuroendocrine features had been reported in two cases.

Conclusions: Mixed small cell undifferentiated squamous carcinoma is an under-recognized subtype of thymic carcinoma associated with poor prognosis. Recognition of this rare subtype should provide for better management of the patients with this aggressive form of thymic carcinoma.

1964 Clinicopathologic Features and Long-Term Outcomes of NUT Midline Carcinoma: An Index Report of the International NMC Registry

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Background: NUT midline carcinoma (NMC) is a poorly differentiated squamous cancer characterized by rearrangement of the *NUT* gene. Research advances have provided opportunities for targeted therapy in NMC, yet the clinical features of this rare disease have not been systematically characterized. An International NMC Registry has been created to identify patient characteristics and treatments correlating with outcome, and to start providing first clinical recommendations.

Design: A clinical database was established first using retrospective demographic and outcomes data available on all known cases of NMC. Questionnaires were completed by treating physicians. Pathologic, demographic, and clinical variables were assessed for 63 patients. Outcome data from 54 patients were available for survival analyses.

Results: The diagnosis of NMC is increasing annually since 2007. A significant increase in age at diagnosis has been observed since 2009 ($p < 0.05$). Geographic distribution of NMC registry patients is heavily concentrated in the United States (N = 41, 65 %). The median overall survival for patients with NMC was 6.7 months. The 2-year progression-free survival (PFS) was 9 % +/- 4 % and 2-year overall survival (OS) was 19 % +/- 6 %. Extent of surgical resection and initial radiotherapy were independently predictors of PFS and OS in a multivariate analysis. Notably, no chemotherapeutic regimen was associated with improved outcome.

Conclusions: NMC portends a particularly poor prognosis among all squamous cell neoplasms. The inadequacy of conventional chemotherapy establishes a pressing need for the development of targeted therapeutics. Intensive local therapies such as gross total resection and radiotherapy are associated with enhanced survival.

1965 Molecular Versus Histopathologic Staging of Lung Adenocarcinoma with Multiple Tumor Nodules

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Background: The AJCC 7th edition staging of lung adenocarcinoma incorporated changes in the staging of multiple tumor nodules. For instance, two separate primary tumors in the same lobe would be staged as T1, while a primary tumor and metastatic lesion in the same lobe would be staged as T3. These decisions are based on histologic similarity. Changes in staging result in different predictions of prognosis and impact therapy. Currently there is no accepted gold standard for determining clonality for staging purposes and determinations are made based on histologic appearance of the tumor. As molecular subtyping, particularly epidermal growth factor receptor (EGFR) and Kirsten-rat sarcoma 2 viral oncogene homolog (KRAS) mutation status becomes more prevalent, this information could be used to more accurately stage adenocarcinoma patients with multiple nodules.

Design: All lung adenocarcinoma cases from CUMC pathology with two or more synchronous or metachronous lesions which also had EGFR and KRAS mutation testing (direct sequencing and ARMS assay) (n = 24) were selected for review. The original reported tumor stage and molecular findings were noted, then tumors were blindly reviewed and scored by percentage of histologic subtype. Staging was done based on the highest percentage histologic subtype, secondary or unusual histologic pattern, and based on mutation analysis. Two cases, which were negative for all mutations tested, were deemed inconclusive by molecular staging and not used in the analysis.

Results: Staging results for original pathologic stage versus molecular stage are 71% (15/21 cases) concordant, primary histologic subtype versus molecular 64% (14/22 cases) concordant, and secondary or unusual histologic pattern versus molecular also 64% (14/22 cases) concordant. Interestingly, the same eight cases were not discordant in both analyses. There is only a 75% (18/24 cases) concordance rate between the two histologic methods.

Conclusions: EGFR and KRAS mutations are activating oncogenic mutations, and therefore early events in tumorigenesis. Thus, they are likely to be effective markers of clonality. In our series, staging by mutation analysis differed from the two histologic staging methods in 36% of cases. Adding molecular staging information to current histologic staging could increase the accuracy in patients with multiple separate tumors thereby improving therapeutic decision making.

1966 Subpleural Fibroblastic Foci Identify a Unique Subset of Patients with Spontaneous Pneumothorax

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Background: Spontaneous pneumothorax (SP) occurs in a heterogeneous group of patients with predisposing factors including inherited to acquired. Newly described entities, like Birt-Hoge-Dube, suggest there are underlying morphologic features which might alert the pathologist to the presence of an as yet unrecognized inherited or developmental disorder. The purpose of this study was to examine cases of SP to identify histopathologic patterns which may correlate with clinical entities.

Design: All cases of SP were selected from the pathology files over a 10 year period (2001-2011). The slides were examined for the presence of fibroblastic foci, eosinophilic pleuritis, pleural fibrosis/elastosis, airspace enlargement/emphysema, intraalveolar macrophages, cholesterol clefts, malignancy, intraparenchymal cysts and lymphangioleiomyomatosis. The significant morphologic features were confimed by another pathologist. The charts were abstracted for sex, age, family history, smoking and asthma history, and radiographic findings.

Results: 92 cases was retrieved. Slide review revealed 18 patients with fibroblastic foci. 6 had chronic lung disease and were excluded. The other 12 demonstrated a distinct lesion of patchy pleural fibrosis with a zonal pattern of dense, collagenized pleural fibrosis peripherally and loose fibroblastic areas at the leading edge. Interstitial fibrosis was absent. The adjacent alveoli ranged from unremarkable to showing mild type 2 hyperplasia. In some of the fibroblastic foci (FF), thick collagen bundles intermingled with the looser myxoid tissue. Ten patients were <25 (16-15) years old, one was 40 and one was 53. Both the 40 and 53 year old were non-smokers. Of the others, 6 were nonsmokers, 5 were smokers, one was unknown. Three had a history of asthma, 3 did not. The others were unknown. All involved the upper lobes. One additionally involved the lower lobe.

Conclusions: The discreet, patchy distribution of lesions and presence in only 13% of cases argue against a non-specific reactive process. These patients do not fulfill criteria for UIP: upper lobe involvement, no honeycomb change, predominantly young, no interstitial fibrosis. Some similarity to the newly described pleuroparenchymal fibroelastosis, an entity also associated with pneumothorax, is noted. Analysis of the elastic structure of the lung may be informative.

1967 p40 (ΔNp63) Is Superior to p63 for the Diagnosis of Pulmonary Squamous Cell Carcinoma

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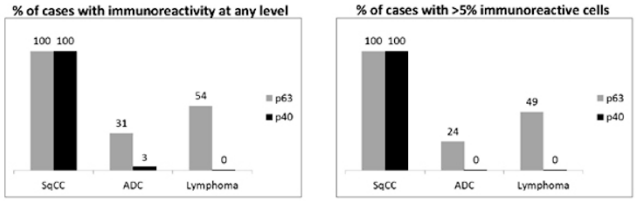
Background: Immunohistochemistry has recently emerged as a powerful ancillary tool for differentiating lung adenocarcinoma and squamous cell carcinoma – a distinction with important therapeutic implications. While the most frequently recommended squamous marker p63 is extremely sensitive, it suffers from low specificity due to its reactivity in a substantial proportion of lung adenocarcinomas and other tumor types, particularly lymphomas. p40 is a relatively unknown antibody that recognizes ΔNp63 – a p63 isoform suggested to be highly specific for squamous/basal cells.

Design: The standard p63 antibody (4A4) and p40 were compared in a series of 470 tumors from the archives of Memorial Sloan-Kettering Cancer Center and The Johns Hopkins Hospital, which included lung squamous cell carcinomas (n=81), adenocarcinomas (n=237), and large cell lymphomas (n=152).

Results: p63 was positive in 100% of squamous cell carcinomas, 31% of adenocarcinomas and 54% of large cell lymphomas (sensitivity 100%, specificity 60%). In contrast, while p40 was also positive in 100% of squamous cell carcinomas, only 3% of adenocarcinomas and none of large cell lymphomas had p40 labeling (sensitivity 100%, specificity 98%). The mean percentage of p63 versus p40-immunoreactive cells in squamous cell carcinomas was equivalent (97% vs. 96%, respectively, p=0.73). Rare

adenocarcinomas with p40 labeling had reactivity in no more than 5% of tumor cells, whereas the mean (range) of p63-positive cells in adenocarcinomas and lymphomas was 26% (1-90%) and 48% (2-100%), respectively.



Conclusions: In summary, p40 is equivalent to p63 in sensitivity for squamous cell carcinoma, but it is markedly superior to p63 in specificity. In effect, any more than minimal (5%) p40 reactivity is entirely specific for the squamous phenotype, eliminating a potential pitfall of misinterpreting a p63-positive adenocarcinoma or unsuspected lymphoma as squamous cell carcinoma. These findings strongly support the routine use of p40 in place of p63 for the diagnosis of pulmonary squamous cell carcinoma.

1968 Evaluation of c-Met FISH on Non-Small Cell Lung Cancer Samples with Known EGFR Mutational Status

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Background: c-MET is a proto-oncogene that encodes hepatocyte growth factor receptor, a member of the receptor tyrosine kinase family. Activation of the signaling pathway results in a variety of cellular responses including proliferation, decreased apoptosis, angiogenesis and increased motility. c-MET can be over-expressed or amplified in a number of epithelial cancers including lung cancer. C-MET amplification has been associated with advanced stage, high histologic grade and EGFR amplification but not activating EGFR mutations and has been observed in less than 5% of non-small cell lung cancer (NSCLC) patients not exposed to EGFR tyrosine kinase inhibitors. We sought to assess MET gene status in a series of NSCLC patients with known EGFR mutational status.

Design: Ninety-one formalin fixed paraffin embedded tissue samples were tested for EGFR mutations using an allele specific PCR which targets the most common mutations in the tyrosine kinase domain of EGFR, (QIAGEN). A subset of samples containing non-mutated, exon 19 deletions, L858R and T790M mutations was selected for c-Met analysis by FISH, (c-MET (7q31)/SE 7 probe, Poseidon/Kreatech Diagnostics, diluted 1:10 with tDenHyb-2 buffer, Insitus Biotechnologies). A cut-off value of c-MET:CEN 7 ratio of 2.0 was used to define amplification. Aneusomy was called when both average c-Met and average CEN 7 counts were greater than 3.8. Deletion of c-MET was called when the c-MET:CEN 7 ratio was ≤ 0.5.

Results: Of the 46 non-mutated samples tested, 3 (6.52%) were amplified, 7 (15.2%) were aneuploid and 3 (6.52%) had a deletion involving the c-MET locus. Of the 21 samples tested with an exon 19 deletion, 1 (4.76%) was amplified and 5 (23.8%) were aneuploid. Of the 20 samples tested with L858R mutations, 0 were amplified, 11 (55%) were aneuploid and 1 (5%) had a deletion involving the c-Met locus. Only 4 T790M mutated samples were tested and 1 (25%) was aneuploidy. Overall amplification, aneuploidy and a deletion involving the c-Met locus was identified in 4.4%, 26.4% and 4.4% of the NSCLC samples tested, respectively.

Conclusions: We confirmed that amplification of c-MET is seen in slightly less than 5% of patients with NSCLC, 6.5% of those without an EGFR mutation and 2.2% of those with harboring an EGFR mutation. Deletion of c-MET was equally as common as c-MET amplification, also seen in 4.4% of samples tested. Aneusomy was significantly more common being present in 26.4% of the samples tested. Further analysis of MET is warranted given its potential as a therapeutic target in lung cancer.

1969 EGFR Mutation Rates in 18246 Consecutive Non-Small Cell Lung Cancer Samples

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Background: Non-small cell lung cancer (NSCLC) is the leading cause of cancer-related deaths worldwide. Two-thirds of patients present with advanced disease and have an average survival of less than 1 year with standard chemotherapy. Studies have demonstrated that exon 19 deletion or L858R substitution in the EGFR gene are the most powerful predictive biomarkers in patients treated with erlotinib or gefitinib. This has led to the recommendation that EGFR mutational status be evaluated prior to initiating chemotherapy. We sought to determine the frequency and distribution of EGFR mutations in our laboratory over the past several years.

Design: From June 2009 to October 2011 we have evaluated the mutational status of EGFR in 18246 formalin fixed paraffin embedded non-small cell lung cancer samples using an allele specific PCR procedure that is capable of detecting 29 of the most prevalent mutations in exons 18-21 of EGFR, (QIAGEN EGFR PCR kit). The assay uses a real-time PCR platform and is capable of detecting mutations at a sensitivity of 1-5% in a background of non-mutated alleles.

Results: Mutations were identified in 2435 (13.3%) of NSCLC samples tested. Of the 2435 samples with a detectable mutation 1252 (51.41%) harbored exon 19 deletions, 84 (3.45%) were mutations at codon 719, 80 (3.29%) were insertions in exon 20, 827 (33.96%) were L858R, 80 (3.29%) were L861Q, 25 (1.03%) were S768I, 24 (0.986%) were T790M, 2 (0.082%) 19 deletion + L858R, 3 (0.12%) exon 19 deletion + T790M, 19 (0.78%) L858R + T790M, 1 (0.041%) T790M + L861Q, 5 (0.21%) T790M + G719, 8 (0.33%) L858R + S768I, 1 (0.041%) L861Q + S768I, 19 (0.78%) S768I + G719 and 5 (0.21%) L861Q + G719.

Conclusions: Analysis of over 18,000 consecutive non-small cell lung cancer specimens sent to our laboratory for evaluation of EGFR mutation status demonstrated that 13.3% harbored an EGFR mutation as detected by our allele specific PCR procedure. Approximately half, (51.4%) harbored an exon 19 deletion and about one third (33.96%) demonstrated the L858R substitution, while 2.6% revealed multiple mutations in the EGFR gene. This represents the largest analysis of EGFR mutational status in a US based population to date.

1970 Evaluation of ALK in Non-Small Cell Lung Cancer Using FISH and RT-PCR

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Background: Translocations involving the kinase domain of the anaplastic lymphoma kinase (ALK) gene have been identified in a number of different malignancies including non-small cell lung cancer (NSCLC). Although ALK has been demonstrated to fuse with a number of different genes, the echinoderm microtubule-associated protein-like 4 (EML4) gene is commonly associated with ALK in NSCLC. Crizotinib was recently approved for the treatment of ALK positive NSCLC patients as determined by the presence of an ALK gene rearrangement based on a companion diagnostic FISH assay. The frequency of ALK related NSCLC reported in the literature varies widely ranging from approximately 2-12%. Part of this variability is likely due to the testing method. We sought to compare FISH with an RT-PCR reaction capable of detecting the 10 most common EML4-ALK variants.

Design: 133 formalin fixed paraffin embedded lung samples were assessed using the Vysis LSI ALK break apart rearrangement FISH probe and a variant specific RT-PCR which is capable of detecting the 10 most common EML4-ALK variants. For FISH, 50 tumor cells were enumerated for the presence of a break apart signal which was considered as present when at least one set of orange and green signals were 2 or more signal diameters apart or when a single orange signal without a corresponding green signal was observed in more than 15% of tumor cells. The RT-PCR reaction uses one-step chemistry and is capable of detecting each of the 10 fusion transcripts at a sensitivity of approximately 1%.

Results: Of the 133 cases, 7 (5.3%) samples had a detectable break apart by the FISH assay. Four of these samples were confirmed as a form of EML4-ALK by the RT-PCR while the other 3 did not generate a signal from any of the 10 EML4-ALK variant specific reactions. The four samples that were confirmed by RT-PCR consisted of 2 variant ones, 1 variant two and 1 variant three. The samples that were not confirmed by the RT-PCR suggest the possibility of an ALK translocation partner other than EML4. In addition to alternative ALK rearrangements, the FISH test identified 3 (2.3%) samples as having ALK gene amplification.

Conclusions: FISH detected a break apart signal in 5.3% of samples, consistent with the reported literature. Attempting to detect the specific EML4-ALK variant by RT-PCR failed to identify 3 (42.8%) of the 7 ALK positive tumors identified by FISH suggesting that this method should not be used as the sole means of identifying patients who may benefit from Crizotinib therapy.

1971 Can ALK Immunohistochemistry Reliably Identify ALK-Translocated Non-Small Cell Lung Cancer?

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Background: Demonstration of *ALK* rearrangement in non-small cell lung cancer (NSCLC) tissue is required for guiding targeted therapy with ALK-inhibitors (e.g. crizotinib). Fluorescence in situ hybridization (FISH) is considered the gold standard method for detecting *ALK* rearrangement. Detection of ALK protein expression by immunohistochemistry (IHC) might be a more rapid and cost effective screening technique for determining which cases to refer *ALK* translocation testing. We evaluated the correlation between ALK protein expression in NSCLC by IHC and *ALK* rearrangement by FISH. Moreover, we quantified protein expression levels relative to ALK+ anaplastic large cell lymphoma (ALCL) to further understand differences from ALCL, in which IHC has supplanted FISH as a surrogate for *ALK* translocation.

Design: A total of 34 NSCLC samples with known *ALK* FISH status (9 positive, 25 negative) were stained using automated IHC (Leica, Bond-Max) with an ALK specific antibody (clone 5A4). In positive lung cancer samples ALK was quantified using quantum dot (QD) nanocrystal fluorescent detection system. Results were compared with a series of ALK+ ALCLs also analyzed by QD using multispectral image analysis (Nuance, v2.10.0)

Results: All 9 cases were positive for ALK expression by IHC. There were no false positive or negative IHC results. By IHC the majority of tumors cells were positive in *ALK* rearranged cases, although intensity of staining varied from weak to moderate. Interestingly, the level of ALK expression was significantly variable among these cases too, and by QD analysis we observed a greater than 20-fold difference between the lowest and highest expression levels. Overall, the levels in lung cancer were 20-fold lower, on average, than those found in primary ALCL ($P < 0.001$, 2-tailed T-test).

Conclusions: IHC with clone 5A4 and polymer detection system performs well in recognizing ALK+ NSCLC. With further validation it should prove useful as either a screening technique or a potential surrogate for FISH testing. Compared to lymphoma, IHC assays for ALK in NSCLC must be highly sensitive since expression levels are much lower in NSCLC. An alternative explanation might be a higher sensitivity of this clone for the EML4-ALK fusion protein.

1972 Adenocarcinoma *In Situ*, Minimally Invasive Adenocarcinoma and Invasive Pulmonary Adenocarcinoma – Evaluation of Interobserver Agreement in 294 Nodules with Survival Analysis

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Background: Recent studies have shown patients with pulmonary adenocarcinoma (PADC) showing entirely lepidic growth (adenocarcinoma in situ, AIS) or invasion ≤5 mm (minimally invasive adenocarcinoma, MIA) have superior outcome compared to patients with conventional invasive adenocarcinoma (IA). This has led to the new IASLC/ATS/ERS classification of lung adenocarcinoma proposed in early 2011. Data on interobserver variability within this new classification system is limited. Further validation of the superior survival of patients with AIS and MIA is also needed, since these tumors are rare and often make up a small minority of tumors in large studies.

Design: Patients were selected from the Mayo Clinic Epidemiology and Genetics of Lung Cancer Study database who underwent surgical resection of PADC at Mayo Clinic from 1997-2010. Cases were enriched for AIS and MIA by giving priority to cases diagnosed as bronchioloalveolar carcinoma (BAC) or adenocarcinoma with BAC features. 294 nodules were reviewed from 254 patients. Existing pathology slides were reviewed by two independent pathologists, who measured sizes of maximum invasion and central scar, if present.

Results: 237 of 294 nodules (81%) were classified into the same invasive category by both observers: 11 AIS, 71 MIA and 155 IA (kappa= 0.62, 95% CI 0.54-0.71). In 9 cases (3%) there was a disagreement between AIS and MIA. In 48 cases (16%) there was a disagreement between MIA and IA. The average difference in invasion measurement between observers was 3.4 mm. The average difference in scar measurement (when present) was 2.6 mm. Overall 5 year survival was significantly different among categories as determined by both observers: for observer 1,100% for AIS, 78% for MIA (relative risk over AIS (RR)=3.3) and 63% for IA (RR=7.6) (p=0.0007); for observer 2, 83% for AIS, 79% for MIA (RR=1.8) and 60% for IA (RR=4.5) (p=0.0001). The 5 year survival for the 48 patients where there was disagreement in classification between MIA and IA was 77%.

Conclusions: Moderate to good agreement was seen between observers (kappa 0.62), with concordance in the determination of AIS, MIA and IA in 81% of cases, and correlation between the combined AIS/MIA group versus IA in 84% of cases. While patients with MIA had better survival than IA, outcome was not as good as patients with AIS. In cases where there was a disagreement between MIA versus IA, survival was similar to the MIA group.

1973 Cytology Samples Are Comparable to Histological Samples for EGFR Mutation Testing in Non-Small Cell Lung Cancer (NSCLC)

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Background: Bronchial washings/brushings and fine-needle aspirations are often used for early screening and cytological diagnosis of lung cancer. The reliability of the different cytology samples for the molecular testing of NSCLC is controversial. Some authorities prefer to restrict molecular testing to histology samples. However, a large number of the diagnostic procedures for NSCLC are performed in community hospitals. In this study we discuss the feasibility of EGFR testing on cytology samples with available cell blocks (CB), as compared to core biopsy samples and resection specimens.

Design: 586 advanced stage NSCLC samples with non-squamous histology were referred by Medical Oncologists for EGFR mutation testing at BCCA Clinical Cancer Genetics Lab within a 14-month period. The formalin fixed paraffin embedded tissue samples were analyzed specifically for the in-frame deletions in exon 19 and the point mutation (L858R) in exon 21 and extensive data on sample characteristics were collected.

Results: Samples were received from 23 centers: 316 samples were from the lung, 78 from lymph nodes and 151 from metastatic sites, most commonly pleura (60), bone (35) and brain (25). A total of 115 cytology CB, 323 biopsy samples and 108 resection samples were analyzed over a 14 months period with an average intralaboratory turn around time of 7.4 calendar days. We analyzed 483 adenocarcinomas and 58 NSCLC not otherwise specified (NOS). Prior to analysis each sample was assessed by a pathologist for adequacy and tumor content and 38 test requests were cancelled (5.11%). Of the 546 tested cases 70 showed exon 19 deletion, 39 exon 21 mutation, 399 were wild type and 37 failed. The EGFR mutation rate was 21.45%. The average sample tumor cellularity and median DNA content for cytology samples (N=112) compared to biopsies (N= 301) and resections (N= 99) were 60%, 76% and 81% and 2.5 ug, 4.6 ug and 34ug. Only 8 of 37 failed tests were cytology samples. Reasons for test failure included low cellularity (16), decalcification (5) and fixation (2). The failure rate for cytology samples (8/115) was almost identical to that of histology samples (29/431) at 6.95% and 6.72% respectively.

Conclusions: Cytology samples are comparable to histology samples in terms of DNA quantity and rate of failure. Therefore, material from cell blocks is suitable for EGFR mutation testing and cytology samples should be preserved as cell blocks whenever possible.

1974 Absence of TTF-1 Immunoreactivity Can Predict EGFR Wild-Type in Non-Small Cell Lung Cancer (NSCLC)

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Background: Activating mutations in the epithelial growth factor receptor (EGFR) are strong predictors of efficacy for tyrosine kinase inhibitors. In-frame deletions in exon 19 and the c.2573T>G(L858R) single-base change in exon 21 together comprise over 90% of these mutations. Thyroid transcription factor 1 (TTF-1) is a tissue-specific transcription factor expressed in epithelial tissues of the lung and thyroid. TTF-1 is an important immunohistochemical marker for diagnosis of pulmonary adenocarcinoma and recent studies also suggest TTF-1 expression is a prognostic factor for increased survival in lung adenocarcinoma.

Design: EGFR tyrosine kinase inhibitor gefitinib is approved in Canada as a first line therapy for patients with stage IIIB/IV non-squamous NSCLC. Since March 2010, 548 patients in British Columbia have been tested for EGFR mutations. Tumour tissue was macrodissected from formalin-fixed, paraffin-embedded cytological samples, biopsies and resections and EGFR mutations in exons 19 and 21 were detected from the purified DNA using PCR fragment size analysis.

Results: EGFR mutations were detected in 109 of 509 samples (21.4%), 70 (13.7%) in exon 19 and 39 (7.7%) in exon 21. Of 323 samples for which TTF-1 immunohistochemistry results were available, 248 (76.8%) were TTF-1 positive and 75 (23.2%) were TTF-1 negative. There were both EGFR mutation status and TTF-1 immunohistochemistry results for 306 samples. TTF-1 expression was detected in 58 of 62 mutation-positive samples; however 178 of 244 TTF-1 positive samples were EGFR wild type. These results demonstrate that TTF-1 IHC is 93.5% sensitive and 27.1% specific for predicting the presence of EGFR activating mutations.

Conclusions: Although TTF-1 immunoreactivity is not specific for the presence of activating EGFR mutations in NSCLC, its absence can reliably predict EGFR wild type with 93.5% sensitivity. Thus, tumour TTF-1 status may be informative in the selection of patients for EGFR mutation testing.

1975 Establishing Quantitative Parameters in the Detection of Somatic Mutations of the Epidermal Growth Factor Receptor Gene in Cytology Samples of Non-Small Cell Lung Carcinomas

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Background: 30-40% Asian and 10-15% Caucasians diagnosed with Non-Small Cell Lung Carcinoma have mutations in the tyrosine kinase domain of the Epidermal Growth Factor Receptor gene (*EGFR*). Two mutations, a short in-frame deletion in exon 19, and a point mutation in exon 21 account for 90% of *EGFR* mutations. Several NSCLC are diagnosed using only cytology, resulting in limited tumoral availability. This study goal is to establish minimal quantitative parameters in cytology for the detection of *EGFR* mutations.

Design: We prospectively analyzed 92 consecutive cases of non-small cell/non-squamous carcinoma (NSC/NSq) based on cytology and immunohistochemical profile. Exon 19 deletions and point mutation in exon 21 were tested using high resolution melting analysis and DNA sequencing. The samples include specimens from BAL/brush, effusions and trans-bronchial/thoracic/esophageal needle aspirations fixed in formalin 10%, centrifuged and aggregated with Histogel®, then classified as Q1 (<50 tumor cells/slide), Q2 (50-100 tumor cells/slide) and Q3 (>100 tumor cells/slide). DNA was extracted from 10x5µm-thick sections.

Results: Out of 92 NSC/NSq cases, 12 cases (13,04%) were positive for *EGFR* mutation (5 in exon 21, 7 in exon 19). Two cases had insufficient DNA for analysis, while in all others 78 cases no mutations were found. Among exon 19 positive cases, all but one were classified as Q3 (85.7%). One case positive for exon 19 was classified as Q1. Among exon 21 positive cases, 4 cases were classified as Q3 (80%), and only one as Q2 (20%). Among wild-type cases, 25 were classified as Q1 (27,1%), 14 as Q2 (15.2%) and 39 as Q3 (42,3%).

Conclusions: Only 1 out of 26 cases (3.84%) classified as Q1, and 1 out of 15 cases (6,66%) classified as Q2 had *EGFR* mutation detected. 49 cases were classified as Q3 (53.2% of cases). In this cohort, *EGFR* mutation was detected in 20.4% of cases. Our study suggests that despite being possible to detect *EGFR* mutations, less than 100 cells/slide appears to limit detection. Additional studies comparing detection in low cellular cytology samples with resected specimens could help establish minimal quantitative guidelines for optmization of *EGFR* mutation testing in cytology.

1976 Testing for 29 EGFR TKI Sensitivity and Resistance Mutations in Lung Cancer Using EGFR RGQ PCR Kit

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Background: Many studies have demonstrated the association between somatic mutations in the epidermal growth factor receptor (EGFR) gene in non-small cell lung cancer (NSCLC) and sensitivity or resistance to the EGFR tyrosine kinase inhibitors (TKIs). In this study we evaluated performance of a commercial kit (EGFR RGQ PCR, Qiagen) for detection of EGFR mutations in a variety of sample types likely to be submitted to a molecular oncology lab.

Design: Qiagen's EGFR RGQ kit detects 29 somatic mutations in the EGFR oncogene using real time PCR and Rotor Gene Q (RGQ) instrument. The kit uses Scorpions and ARMS® technologies to detect these mutations in the background of wild type genomic DNA. Sixty two confirmed NSCLC cases were tested in parallel using EGFR RGQ PCR and laboratory developed assays (LDA) EGFR exon 19 deletion/exon 21 L858R point mutation. Sample types were as follows: 42 lung biopsies/resections, 3 lymph node biopsies, 3 brain biopsies/resections, 3 liver biopsies, 1 gastric biopsy, 1 skin biopsy, 4 pleural cytology fluid cell blocks, 1 pleural cytology slide smear, 2 pericardial fluid cytology cell blocks, 1 scapula resection, 1 paraspinal mass. Manual microdissection was performed when tumor content was less than 50% of total cells (20/62 cases).

Results:

Table 1: Analytical sensitivity of the EGFR RGQ assay

Mutation	Sensitivity
T790M	10%
Deletions	1.25%
L858R	2.5%
L861Q	0.3125%
G719X	0.625%
S768I	1.25%
Insertions	2.5%

Analytical sensitivity for LDA is 5% for both the exon 19 deletion/exon 21 L858R point mutation. Exon 19 deletion was detected in 14/62 (22.6%) of cases by both methods. Exon 21 mutation was detected in 8/62 (12.9%) of cases with the LDA and 9/62 (14.5%) with the EGFR RGQ method. The EGFR RGQ method detected 3 additional mutations: one insertion, one G719X mutation, and one T790M mutation. Total cost of testing per sample is USD 48.24 for LDA and USD 628.62 for EGFR RGQ. The EGFR RGQ method has more manual steps at the set up (more tubes and pipetting) but is overall less technically challenging to run and interpret. Total run time (DNA to results) is 7 hours for LDA and 2 hrs 45 min for EGFR RGQ. The EGFR RGQ assay does not allow size determination for exon 19 deletions.

Conclusions: Although more expensive than LDA, EGFR RGQ assay offers an evaluation of 29 EGFR mutations in exons 18 to 21 currently known to be associated with sensitivity and resistance (T790M) to TKIs in NSCLC compared to only 2 mutations detected by LDA. Being able to test for more mutations at a greater sensitivity makes this assay more attractive to oncologists at our institution.

1977 Microaspiration Is Distinct from Aspiration Pneumonia

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Background: Chronic microaspiration due to gastroesophageal reflux (GER) has been suggested to play a role in worsening of asthma and chronic obstructive pulmonary disease, in the pathogenesis of idiopathic pulmonary fibrosis, and in precipitating acute and chronic rejection with development of obliterative bronchiolitis in lung transplant patients. Frequent subclinical aspiration (microaspiration) of small droplets in patients of GER disease can cause aspiration/chemical pneumonitis. There is confusion in the literature due to the use of the term aspiration pneumonia for both microaspiration and pneumonia that is caused by aspiration of large amounts of gastric contents with subsequent bacterial superinfection in obtunded or intubated patients.

Design: 13 cases of microaspiration (aspiration pneumonitis) were retrieved from pathology archives. The slides were reviewed and histological and clinical findings were compared with 14 cases of aspiration pneumonia.

Results: Patients with microaspiration were 39 to 78 years in age and were diagnosed on biopsy specimens (7 transbronchial and 6 wedge biopsies). Four were lung transplant recipients who underwent routine surveillance biopsies. 7/15 cases involved lower lobes (5 right and 2 left), 3 cases right middle lobe and 4 cases upper lobes (3 right and 1 left). Nine of 13 patients were treated with proton pump inhibitors. The biopsies showed peribronchiolar poorly formed granulomas with multinucleated giant cells; 10 of 14 cases had exogenous lipoid pneumonia and one had organizing pneumonia. In contrast, almost all cases of aspiration pneumonia were diagnosed at autopsy in severely ill patients with bilateral and multifocal involvement. Patients with aspiration pneumonia ranged from 4 months to 78 years old. The characteristic pathologic findings of aspiration pneumonia included accumulation of neutrophils within airway and adjacent alveoli with abscess formation, destruction of lung parenchyma with replacement of acute inflammation and necrosis, and the presence of vegetable material or skeletal muscle in alveolar spaces. Bacterial and fungal organisms were identified in up to half of the cases either by tissue or blood culture or by histology alone.

Conclusions: Microaspiration is distinct from aspiration pneumonia both clinically and histologically. In the literature, the term aspiration pneumonia has been used for both these conditions. In view of the different clinical significance and treatment, the term microaspiration reflects the underlying pathogenesis of this disease.

1978 Disease-Free Survival of Patients with NSCLC after Surgical Resection and Correlation with ERCC1 Expression and Genotype

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Background: Excision repair cross-complementation group 1 (ERCC1) expression is recognized as a favorable prognostic marker in cases of surgically-resected NSCLC. However, in patients treated with adjuvant chemotherapy after surgical resection, ERCC1 correlated with poor prognosis. Class III beta tubulin (TUBB3) is also known to be a predictive marker of efficacy after treatment with taxanes or vinorelbine.

Design: We retrospectively analyzed 363 tumor tissues from patients with surgically resected NSCLC. Tissue samples were labeled with ERCC1 and TUBB3-specific antibodies. With genomic DNA from 262 patients, single nucleotide polymorphisms of the ERCC1 gene (T19007C and C8092A) were studied via PCR-RFLP analyses.

Results: Only 5.9% of stage I (14/238) and 61.6% of stage II-III patients (77/125) received adjuvant chemotherapy. Relapses were noted in 30.6% (111), and among them 31 patients ultimately succumbed. The relapse rate (RR) was 24.8% in stage I, and 41.6% in stage II-III. The RR was significantly lower in the ERCC1-positive group (24.3%) as compared to the negative group (36.3%, p=0.014), and was lower in the AA/CA genotype of the ERCC1 C8092A locus (29.5%) compared to the CC genotype (42.1%, p=0.034). The median disease-free survival (DFS) time was 62.3 months. DFS was significantly longer in ERCC1-positive group (62.3m) compared to the negative group (48.0m, p=0.042). In a multivariate analysis, ERCC1 expression and the C8092A polymorphism were independent prognostic factors in the chemonaïve, stage I group.

Conclusions: ERCC1 expression and the AA/CA genotype of the C8092A locus in surgically resected NSCLC were correlated with good prognosis.

1979 Optimizing Lung Carcinoma Diagnosis: FNA, Core, or Both

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Background: A greater number of minimally-invasive procedures are being performed to diagnose lung cancer. Institutions perform CT-guided fine needle aspirate (FNA), core biopsy (CB), or both (B). Due to the advanced stage of disease at the time of sampling, these procedures may often provide the only diagnosis to guide prognosis and treatment. The aim of this study was to determine which modality (FNA, CB, or B) provides sufficient tissue to render a specific diagnosis and pursue ancillary studies to guide tumor-specific treatment.

Design: A retrospective search was performed for CT-guided lung FNA, CB, or B with on-site assessment by a cytopathologist. All cases positive for carcinoma were grouped by modality and according to whether or not the diagnosis was 'specific', defined as refined enough to subtype a primary carcinoma or to determine the site of origin of a secondary carcinoma, achieved by performing immunostains when necessary. In cases of poorly differentiated carcinomas, where immunostains were extensively performed but a specific diagnosis was not achievable due to the nature of the tumor rather than the limitations of the sample, the diagnosis was considered specific. Cases of primary lung adenocarcinoma were further assessed according to which modality provided sufficient tissue to pursue molecular studies (MS).

Results: Of 125 cases retrieved, 76 were positive for neoplasm. Of the neoplastic diagnoses, 68 were carcinomas.

Carcinoma: Diagnostic Specificity of FNA, CB, and B

	FNA, n=40	CB, n=16	B, n=12
Specific	35 (88%)	15 (94%)	10 (83%)
Non-Specific	5 (12%)	1 (6%)	2 (17%)

No statistical differences were found in the ability of any of the modalities to reach a specific diagnosis (Fisher's Exact Test, p=0.67).

Primary Lung Adenocarcinoma: MS from FNA, CB, and B

	FNA, n=19	CB, n=7	B, n=8
Sufficient	14 (74%)	7 (100%)	5 (62%)
Insufficient	1 (5%)	0 (0%)	2 (25%)
N/A	4 (21%)	0 (0%)	1 (13%)

Sufficient = sufficient tissue to perform MS for EGFR/KRAS/ALK; Insufficient = insufficient tissue to perform MS; N/A = MS not routine protocol at time of diagnosis; No statistical differences were found in the ability of any of the modalities to provide sufficient tissue for MS (Fisher's Exact Test, p=0.27).

Conclusions: The results of this study suggest that FNA, CB, and B are comparable in reaching a specific diagnosis and in having sufficient tissue for MS, though the study is limited by the number of CB and B cases that met inclusion criteria. However, the results demonstrate that FNA is as capable alone as when combined with a concurrent CB in reaching the diagnostic and prognostic goals of minimally-invasive procedures for lung carcinoma.

1980 Immunohistochemistry May Not Be a Reliable Screening Tool for Identification of ALK Rearrangement (ALKR) in Non-Small Cell Lung Carcinoma (NSCLC)

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Background: The discovery of EML4-ALK fusion gene in patients with NSCLC was a breakthrough in targeted therapy for lung cancer with significant clinical implications. ALKR is however only seen in a small percentage of NSCLC making identification of these patients challenging and costly.

Design: Using immunohistochemistry (IHC) with mouse monoclonal 5A4 antibody (Ab) from Nikirei Biosciences and fluorescence in situ hybridization (FISH) we screened a tissue microarray built from 593 resected surgical specimens from stage I NSCLC (243 lung adenocarcinoma (ACA), 272 squamous cell carcinoma (SQC), 35 large cell carcinoma, 32 non-small cell carcinoma NOS, and 6 other). IHC was scored as 0 (no staining), 1+ (faint cytoplasmic staining), 2+ (moderate, smooth cytoplasmic staining) and 3+ (intense, granular cytoplasmic staining) in >10% of tumor cells. IHC positive cases were 3+ only. Suspicious and positive cases were confirmed by IHC and FISH on whole section (WS).

Results: Results by FISH were available on 273 cases and by IHC on 385 cases. A total of 11 cases, either positive (N=2) or suspicious (N=9) by at least one methodology, were identified. Cases suspicious by FISH (N=5) were however not suspicious by IHC and cases suspicious by IHC (N=4) were not suspicious by FISH. One case was positive on TMA as well as on WS by IHC and FISH. The only other unequivocally positive FISH case, with an atypical pattern (loss of 5'ALK signal), was not positive or suspicious by IHC. The average age of our 5 males and 5 females was 63 years. There were 4 ACA, 5 SQC and 1 NSCLC NOS, and 3 of all were positive for TTF1.

Conclusions: IHC screening for ALKR in NSCLC with 5A4 antibody may not necessarily identify all cases with gene rearrangement by FISH. As small biopsy/cytology samples are inherently limited for molecular testing the question of finding the best strategy to identify ALKR as well as other clinically relevant molecular anomalies is critical, both in terms of time and cost. These results are currently in the process of being compared to other primary antibody clones (ALK-1 by Dako, 5A4 by Novocastra and D5F3 by Cell Signaling Technology) and revelation systems (FLEX by Dako, CSA II by Dako and ADVANCE by Dako).

1981 Bleomycin and Paraquat Mediated Pulmonary Fibrosis Is IL-17 Independent

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Background: Pulmonary fibrosis is a destructive process that in many cases are of unknown cause such as idiopathic pulmonary fibrosis. Better characterization of the immunological mechanisms of pulmonary fibrosis is needed to identify new therapeutic modalities for these diseases. The aim of the current study was to characterize the mechanisms of pulmonary fibrosis in the late phase and to determine whether IL-17A plays an important regulatory role.

Design: The pulmonary fibrosis was induced in distinct animal models, including Bleomycin (B-BLM) and Paraquat (B-PQ) in Balb/c mice. Additionally, IL17-RA Knockout (IL17KO) and wild C57 mice (C-BLM) were included. We used the picrosirius-polarization method and weigert's resorcin-fuchsin stain to morphometric study the peribronchiolar collagen and elastic fibers, respectively. By immunohistochemistry, we evaluate TGF-β, IL-21, IL-6, CD83, IL-17, IL-23, IL-13, STAT3, PDGF, FGF, TNF-α and limphocytic markers expression, using stereology method. The sacrifice of mice was performed 21 day after induction.

Results: We report here that late PQ and BLM-mediated peribronchiolar fibrosis is IL-17 independent, as IL17-RA -/- mice developed similar peribronchiolar fibrosis after induction when compared to B-BLM, B-PQ and C-BLM (Fig left). We found that only B-BLM and B-PQ presented increase of elastic fibers and they show a positive correlation with the deposition of collagen fibers. In IL-17KO group TGF and collagen correlated. The B-BLM showed marked increase in dendritic cells (CD83) and T lymphocytes (CD3), but the IL-17 axis is suppressed by low expression of IL-23, IL-13 and IL-17 (tendency). In contrast, the mice without IL-17A receptor (IL17KO) showed overexpression of IL-17 axis by high expression of IL-17, IL-6, IL-21, STAT3 and CD8+ T cell. The B-PQ group has only increased FGF-β.

Conclusions: Bleomycin and paraquat-mediated peribronchiolar fibrosis is IL-17 independent in the late phase. However, distinct mechanisms of peribronchiolar fibrosis may be involved, such as IL-17 response in C57 mice and presentation and processing of antigens in Balb/c mice. More studies are necessary to validate the regulatory role of IL-17 on pulmonary fibrosis process.

Financial Support: FAPESP, CNPq

1982 Ki-67 Is a Strong Prognostic Marker for Non Small Cell Lung Cancer When Tissue Heterogeneity Is Considered

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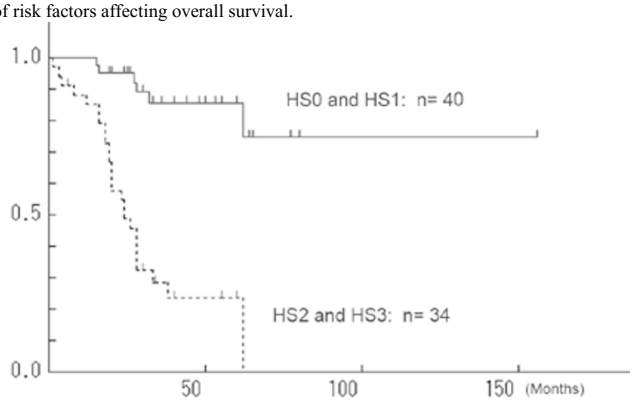
Background: The use of tissue microarray for biomarker validation is powerful. However, heterogeneity within certain tumors may complicate the interpretation of small core stainings. Multiple coring with biased site selection is artificial and may not be the best solution for this issue. We recently developed new technique, Spiral Array, which observes the side of thick reeled sections that enable to find whole morphology included in the one axis of paraffin sections. Using Spiral Array, we investigated prognostic significance of Ki-67 staining from the view of staining heterogeneity.

Design: One hundred cases of surgically removed lung cancer were collected. Spiral Array blocks were generated out of 100 cases using paraffin sections cut at 100 μm thick. Four μm thick sections of the array block were stained for Ki-67. Staining results in the each reel were scored for areas with lowest (LS), highest (HS), and dominant (DS) expression frequencies exclusively in the cancer cells. The scores were divided into four grades (0, <1%; 1, 1-10%; 2, 11-30%; 3, >30%). Clinical records including follow up status were collected, and prognostic significance of Ki-67 was analyzed using Log rank test.

Results: Seventy eight cases had clinical data including follow up status. Pathological stage was available for 91 patients including 43 stage IA, 22 stage IB, 2 stage IIA, 9 stage IIB, 13 stage IIIA, 1 stage IIIB, and 1 stage IV. The proportion of Ki-67 staining was 18 score 3, 28 score 2, 29 score 1, and 21 score 0. Numbers obtained by subtraction of LS from HS were considered as heterogeneity score (HeS) where more than HeS2 were seen in 23 cases. Cases with score 2 and 3 of HS and HeS showed significant poorer prognosis (both $P<.001$), whereas LS or DS did not show any prognostic values. The results were identical when analysis was limited to adenocarcinoma and squamous cell carcinoma. COX multivariate analysis showed that both HS and HeS to be independent of risk factors affecting overall survival.



Conclusions: Ki-67 is a strong prognostic marker for non small cell lung cancer when highest staining frequency or levels of staining heterogeneity are considered. Considering tissue heterogeneity is important for the establishment of tissue-based biomarkers.

1983 Lung Cysts in Birt-Hogg-Dube Syndrome: Unique Histopathological Features and Accelerated mTOR-Mediated Signaling

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Background: Birt-Hogg-Dube syndrome (BHD) is an autosomal dominant disorder characterized by fibrofolliculomas, renal cell tumors and pulmonary cysts with recurrent pneumothoraces. Pulmonary manifestations often constitute the initial clinical presentation, and the correct diagnosis is mandatory for proper patients' care. Histopathology of the BHD lung cysts, however, is poorly understood. BHD is caused by mutations in the *BHD* gene, which encodes folliculin (FLCN). FLCN is regarded as a tumor suppressor, regulating cellular activity through the mammalian target of rapamycin (mTOR) pathway.

Design: We investigated the histopathology of BHD lung cysts, using specimens resected for pneumothoraces in 12 patients of 9 BHD families. The patients were in their late 30s to 60s with a female predominance. Conventional and immuonohistochemical stainings were performed with special reference to FLCN and related proteins involved in the mTOR pathway. Genetic analysis of the germ line *BHD* gene mutation was also performed.

Results: Genetic tests confirmed all the patients examined had heterozygous BHD gene mutations. Histopathologically, the BHD lung cysts showed unique features: 1) part of the cyst wall is often abutting or incorporated within the the pleura, interlobular septa or bronchovascular bundles; 2) the cyst is lined by alveolar pneumocytes, attenuated or with a predominance of type II pneumocyte-like cuboidal cells; and 3) some cysts show internal septation by alveolar walls, or a complex alveolar pattern. Nonspecific blebs and bullae were also seen, but were thought to be secondary changes due to pneumothoraces or intrapulmonary rupture. Immunohistochemically, the lining cells of the cysts expressed pneumocyte markers and FLCN. These cells also expressed phospho-S6 strongly, suggesting activation of the mTOR pathway.

Conclusions: The BHD lung cysts have unique histopathological features that help render the correct diagnosis. They may be regarded as hamartoma-like aberrant cystic alveolar formation, possibly under activation of mTOR pathway due to haploinsufficiency of FLCN.

1984 Frequency of ALK Translocations in 2560 Non-Small Cell Lung Cancer Samples

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Background: The anaplastic lymphoma kinase (ALK) gene codes for a transmembrane receptor tyrosine kinase (RTK) in the insulin receptor superfamily. It has been identified as the fusion partner in a number of different malignancies and has long been suspected as a driver oncogene in these conditions. Although a number of studies have been conducted, the frequency of EML4-ALK in NSCLC ranges from 2-7% likely due to variability of patient selection and testing platforms. Crizotinib was recently approved for the treatment of ALK positive NSCLC patients as determined by the presence of an ALK gene rearrangement based on a companion diagnostic FISH assay. We assessed a large series of patients to determine the positivity rate of this assay in a US based population.

Design: 2560 consecutive formalin fixed paraffin embedded non-small cell lung cancer samples sent to our laboratory for determination of ALK rearrangement status were assessed using the Abbott LSI ALK break apart rearrangement FISH probe. Fifty tumor cells were enumerated for the presence of a break apart signal which was considered as present when at least one set of orange and green signals were 2 or more signal diameters apart or when a single orange signal without a corresponding green signal was observed in more than 15% of the tumor cells.

Results: Of the 2560 samples tested, 163 did not contain sufficient tumor content to conduct testing and 39 (1.5%) samples demonstrated suboptimal hybridization. 79 (3.35%) samples demonstrated an ALK rearrangement and 2279 had no detectable alteration.

Conclusions: The Abbott LSI ALK break apart FISH probe has excellent performance characteristics with a failure/suboptimal hybridization rate of approximately 1.5% in our hands. Although the ALK break apart probe does not specifically identify EML4 as the ALK fusion partner, the frequency of ALK rearrangements was 3.35% in our series of NSCLC patients. This number agrees with the expected rates based on previous studies and helps define the positivity rate in a US based population.

1985 Assessment of the ALK Antibody, 5A4 in Detecting ALK Rearrangments in Non-Small Cell Lung Cancer Specimens

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Background: Translocations involving the kinase domain of the anaplastic lymphoma kinase (ALK) gene have been identified in a number of different malignancies including

non-small cell lung cancer (NSCLC). Although ALK has been demonstrated to fuse with a number of different genes, the echinoderm microtubule-associated protein-like 4 (EML4) gene is commonly associated with ALK in NSCLC. Crizotinib was recently approved for the treatment of ALK positive NSCLC patients as determined by the presence of an ALK gene rearrangement based on a companion diagnostic FISH assay. We sought to determine if a commercially available ALK antibody could identify tumors assessed as ALK positive by the approved FISH assay.

Design: 100 formalin fixed embedded tissue sections, 50 from tumors assessed as ALK positive by FISH and 50 tumors assessed as ALK negative by FISH were pulled from our archives. 4 micron sections were stained with a monoclonal antibody, 5A4 (Leica, Buffalo Grove, Il) 1:50, following heat-induced epitope retrieval, 100° C, pH9, 20 minutes. FISH was performed using the Abbott LSI ALK break apart probe. 50 tumor cells were enumerated for the presence of a break apart signal which was considered as present when at least one set of orange and green signals were 2 or more signal diameters apart or when a single orange signal without a corresponding green signal was observed in more than 15% of tumor cells.

Results: 41 (82%) of the 50 tumors assessed as ALK positive by FISH showed expression of ALK protein with the 5A4 clone ranging from 1+ to 3+ intensity. The remaining 9 tumors showed no expression with the ALK antibody. All 50 tumors assessed as ALK negative by FISH showed no expression of ALK protein.

Conclusions: When ALK expression was noted with the 5A4 clone, the tumor was assessed as ALK positive by FISH; however, 9 (18%) of the tumors assessed as ALK positive by FISH lacked expression of the ALK protein. The 5A4 clone lacks the sensitivity to replace the FISH assay in clinical practice based on this series.

1986 Rationale for Treatment of Metastatic Squamous Cell Carcinoma of the Lung Using FGFR1 Inhibitors

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Background: We previously identified amplification of the fibroblast growth factor receptor 1 (FGFR1) gene as a potential therapeutic target for a small molecule inhibitor therapy in squamous cell lung cancer (L-SCC). Currently, clinical phase 1 trials are running in order to test if patients with FGFR1-amplified L-SCC benefit from a targeted therapy using small molecule inhibitors against FGFR. As most lung cancer patients present with metastatic disease, we investigated if the FGFR1 amplification also occurs in lymph node metastatic tissue of FGFR1-amplified and non-amplified primary tumors. Our study aims to give a rational whether to include patients with a metastatic L-SCC in a targeted small molecule inhibitor therapy.

Design: Our study cohort consists of 72 patients suffering from L-SCC. Of these, 39 patients presented with regional lymph node metastasis. Tissue microarrays were constructed from formalin-fixed paraffin-embedded tissue of the primary tumors and, where present, of the corresponding lymph node metastasis. A biotin-labelled target probe spanning the FGFR1 locus (8p11.22-23) was used to determine the FGFR1 amplification status by fluorescence in-situ hybridization (FISH).

Results: 16% (12/72) of all primary tumors and 18% (7/39) of the lymph node metastasis displayed a FGFR1 amplification. Of interest, FGFR1 lymph node metastasis developed from FGFR1 amplified primary tumors. Furthermore, non-amplified tumors never displayed FGFR1 amplification in their corresponding lymph node metastases.

Conclusions: We could confirm that the FGFR1 amplification is a common genetic event in L-SCC. On top of that, we are the first to discover FGFR1 amplification in lymph node metastasis. Interestingly, the FGFR1 amplification status always transfers from primary L-SCC into the corresponding lymph node metastasis. Therefore, we suggest that the FGFR1 amplification is a clonal event in tumor progression. Beyond this biologically relevant observation, our finding is the basis for a rational of treating patients suffering not only from primary but also from metastatic FGFR1 amplified L-SCC with a targeted small molecule inhibitor therapy using FGFR inhibitors.

1987 Morphologic and Molecular Features of Primary Lung Adenocarcinomas That Metastasize to Brain

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Background: Lung cancer, the leading cause of cancer-related deaths worldwide, has a 15% incidence of brain metastases within the first year of diagnosis. While morphologic and molecular heterogeneity occurs in lung adenocarcinomas, it is not known whether certain features predict the propensity to metastasize to the central nervous system (CNS). We hypothesize that lung adenocarcinomas which metastasize to the CNS show distinct morphologic and molecular features, when compared to those that are locoregionally confined.

Design: Primary lung adenocarcinoma resection specimen slides were retrieved (2001-2010) and studied retrospectively for morphologic differences between tumors that developed CNS metastases (n=16) and those that had locoregional spread only (n=149). The morphologic patterns of primary lung adenocarcinomas with and without brain metastases were classified according to the percentage of different morphologic patterns and then given a histologic score based on the two most predominant patterns [Am J Surg Pathol. 2010; 34(8):1155-62]. A Fischer-exact test was used to compare the morphologies of case and control groups. Adenocarcinomas that developed CNS metastases were analyzed by PCR for *KRAS* and *EGFR* mutations and by FISH for *EML4-ALK* translocations.

Results: Regardless of pathologic stage, lung adenocarcinomas which developed CNS metastases were more often histologic score 6 (6/16 cases) compared to control cases (3/149; p<0.001). No difference was observed between the number of histologic score 5 [(3,2) and (2,3)] cases in the CNS metastasis group (10/16) compared to control cases (91/149; p=0.6). Lung primaries which developed CNS metastases were more likely to have predominant solid/micropapillary pattern (12/16), score 5(3,2) or score 6, compared to controls (34/149; p<0.01). *KRAS* mutations were detected in 7 out of 16 lung adenocarcinomas with brain metastases; 4 of these 7 were *KRAS* G12C mutations. No *EGFR* mutations or *EML4-ALK* translocations were detected.

Conclusions: Lung adenocarcinomas which developed CNS metastases are more likely to be of higher histologic score 6(3,3) or 5(3,2), and preliminary data suggest they may be more likely to harbor KRAS mutations. The presence of a predominant solid or micropapillary pattern may morphologically predict primary lung adenocarcinomas that metastasize to the CNS, regardless of pathologic stage.

1988 Sarcoid Lung Disease: Pathologic Findings at Explant with Imaging Correlation

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Background: Sarcoid granulomas typically occur in a lymphatic distribution in the lung. A subset of patients may develop lung failure due to fibrosis. CT findings of patterns of fibrosis have not been studied in detail.

Design: We retrospectively evaluated histologic sections from 10 lung explants in a patient with a clinical diagnosis of sarcoid lung disease (SLD). Each lung was sectioned in a systematic fashion by the same pathologist sampling central and peripheral sections from each lobe. In 9 patients, high-resolution CT scans were available for correlation.

Results: Histologically, 7 of 10 explants showed findings typical of SLD (granulomas and fibrosis in a lymphatic distribution); 2 showed dense scarring with elastosis in a sarcoid-like distribution without granulomas (atypical sarcoid). The 10th case showed incidental SLD (granulomas without fibrosis) in a setting of organizing pneumonia interstitial lung disease (cryptogenic organizing pneumonia, COP). The 9 patients with SLD were 7 women (51 ± 12 years) and 2 men (48 ± 4 years). Of the 7 cases with typical sarcoid, 4 had numerous granulomas, in the bronchial submucosa, peribronchial regions, and interstitium. Three had relatively sparse granulomas embedded in dense scar. Of the 9 cases with typical or atypical sarcoid, 6 cases showed microscopic areas of NSIP up to 1 cm in size, and 2 peripheral honeycombing; however, none showed an overall pattern resembling NSIP or usual interstitial pneumonia (UIP). Bronchiectasis was seen in every case and large cysts (up to 7 mm) lined by respiratory epithelium and surrounded by scar were characteristic and seen in 6 of the 9 cases.

Of the 7 lungs with typical sarcoid, 3 CTs were evaluated as definite sarcoid, and 3 probable (one CT not available). The 2 atypical sarcoid cases were evaluated by CT as definite, and one probable sarcoid. The case of COP was evaluated as probably not sarcoid on CT. Imaging demonstrated honeycombing bronchiectasis in all cases of definite or probable sarcoid, with possible honeycombing in 2 (one correlating with histologic findings).

Conclusions: SLD shows a fibrotic pattern that is distinct from UIP or NSIP, and is composed of dense fibrosis with bronchiectasis and large cysts lined by respiratory epithelium. There is a good correlation with high resolution CT. Granulomas amenable to biopsy (in the peribronchiolar interstitium or bronchial submucosa) were present in 4 of 9 cases of presumed sarcoid; in the others they were scarce or absent, related to advanced scarring.

1989 Morphological Characteristics of Pleural Mesothelioma Cells Harboring 9p21 Homozygous Deletion Detected by Fluorescence *In Situ* Hybridization (FISH) in Effusion Cytology

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Background: In malignant pleural mesothelioma (MPM), cytologic analysis is the primary diagnostic approach, since most patients first present with pleural effusion. However, the cytologic distinction between MPM and reactive mesothelial cells (RMC) in effusions can be impossible sometimes due to the lack of both well-established immunocytochemical markers and definite cytological criteria for MPM. Homozygous deletion of the 9p21 locus, the site of the *CDKN2A/p16* gene, frequently occurs in MPM but so far has never been reported in RMC. The aim of this study was to define the cytomorphological characteristics of MPM cells, identified by the presence of 9p21 homozygous deletion by FISH.

Design: For the above purpose, cells on smear preparations were recorded using a virtual microscope system, and then subjected to FISH analysis. Thereafter, 9p21 homozygous deletion-positive cells (about 100 cells each case) were identified in the recorded virtual slides, followed by analysis of their morphological characteristics. The study material comprised 61 histopathological (45 MPM + 16 RMC) and 13 cytological (7 MPM + 6 RMC) cases.

Results: Cut-off values (>10% for homozygous deletion and >52% for heterozygous deletion) were set based on the results of 9p21 FISH in tissue sections. According to the cut-off values, all effusion cases of MPM were 9p21 homozygous deletion-positive, whereas none of RMC cases was deletion-positive.

Conclusions: The 9p21 homozygous deletion-positive MPM cells exhibited significantly more frequent cell-in-cell engulfment, multinucleation, especially more than three nuclei, and larger multicellular clusters composed of more than 10 cells, compared with 9p21 deletion-negative RMC, indicating that these findings are useful for cytological differentiation of MPM cells from RMC.Our study also introduced a new methodology to analyze the morphology of a tumor using the combination of the virtual microscope system and FISH.

1990 The Influence of the Bronchioloalveaolar Component of an Invasive Adenocarcinoma on Survival

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Background: The literature has been divided as to whether the adenocarcinoma in situ (AIS) component of an invasive adenocarcinoma has an influence on prognosis. We were able to study a cohort of Norwegian patients, who had resections for adenocarcinoma to answer this question.

Design: 137 cases with an AIS component were selected from a series of invasive adenocarcinomas. Some cases were reclassified as different tumor types and discarded. The cases were studied by one pathologist (PSH) who had no knowledge of the clinical outcome. The percentage of the AIS component was calculated on the total number of tumor sections and a mean calculated. The presence of lymphatic, vascular and pleural invasion, when present, were noted. A final diagnosis as to the predominant cell type in the tumor was recorded. Age, sex, tumor size, pTNM grade and pathology variables were adjusted for in a Cox regression analysis.

Results: 103 patients were identified with an AIS component, five cases had 'pure' AIS and two further cases had more than a 95% component. The remaining 96 cases had invasive adenocarcinoma with an AIS component. 56.3% of the patients were aged 50 - 69 years. There were 55 females and 48 males. 64% of cases were p stage I (TNM7). Correlations with age, sex, pTNM stage, side of the tumor had no significant correlations. Patients with a greater AIS component had superior survival ($p = 0.019$). However mucinous tumors had a worse prognosis than non-mucinous ($p=0.022$). As expected, patients with lymph node metastases as well as pleural invasion and lymphatic and vascular involvement had poorer survivals than those without.

Conclusions: The percentage of the AIS component plays an independent significant prognostic factor in adenocarcinomas of lung with this component. It is recommended that histopathologists routinely calculate this percentage and incorporate the information into their final report, as this is a significant factor relating to survival. It does not take long to calculate the percentage of the AIS component. This data needs an interobserver verification study. The present data is compared with that in the literature.

1991 PLZF Immunstaining Inversely Correlates with Aggressiveness in Pulmonary Neuroendocrine Tumors

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Background: Pulmonary neuroendocrine tumors (PNT) include the low grade typical carcinoid (TC) the intermediate grade atypical carcinoid (AC), and the two high grade tumors, large cell neuroendocrine carcinoma (LCNEC) and small cell carcinoma (SCLC). While these tumors exhibit differing biologic behavior, they may be problematic to separate on small biopsy or cytology specimens. We surveyed a spectrum of PNTs for immunohistochemical detection of PLZF (Promyelocytic leukemia zinc finger), a transcriptional repressor with tumor suppressor-like activity. PLZF has been most extensively studied in leukemia; very few studies in solid tumors have been performed.

Design: Immunohistochemical staining for monoclonal anti-PLZF (D-9, 1:1000, Santa Cruz Biotechnology, Santa Cruz, CA) was performed on a total of 60 representative blocks from archival cases of carcinoid tumorlets (n=13); TC (n=18); AC (n=7); SCLC (n=18), LCNEC (n=4). Immunohistochemical staining intensity (0 to 3+), intracellular localization, and percent of immunoreactive tumor cells were recorded.

Results: Normal Type 1 alveolar epithelial cells displayed nuclear staining, serving as an internal positive control. All 13 tumorlets had nuclear immunoreactivity with 2-3+ intensity and staining in 95% to 100% of cells; 8 also displayed cytoplasmic immunoreactivity (1+ intensity; 95 to 100% of cells). Of the 18 TC, 17 had nuclear immunoreactivity with 2-3+ intensity staining of 10-100% of tumor cells (average = 70%). 16 also had cytoplasmic immunoreactivity, 1-2+ in intensity, involving 10-100% of cells (average=63%). Of 7 AC, 5 had nuclear immunoreactivity, intensity 2-3+, involving 20-80% of cells, (average=35%). 6 of these had cytoplasmic immunoreactivity with 1-2+ intensity, involving 30% to 100% of cells (average= 55%). All cases of SCLC and LCNEC were completely unreactive.

Conclusions: The percent of nuclear expression of PLZF in PNTs has an inverse correlation with grade. No staining was observed in either LCNEC or SCLC, suggesting that dysregulation of this transcriptional repressor may play a role in the aggressive behavior of the high grade PNTs. Further, the differential expression of PLZF appears to have utility in classification of PNTs. In particular, positive PLZF staining appears to exclude a diagnosis of SCLC or LCNEC.

1992 ALK Rearrangement Detected by FISH and Inmunohistochemistry Methods. Prevalence and Clinical Outcomes in a Selected Population of Advanced Non Small Cell Lung Cancer Patients

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Background: ALK rearrangement represents a novel molecular target in a subset of non small cell lung cancers (NSCLC). Our aim is to explore fluorescence in situ hibridation (FISH) and immunohistochemistry (IHC) as diagnostic methods, prevalence and clinical outcomes of ALK rearrangement patients in a selected population of NSCLC.

Design: Patients with NSCLC previously screened for EGFR mutation at our institution between June 2006 and January 2010 were selected. ALK rearrangement was identified by using FISH and the value of IHC (D5F3 monoclonal antibody-mAb) was explored. For IHC ALK protein expression positivity was defined as tumor-specific staining of any intensity in ≥10% of the tumor cells.

Results: 99 patients were identified with median age was 61.5 years (range 35-83), 80% were adenocarcinomas, 7% squamous and 13% NOS carcinomas. 51% patients were female. All were caucasian. 32% of the patients were never smokers and 30% former smokers. 7 (7%) patients were ALK rearranged positive by FISH, 13 (13%) were EGFR mutant, and 65 (65.6%) were wild type (WT/WT) for both ALK and EGFR. ALK rearrangements and EGFR mutations were mutually exclusive. ALK rearranged patients tend to be younger than EGFR mutated or WT/WT patients (median age of 56.7, 63 and 62.3 years, respectively). Patients with ALK positive tumors were predominantly never smokers (71.4%) and adenocarcinomas (71.4%). ALK positive and EGFR mutant patients have a better survival than WT/WT. All patients with ALK FISH negative tumors were negative for ALK IHC. Out of 7 patients positive for ALK FISH, 5 were also positive for ALK IHC, 1 negative and in the other there was not enough tissue to perform the analysis. The ALK FISH positive cases were analyzed by IHC with 5A4 mAb obtaining the same results.

Conclusions: The prevalence of ALK rearrangement is 8.5% in a caucasian selected population of NSCLC. ALK positive patients have different clinical features and a better prognostic than EGFR WT and ALK negative patients. IHC with D5F3 mAb against ALK is a promising method for detecting ALK rearranged NSCLC patients.

1993 Molecular Testing for Lung Adenocarcinoma: Concordance between Cytology and Histology

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Background: Lung cancer is a leading cause of mortality, and patients often present at a late stage. Targeted therapies have become available for adenocarcinoma (ADCA) with epidermal growth factor receptor (EGFR) mutation. Greater numbers of minimally invasive procedures with smaller sample size, including transthoracic CT- and endobronchial ultrasound (EBUS)-guided FNAs, are being performed for diagnosis and determination of EGFR as well as KRas status. These markers can be evaluated on cytology specimens, but no comparison of results between cytology and corresponding histology specimens has been performed. This study aims to establish the validity of molecular testing (MT) on cytology samples by comparing their results with those on histology samples.

Design: Patients for whom both cytology and histology samples from the same or different site contain ADCA were identified retrospectively. MT was performed on formalin-fixed, paraffin-embedded samples. Exons 18-21 were amplified by PCR to detect EGFR mutations, and real-time PCR with allele-specific primers was used to detect common KRas mutations.

Results: Sixteen cytology – 9 EBUS- and 7 CT-guided FNAs – and 20 histology – 4 bronchoscopic, 4 other endoscopic, and 1 CT-guided core needle biopsies, as well as 11 resection specimens were identified from 14 patients. Sites sampled include lymph nodes (LN, n=8), lung masses (n=23), and other (n=5). Concordant results were obtained for all KRas (6/6) and 7/8 EGFR analyses performed on corresponding cytology and histology samples.

Concordance Between Cytology and Histology

Cytology	Histology	EGFR		KRas	
specimen	specimen	+	-	+	-
1°	1°	1	4	0	4
LN	LN	0	1	1	0
LN	1°	0	1	0	0
LN	M	Discordant (1)		0	1

1°: Primary tumor; LN: Lymph node metastasis; M: Distant metastasis; EGFR: Epidermal growth factor receptor; KRas: Kristen-Rous sarcoma virus; (+): Positive for mutation; (-): Negative for mutation

The one discordant case came from a LN cytology sample (EGFR+) and a histology sample of a distant metastasis (EGFR-).

Conclusions: Identification of mutation in lung ADCA affects clinical decision-making, and it is important that results from small samples be accurate. Although this study is limited by low sample number, it shows that MT on cytology is as sensitive as that on histology. Differences in MT emphasize the need to perform testing on subsequent specimens.

1994 The Diagnostic Utility of *p16/CDKN2A* FISH in Distinction between Sarcomatoid Mesothelioma and Fibrous Pleuritis

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Background: In sarcomatoid mesotheliomas, the distinction from fibrous pleuritis is sometimes difficult, especially when the amount of tumor in the biopsy is small, cells are bland-appearing, immunohistochemistry is inconclusive, or tumor invasion of the stroma cannot be assessed. However, the correct diagnosis is crucial to patient care and compensation. It is reported that most mesotheliomas have deletion of *p16/CDKN2A* and methylated *p16/CDKN2A* promoters. Homozygous *p16/CDKN2A* deletion detected by FISH is reported to be a powerful technique for confirming the diagnosis of mesothelioma over reactive mesothelial cells. We studied the diagnostic utility of *p16/CDKN2A* FISH and detection of the methylation status of *p16/CDKN2A* for the diagnosis of sarcomatoid mesothelioma.

Design: A retrospective analysis of 69 surgical biopsies from 67 patients with histologically confirmed malignant pleural mesothelioma (epithelioid 33, biphasic 12, sarcomatoid 22) was performed. Methylation specific PCR were attempted in 62 cases. FISH for deletions of *p16/CDKN2A* was performed in 22 cases of mesothelioma and in 3 cases of fibrous pleuritis. The diagnosis of mesothelioma was rendered based on histology, results of immunohistochemistry, image studies, and clinical course.

Results: Homozygous deletion of *p16/CDKN2A* was observed in 11% (1/9) of epithelioid mesotheliomas and in 56% (5/9) of sarcomatoid mesotheliomas.

Heterozygous deletion was observed in 67% (6/9) of epithelioid mesotheliomas, 100% (3/3) of biphasic mesotheliomas, and 44% (4/9) of sarcomatoid mesotheliomas. Of fibrous pleuritis cases, none had a deletion of *p16/CDKN2A*. Methylation of *p16/CDKN2A* was observed in 13 of 59 informative cases (22%). It was observed in 17% (5/30) of epithelioid mesotheliomas, in 27% (3/11) of biphasic mesotheliomas, and in 22% (4/18) of sarcomatoid mesotheliomas. None of the tumors with homozygous deletion of *p16/CDKN2A* was methylated. Unmethylated epithelioid mesothelioma had changed to methylated pleomorphic mesothelioma during the course of chemotherapy for three years in one patient.

Conclusions: Our results demonstrated that homozygous deletion of *p16/CDKN2A* is more common in sarcomatoid mesotheliomas than in epithelioid mesotheliomas, and *p16/CDKN2A* FISH analysis can be a reliable test for the distinction between sarcomatoid mesothelioma and fibrous pleuritis.

1995 Cribriform Pattern Identifies a Poor Prognostic Subset of Acinar Predominant Tumors in Stage I Lung Adenocarcinoma Patients

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Background: A newly proposed IASLC/ATS/ERS lung adenocarcinoma classification emphasizes the prognostic significance of histologic subtypes. In this classification, however, one limitation is that the majority of patients (approximately 40%) are classified into the acinar predominant subtype. We investigated whether cribriform pattern can further stratify the prognosis of histologic subtypes in stage I lung adenocarcinoma.

Design: H&E stained slides of 540 stage I lung adenocarcinoma patients (2002-2009) were reviewed. Tumors were classified into histologic subtypes according to the IASLC/ATS/ERS classification: adenocarcinoma in situ (AIS), minimally invasive adenocarcinoma (MIA), lepidic, papillary, acinar, micropapillary, solid predominant, invasive mucinous and colloid adenocarcinoma. The percentage of cribriform pattern was recorded in 5% increments, and we added cribriform predominant subtype. Log-rank test was used to analyze the association between histologic variables and recurrence-free probability (RFP).

Results: Patients with AIS/MIA (n=26) experienced no recurrence (3-year RFP: 100%). Patients with lepidic predominant tumors (n=76) had the low-risk of recurrence (3-year RFP: 97%). Patients with acinar (n=193), and papillary predominant tumors (n=102) had the intermediate-risk of recurrence (3-year RFP: 90% and 91%, respectively). Patients with micropapillary (n=46), solid predominant (n=71), and invasive mucinous/colloid adenocarcinoma combined group (n=26) had the high-risk for recurrence (3-year RFP: 59%, 72%, and 62%, respectively). The 3-year RFP of cribriform predominant subtype (n=16, 70%) was comparable to the high-risk group for recurrence. When looking at RFP according to cribriform pattern percentage in all cases, patients with ≥30% cribriform pattern (n=31) had marginally lower RFP (3-year RFP: 77%) than <30% cribriform pattern (n=509, 86%, p=0.047). Within patients with acinar predominant subtype, patients with ≥30% cribriform pattern (n=25) had significantly lower RFP (3-year RFP: 75%) than <30% cribriform pattern (n=168, 92%, p=0.001).

Conclusions: Cribriform pattern further stratified the acinar predominant tumors into two prognostically distinct subsets. In addition, cribriform predominant tumors may be considered as a poorly differentiated or high grade category with a high-risk for recurrence.

1996 Thyroid Transcription Factor-1 Expression Correlates with Predominant Histologic Subtypes and Recurrence in Stage I Lung Adenocarcinoma Patients

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Background: The majority of lung adenocarcinoma express thyroid transcription factor-1 (TTF-1), but lack of TTF-1 expression correlates with worse prognosis. We investigated whether TTF-1 expression can correlate with a newly proposed IASLC/ATS/ERS lung adenocarcinoma classification and further stratify prognosis of histologic subtypes in stage I lung adenocarcinoma.

Design: H&E stained slides of 506 stage I lung adenocarcinoma patients (1995-2005) were reviewed. Tumors were classified into histologic subtypes according to the IASLC/ATS/ERS classification. We constructed a tissue microarray and performed immunohistochemistry for TTF-1. In all, 459 cases with adequate cores were available for TTF-1 expression analysis. Intensity of staining was scored as 0 (no expression), 1 (mild), 2 (intermediate), and 3 (strong). According to the intensity score, TTF-1 expression was divided into two groups: low (0-1) or high (2-3). Log-rank test was used to analyze the association between histologic variables and recurrence-free probability (RFP).

Results: High TTF-1 expression was identified in 100% (8/8) of adenocarcinoma in situ and minimally invasive adenocarcinoma, 96% (25/26) of lepidic predominant, 88% (113/129) of papillary, 87% (188/215) of acinar, 75% (9/12) of micropapillary, 77% (44/57) of solid, 44% (4/9) of invasive mucinous, and 33% (1/3) of colloid adenocarcinoma. High TTF-1 expression was more frequent in tumors with low architectural grade (p<0.001). The 5-year RFP of tumors with low TTF-1 expression (n=67, 69%) was lower than high TTF-1 expression (n=392, 85%, p=0.001). Among tumors with the acinar subtype, low TTF-1 expression (n=27) was associated with lower RFP (5-year RFP: 70%) compared to high TTF-1 expression (n=188, 86%, p=0.013). Among patients with other each histologic subtype, there was no significant difference of RFP between low and high TTF-1 expression.

Conclusions: TTF-1 expression was significantly correlated with architectural grade based on histologic subtype. In addition, TTF-1 expression correlated with recurrence, and further stratified the acinar predominant tumors into two prognostically distinct subsets.

1997 Feasibility of Molecular Testing in Patients with Chemorefractory Non-Small Cell Carcinoma

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Background: The majority of patients with advanced Non-Small Cell Carcinoma (NSCLC) experience relapse or disease progression on chemotherapy. Targeted therapies based on tumor biomarker profile may be beneficial in these patients. Obtaining adequate tissue for molecular profiling in patients who are heavily treated may be challenging. This study was conducted to evaluate the feasibility of biopsy for molecular testing in patients with chemorefractory NSCLC.

Design: We analyzed the database for BATTLE trials (Biomarker-integrated Approaches of Targeted Therapy for Lung Cancer Elimination), including first BATTLE trial and the data from the ongoing BATTLE II trial. Biopsies were collected in 324 patients from BATTLE I and 46 patients from BATTLE II. The biopsy material included core needle biopsies (CNB) for BATTLE I and CNB together with Fine Needle Aspiration (FNA) for BATTLE II. Immediate assessment for tissue adequacy was performed by an onsite cytopathologist for all the BATTLE II cases. Tissue source included lung and metastatic sites including lymph node, liver, adrenal gland, skin, bone and mediastinum. Mutation analysis for EGFR and KRAS was performed in all the cases with adequate material within 2 weeks after the biopsy.

Results: Of 324 patients enrolled in BATTLE-1 trial, CNBs with adequate (≥200 cells/slide) content for molecular testing were obtained in 271 (84%) cases. Tissue fibrosis and necrosis were the major histological findings in the cases with non-adequate tissues. The specimen adequacy did not correlate with tissue source. *KRAS* and *EGFR* mutation analyses were successfully performed in 270 cases and were detected in 18% (n=48) and 17% (n=46), respectively. Of the 46 patients enrolled in the ongoing BATTLE-2 trial, adequate tissue was obtained in all cases except one. Similar rates of *EGFR* (11%) and *KRAS* (16%) mutations have so far been detected in this ongoing trial.

Conclusions: We have studies the diagnostic yield of CNB for molecular testing in patients with advanced, chemorefractory NSCLC. Successful molecular testing, utilizing CNB is feasible in patients in this clinical setting. Combined CNB and FNA may have a role in reducing the failure rate.

1998 BRAF Mutation Analysis in Pulmonary Langerhans Cell Histiocytosis

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Background: Pulmonary Langerhans Cell Histiocytosis (PLCH) refers to an interstitial proliferation of Langerhans cells in the lung. PLCH is currently regarded as part of the spectrum of diseases associated with cigarette smoking. The nature of PLCH is not clear. Some regard it as a reactive process while others favor a true neoplastic process. Recently BRAF mutations have been identified in systemic and osseous variants of the disease. This study aims to analyze a series of PLCH patients for the BRAF mutation in the lung.

Design: Histopathologically documented cases (n= 10) of PLCH were evaluated for BRAF mutations. A total of 18 paraffin embedded tissue blocks from 10 cases were analyzed for BRAF mutation (some cases have multiple tissue blocks). Lesional areas were identified and microdissected. BRAF specific PCR reactions were performed with a peptic nucleic acid (PNA) clamp designed to block amplification of wild-type sequences between codons 598 and 602. Amplicons were analyzed by real time PCR. All results were confirmed by Sanger sequencing using capillary electrophoresis. The obtained sequencing data was then compared to a wild-type BRAF reference file to identify possible mutations.

Results: BRAF mutations were found in 5 cases out of 7 qualified cases (71.4%). One of 10 cases had no diagnostic tissue left in the block and two cases were rejected due to suboptimal DNA quality. Cases with multiple blocks showed (GTG>GAG) V600E mutation in all blocks tested. One of the cases showed an unexpected (ACA>ATA) T599I mutation in the BRAF gene.

Conclusions: Our findings suggest that BRAF mutations are common in PLCH, supporting the view that PLCH may be a neoplastic proliferative disorder. Our findings further suggest a potential benefit from FDA-approved drug vemurafenib targeting BRAF mutation. Deep sequencing for other possible mutations involving BRAF or other oncogenes may be useful in BRAF codon 600 wild type PLCH cases.

1999 PAX2, PAX5, and PAX8 Expression in Pulmonary Neuroendocrine Tumors

S Kirby, W Frankel, W Marsh, F Junya, J Jen, T Franks, W Travis, K Shilo. The Ohio State University Medical Center, Columbus, OH; Toyama University Hospital, Toyoma, Japan; Mayo Clinic, Rochester, MN; JPC, Silver Spring, MD; Memorial Sloan-Kettering Cancer Center, New York, NY.

Background: PAX genes 1-9 are transcription factors with DNA-binding capabilities, whcih play key roles in fetal development, tissue maintenance, and repair. Recent studies have documented PAX gene expression in a wide variety of cancers with preferential expression of PAX2 in mullerian and renal carcinomas; PAX5 in pulmonary small cell lung carcinoma (SCLC) cell lines, mullerian tumors, and B-cell lymphomas; and PAX8 in mullerian, renal, and thyroid carcinomas, as well as in pancreatic/duodenal well differentiated neuroendocrine tumors (NETs). Based on patterns of staining, these markers have been suggested as useful site specific tumor markers. Since the biological and diagnostic significance of PAX genes in NETs is not well elucidated, we investigated their expression and correlation with clinicopathological findings in a wide spectrum of pulmonary NETs.

Design: Tissue microarray based samples from 178 patients with pulmonary NET were studied for PAX8 (rabbit polyclonal, Cell Marque), PAX5 (clone 1EW, Novocastra),

and PAX2 (rabbit polyclonal, Invitrogen) expression by immunohistochemistry. Nuclear expression was recorded as negative (0, absent), or positive with 1+ (weak), 2+ (moderate), and 3+ (strong) intensity. PAX expression was correlated with clinical-pathological variables including patients' age, gender, overall survival and tumor type, grade and stage.
Results: PAX8 expression was identified in 16.0% (23/144) of pulmonary NETs, and included 2.4% (1/42) of typical carcinoids, 11.1% (3/27) of atypical carcinoids, 33.3% (8/24) of large cell neuroendocrine carcinomas (LCNECs), and 21.6% (17/51) of SCLCs. PAX8 expression correlated with tumor grade ($p<.05$), but not with other clinical pathological variables. PAX2 expression was detected in 1 SCLC representing 0.5% (1/181) of pulmonary NETs. PAX5 expression was detected in 1 SCLC representing 0.7% (1/145) of pulmonary NETs.
Conclusions: PAX8 expression correlates with NET differentiation/grade and is observed in a significant percentage of LCNEC and SCLC. Since PAX8 expression is also seen in rare pulmonary carcinoids, its suggested site specificity for pancreatic/duodenal NETs should be used with caution. The vast majority of pulmonary NETs is negative for PAX2 and PAX5 (clone 1EW). The rarity of PAX5 expression in SCLCs in this study may be related to the choice of antibody clone.

2000 Histone 1.5 (H1.5) Staining Directly Correlates with High Grade in Pulmonary Neuroendocrine Tumors

H-BM Ko, JF Hechtman, Y Kinoshita, DE Burstein, MB Beasley. Mount Sinai School of Medicine, New York.
Background: Pulmonary neuroendocrine tumors (PNT) encompass a spectrum of tumors including the low and intermediate grade typical carcinoid (TC) and atypical carcinoid (AC) as well as the high grade large cell neuroendocrine carcinoma (LCNEC) and small cell carcinoma (SCLC). These tumors may pose diagnostic challenges on small biopsy specimens. Histone 1.5 (H1.5) is a member of the linker Histone 1 (H1) family. Levels of H1 are maximally phosphorylated in late G2 of the cell cycle, loosening the chromatin to allow transcription factors to bind. H1.5 has been shown to be the predominant H1 variant on active chromatin and chromatin poised for transcription. H1.5 has not been specifically evaluated in the spectrum of PNT. We evaluated Histone 1.5 staining in PNTs.
Design: 55 cases of PNT were retrieved, including carcinoid tumorlet (n=11), TC (n=17), AC (n=7), SCLC (n=16), and LCNEC (n=4). Immunohistochemical staining for H1.5 was performed (*Abcam, 1:800*). The staining was evaluated based on intensity (0 to 3+), and proportion of positive tumor cells (0-100%).
Results: H1.5 staining was universally found to be strictly nuclear, with no evidence of cytoplasmic staining in all 55 of the cases studied. Of 11 tumorlets, 1 showed nuclear immunoreactivity with 1+ intensity in 1% of the tumor cells; 2 showed weak/equivocal staining. Of 17 TC, 1 had nuclear immunoreactivity, intensity 2-3+, involving 15% of the tumor cells; 1 showed weak/equivocal staining involving 20% of cells. Of 7 AC, 2 cases had nuclear immunoreactivity, intensity 1-2+, involving 20% of cells; 3 showed weak/equivocal staining involving 5-20% of cells (average=12%). All 16 of the SCLC showed nuclear immunoreactivity, 1-3+ intensity, involving 30-100% of cells (average= 84%). All 4 of the LCNEC had nuclear immunoreactivity, intensity 1-3+, involving 40-100% of cells (average = 84%).
Conclusions: High nuclear expression of H1.5 in PNTs is directly correlated with high tumor grade. Low and intermediate grade TC and AC have a low incidence of positive staining for H1.5, with staining being weak and/or involving a small percentage of cells. In contrast, all high grade LCNEC and SCLC tumors showed positive staining for H1.5, with a high percentage of cells staining positive. These results are compatible with the theory that high expression of H1.5 reflects a constant state of DNA replication in high grade PNTs. In addition, staining for H1.5 may be useful in differentiating carcinoid tumors from high grade neuroendocrine tumors on small pulmonary biopsies.

2001 Characterization and Clinical Validation of an Immunohistochemical Assay for Met in Non-Small Cell Lung Cancer

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Background: The *MET* gene encodes a transmembrane receptor tyrosine kinase, which is the receptor for hepatocyte growth factor and is expressed on a variety of normal epithelial cells and carcinomas. For some tumor types, expression of Met has been correlated with poor clinical outcome. To develop a standardized Met IHC assay for FFPE tissue needed to definitively determine the prognostic and predictive implications for Met expression we evaluated the performance of a rabbit monoclonal anti-Met Ab (SP44).
Design: Immunohistochemistry (IHC) for Met was performed on formalin-fixed, paraffin-embedded cell pellets and tissues on an automated platform (Ventana Benchmark XT) using a rabbit monoclonal antibody (SP44; Ventana Medical Systems) generated against the intracellular domain of Met. The cell staining intensity for Met was scored as none, weak, moderate or strong. For NSCLC tumor samples a comprehensive clinical scoring system was developed to account for the heterogeneity of Met expression seen in tumor tissue. The scoring system evaluates both, staining intensity and percent cells staining at a given level. NSCLC cases expressing moderate or strong levels of Met in ≥50% of tumor cells (clinical IHC score 2+ or 3+) were classified as Met diagnostic positive (Met Dx+).
Results: IHC for Met on FFPE sections of cell lines expressing Met at various levels (endogenously and manipulated through ectopic transfection of *MET* or after RNA-interference knockdown) showed excellent correlation between IHC scores and Western blot results. The pre-clinical evaluation demonstrated excellent specificity and sensitivity of the SP44 antibody and its suitability for determining Met protein expression on FFPE tissue. Expression of Met was variable and heterogeneous in benign respiratory mucosa and pneumocytes. In a cohort of NSCLC patients (n=128) from a phase II trial evaluating MetMAb (onartuzumab), a one-armed anti-Met antibody, for the treatment of NSCLC, 54% of cases were classified as Met Dx+. A statistically significant benefit in progression-free (PFS) and overall survival (OS) was observed for patients with Met Dx+ tumors treated with the MetMAb antibody onartuzumab (PFS: Hazard ratio=0.53, 95% CI=0.28-0.99, p=0.04; OS: Hazard ratio=0.37, 95% CI=0.19-0.72, p=0.002.
Conclusions: We have characterized and validated an IHC assay for Met in FFPE tissues, which identifies a population of NSCLC patients who may derive benefit from Met-targeted antibody therapy with MetMAb (onartuzumab).

2002 C-Kit Expression Is Associated with *KRAS* Mutation in Lung Adenocarcinoma

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Background: Effective treatments for primary lung adenocarcinoma with *KRAS* mutation remain elusive. Further investigation into the biology of these tumors is needed, including identification of associated markers. The proto-oncogene *c-kit* that encodes a tyrosine kinase receptor c-kit is not commonly mutated or amplified in lung adenocarcinoma, but our experience with a small number of cases suggests that c-kit protein overexpression may be associated with *KRAS* mutation. We sought to examine c-kit expression levels in a large cohort of lung adenocarcinomas in the context of *KRAS* mutation status, clinical profiles, and patient outcomes.
Design: Three-hundred nine cases of stage I and II primary lung adenocarcinoma resected at our institution were immunohistochemically stained with c-kit on tissue microarrays. c-kit cytoplasmic and/or membranous intensity was scored as 2 (strong), 1 (weak), or 0 (negative) and multiplied by the percentage of positive cells, for overall scores of 0 to 200; cases scoring 25 or greater were deemed positive. For the 148 most recent cases (resections since 2008, cohort 1), mutational analysis data was available from an in-house clinical SNaPshot assay of common cancer-associated genes, and these results were compared with c-kit expression. Detailed clinicopathologic and outcome data were obtained for the additional 161 cases (resected before 2007, cohort 2) and compared with c-kit expression.
Results: In cohort 1, 38 cases had *EGFR* mutations, 46 had *KRAS* mutations, and 64 were wild-type for both (NENK). c-kit was positive in 2/38 *EGFR* mutants (5%), 20/46 *KRAS* mutants (43%), and 15/64 NENK (23%). c-kit expression was more common in cases with *KRAS* mutation compared to those with *EGFR* mutation ($p<0.0001$), NENK ($p=0.026$), or wild type for *KRAS* ($p=0.0009$). Cohort 2 consisted of 57% females, mean age at diagnosis of 67 years, 87% clinical stage I, and an acinar pattern as the most common predominant histology (51%). The average follow-up time was 5.3 years (range 0-16 years). The 5-year disease-free survival for c-kit-positive versus negative cases was 39% and 40%, respectively. Gender, age, clinical stage, and predominant histology also appeared unrelated to c-kit expression.
Conclusions: Lung adenocarcinoma with *KRAS* mutation appears enriched in c-kit overexpression by immunohistochemistry, raising the possibility of inhibiting c-kit signaling pathway as a treatment option for this population. The finding that c-kit expression was not associated with survival may be a function of the generally favorable prognosis of this early-stage cohort, and further investigation is needed in advanced stage disease.

2003 The Performance of an E746-A750del Mutation Specific EGFR Antibody in Non-Small Cell Lung Cancer Specimens

A Kyshtoobayeva, KJ Bloom. Clarient, A GE Healthcare Company, Aliso Viejo, CA.
Background: Non-small cell lung cancer (NSCLC) is the leading cause of cancer-related deaths worldwide. Two-thirds of patients present with advanced disease and have an average survival of less than 1 year with standard chemotherapy. Studies have demonstrated that exon 19 deletion or L858R substitution in the EGFR gene are the most powerful predictive biomarkers in patients treated with erlotinib or gefitinib. This has led to the recommendation that EGFR mutational status be evaluated prior to initiating chemotherapy. Currently mutational status is assessed by sequencing of the EGFR gene or allele specific PCR. Approximately half of all EGFR mutations harbor deletions in exon 19. We sought to assess the performance of a E746-A750del mutation specific EGFR antibody purported to identify cells harboring the exon 19 deletion.
Design: 100 formalin fixed embedded tissue sections, 50 harboring an exon 19 deletion and 50 assessed as non-mutated or harboring a mutation other than an exon 19 deletion were pulled from our archives. 4 micron sections were stained with a rabbit monoclonal E747-A750del mutation specific antibody, 6B6, (cell signaling, Danvers, MA) 1:300, following heat-induced epitope retrieval, 100°C, pH9, 20 minutes.
Results: 31 (62%) of the 50 tumors harboring an exon 19 deletion showed expression of the 6B6 antibody ranging from 1+ to 3+ intensity. The remaining 19 tumor showed no expression. All 50 tumors lacking an EGFR mutation or harboring a mutation other than an exon 19 deletion showed no expression of the 6B6 antibody.
Conclusions: The E746-A750del mutation specific EGFR antibody 6B6 identified 62% of the tumors harboring an exon 19 deletion and showed no expression in tumors lacking and EGFR mutation or harboring a mutation other than an exon 19 deletion. The poor sensitivity of this antibody limits its usefulness in replacing or supplementing mutational testing by sequencing or allele specific PCR.

2004 The Performance of an L858R Mutation Specific EGFR Antibody in Non-Small Cell Lung Cancer Specimens

A Kyshtoobayeva, KJ Bloom. Clarient, A GE Healthcare Company, Aliso Viejo, CA.
Background: Non-small cell lung cancer (NSCLC) is the leading cause of cancer-related deaths worldwide. Two-thirds of patients present with advanced disease and have an average survival of less than 1 year with standard chemotherapy. Studies have demonstrated that exon 19 deletion or L858R substitution in the EGFR gene are the

most powerful predictive biomarkers in patients treated with erlotinib or gefitinib. This has led to the recommendation that EGFR mutational status be evaluated prior to initiating chemotherapy. Currently mutational status is assessed by sequencing of the EGFR gene or allele specific PCR. Approximately one third of EGFR mutations are the L858R substitution. We sought to assess the performance of an L858R EGFR mutation specific antibody.

Design: 100 formalin fixed embedded tissue sections, 50 harboring the L858R substituion and 50 assessed as non-mutated or harboring a mutation other than L858R were pulled from our archives. 4 micron sections were stained with a rabbit monoclonal L858R mutation specific antibody, 42B2, (cell signalling, Danvers, MA) 1:50, following heat-induced epitope retrieval, 100°C, pH9, 20 minutes.

Results: All 50 NSCLC tumors harboring the L858R mutation, showed expression with the 42B2 antibody ranging from 1+ to 3+ expression. Expression was limited to tumor cells but occasionally extended to atypical cells lining alveolar spaces adjacent to tumor. No expression was noted in any of the tumors lacking the L858R mutation, including all non-mutated tumors as well as those tumors harboring a mutation other than L858R.

Conclusions: The rabbit monoclonal antibody 42B2, directed against the EGFR L858R substitution demonstrated 100% sensitivity and 100% specificity for the detecetion of the L858R substitution. The usefulness of this antibody is limited since only about one third of NSCLC demonstrating an EGFR mutation harbor an L858R substitution.

2005 Association of KRAS Mutation in Non Small Cell Lung Cancer and 18F-FDG Uptake in PET/CT

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Background: Non small cell lung cancer (NSCLC) tumors with KRAS mutations display primary resistance to EGFR TKIs, molecular evaluation of KRAS is important to predict clinical treatment outcomes and to decide treatment. Imaging studies such as CT scan and Positron emission tomography (PET) scan have been used in lung cancer for an accurate initial staging, response monitoring, and follow – up. Many factors can influence FDG uptake behaviour and the underlying mechanisms for FDG accumulation in tumours are still unknown. Mutation of the KRAS gene is one of the most important events in carcinogenesis of the lung. The objective of this study is to determine differences in 18F-FDG uptake between patients with NSCLC who harbor KRAS mutation.

Design: We include 55 patients (39 M, 16 F, median age 61) with diagnosis of NSCLC by cytologic procedure (Bronchoscopy, EBUS and CT guided FNA). They also underwent PET-CT for staging performed by a PET-CT Biograph LSO-DUO scanner (SIEMENS™).KRAS mutations in codons 12 and 13 in exon 2 were analyzed using conventional DNA sequencing using ABI PRISM TM 310XL.

Results: KRAS mutations were identified in 24 patients (17 M, 7 F, median age 61). All of them were smokers. Regard histologic subtype 18 (75%) of them were adenocarcinoma, 5 (20.8%) squamous cell carcinoma and 1 cases (4.2%) NSCLC- NOS. Patients with wild type and mutated KRAS had the same age, gender and histologic subtype distribution. [18F] FDG uptake was higher in patients with KRAS mutated than wild-type in primary tumour (SUVmax 11.5 ± 6.7 vs 8.3 ± 3.8) (p 0.03) and nodal metastases (SUVmax 9.15 ± 6 vs, 6.19 ± 3.1) (p0.04) Distant metastases were observed in 33 patients and the SUVmax was higher in mutant KRAS cases than wild-type but without statistically significant differences.

Conclusions: Among patients with advanced lung cancer, those smokers, men and with higher SUVmax on primary tumour and nodal metastases in 18F-FDG PET/CT are more likely to carry KRAS mutations. 18F-FDG uptake might be helpful to discriminate patients who harbor KRAS mutations with poorer prognosis.

2006 CD146 Expression in Pleural and Peritoneal Mesothelioma

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Background: Malignant mesothelioma (MM), of either peritoneum or pleura, is an uncommon cancer. The diagnosis is often difficult to make, in part because of the overlapping morphology of reactive and malignant mesothelial cells. CD146 (also referred to as MUC18) is a transmembrane glycoprotein involved in intercellular adhesion. It has recently been reported to be a highly sensitive and specific marker of malignant, but not reactive, mesothelial cells in a small study of effusion cytology specimens. The staining patterns of CD146 in surgical pathology specimens in either pleural or peritoneal mesothelioma have not been reported.

Design: Tissue microarrays containing 178 mesotheliomas were stained with an antibody to CD146. These cases consisted of 26 pleural mesotheliomas and 152 peritoneal mesotheliomas. Fifty-four whole slide sections with reactive and or hyperplastic mesothelial cells were stained as controls. Cases were considered positive when 5% of the lesional cells showed membranous immunoreactivity. As CD146 is a transmembrane protein, cytoplasmic staining was considered non-specific, and was counted as negative.

Results: Overall, 104 of 178 malignant cases showed membranous CD146 expression, overall sensitivity **58%.** Sensitivity was similar amongst the two sites (pleura 50%, peritoneum 60%). The median amount of tumor cells showing immunoreactivity was 40%. Twenty-five of 26 cases with reactive/hyperplastic mesothelial cells in the abdomen were negative for membranous CD146 as were 25 of 28 cases in the thorax. Three of 54 benign cases showed foci of cytoplasmic positivity which was interpreted as negative. Overall specificity was **93%**. The positive predictive value was **96%**. The negative predictive value was **40%**.

Conclusions: The positive predictive value of 96% demonstrates the utility of a positive result for CD146 immunohistochemistry in the distinction of malignant from benign mesothelial cells. There are limitations, however, including weak intensity in some positive cases and rare cytoplasmic staining of unclear significance.

2007 Epigenetic Regulation of BCL2-Associated X Protein in Neuroendocrine Lung Tumors

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Background: The antiapoptotic protein, Bcl2, and it antagonist proapototic, BCL2-Associated X Protein or Bax have been studied in neuroendocrine lung tumors showing that small cell carcinoma of the lung (SCLC) have increased expression of Bcl2, and less expression Bax, than carcinoid tumors (CT). The following study was undertaken to compare epigenetic down-regulation of BAX in both carcinoid tumors and small cell tumors of the lung.

Design: Formalin fixed paraffin embedded tissues from 103 cases of primary lung neuroendocrine lung tumors including 89 small cell carcinomas of the lung, 11 typical carcinoids (TC) and three atypical carcinoids (AC) were used for this study. DNA extracted from microdissected tumor tissue was treated with sodium bisulfite and amplified by PCR using primers to the BAX gene promoter, followed by pyrosequencing. Percent methylation of the BAX promoter region was compared between SCLC and carcinoid tumors. Data was analyzed by the independent samples T-test using SPSS statistical software.

Results: BAX methylation ranged from 3 to 24% in TC, 2 to 7% in AC, and 0 to 19% in SCLC. The average methylation level was lower in SCLC (7.44%) than in TC (10.45%) (p=0.118) and the average methylation level in AC (5%) was lower than in the TC (P=0.156).

Conclusions: Small cell carcinoma of the lung has lower BAX promoter methylation than carcinoid tumors which would predict a higher expression of this pro-apoptotic protein coded by BAX gene. These findings are in contrast to previous observations of lower expression of Bax protein in SCLC compared to AC and TC. Thus, these studies do not support epigenetic control of BAX as a significant regulatory mechanism in these tumors. More studies are needed to better understand the regulation of the apoptotic pathway of small cell carcinoma which is a promising era for better chemotherapeutic agents.

2008 Loss of PTEN Expression and Gene Copy Number in Non Small Cell Lung Cancer

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Background: PTEN (Phosphatase and Tensin homolog deleted on chromosome ten) which suppresses the pro-survival Akt pathway, is known to be inactivated in multiple cancers. To characterize the role of PTEN in non-small cell lung carcinoma (NSCLC), we examined PTEN expression and gene copy number loss in a large cohort of NSCLC patients and compared findings to published genomic imbalance datasets.

Design: Tissue microarrays from 153 formalin fixed paraffin embedded tumors were constructed and sections were stained with PTEN monoclonal antibody clone 138G6 (Cell Signaling Technology, Danvers, MA). Tumors with absence of immunostaining were considered PTEN negative. *PTEN* copy number loss was evaluated by dual-colour fluorescence in situ hybridization (FISH) in 100 tumor nuclei with in-house *PTEN* probe labeled with Spectrum Green and Vysis® Chromosome Enumerating Probe for chromosome 10 (Abbott Laboratories, Abbott Park, IL) labeled with Spectrum Aqua. Tumors were considered *PTEN* deleted if 65% of cells had < 2 copies of *PTEN*. Single nucleotide polymorphism (SNP) array data was mined from 757 NSCLC published datasets. The *PTEN* region was analyzed with the rank segmentation algorithm with thresholds for copy gain and loss of 0.4 and -0.4, respectively.

Results: Immunostaining for PTEN was negative in 41.2 % (n=63/153) of cases, more frequent among squamous cell carcinomas (26/44 or 59%) than adenocarcinomas (32/94 or 34%) (p=0.0095). Among the 133 cases with interpretable FISH results, 7 (5.2%) were PTEN deleted, including hemizygous loss in 4 cases, monosomy in 2 cases, and homozygous loss in one case. These results are consistent with the analysis of SNP datasets which also identified a low PTEN deletion rate of 5.3% (40/757). Disease-free survival was significantly poorer in patients with PTEN negative tumors by immunostaining, as compared to PTEN positive immunostaining (HR=1.71; 95% confidence interval=1.01-2.92; p=0.045).

Conclusions: Up to 40% of NSCLC do not express PTEN protein, while *PTEN* deletion was detected in only 5.2% of tumors by FISH. These results suggest that loss of PTEN expression in NSCLC is primarily by epigenetic or mutation. Silencing of PTEN expression appears to be a poor prognostic marker for NSCLC patients.(This work was supported by grants from the Canadian Cancer Society, Canadian Institute of Health Research and Ontario Institute of Cancer Research.)

2009 Histological Patterns and Tumor Necrosis Have Significant Prognostic Implications in Stage I Lung Adenocarcinoma

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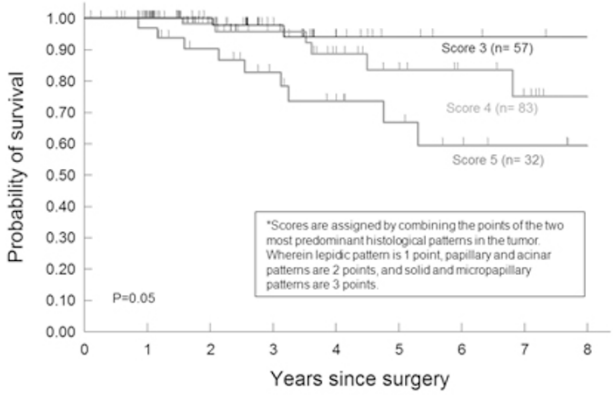
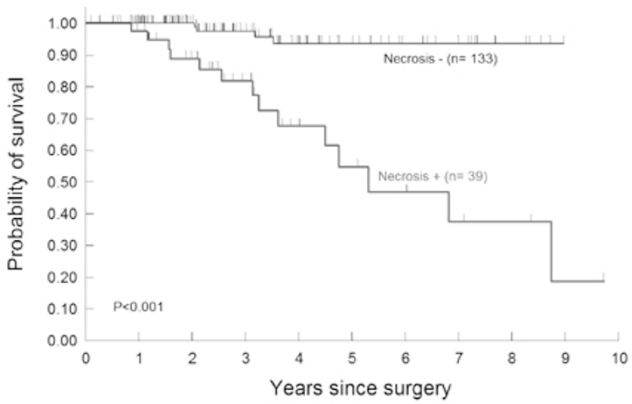
Background: Histomorphological patterns of lung adenocarcinoma have been found to play a distinctive role in its prognosis, based on which new classification and grading schemes have recently been proposed. The purposes of this study are to validate the recommendations, and try to analyze possible prognostic factors.

Design: We retrospectively reviewed histological slides of 172 patients with stage I lung adenocarcinoma who received surgery and follow-up at our institute. The respective percentages of the six histological subtypes, i.e. lepidic, papillary, acinar, micropapillary, and solid, were determined. The presence of lymphovascular invasion and/or necrosis was also reviewed.

Results: Of the 172 patients, 58(34%), 72(42%), and 42(24%) were stage T1a, T1b, and T2, respectively. Seven(5%) were minimally invasive carcinoma (invasive tumor <= 5mm). Thirty-five(20%) were lepidic predominant, 47(27%) papillary, 68(40%) acinar, 19(11%) solid, and 3(2%) micropapillary. The 5-year survival rate was 93% in lepidic predominant cases, and 45% in solid predominant cases. A 3-tier scoring system was applied, which stratified the patients into 3 subgroups with distinctive prognoses.



Tumor necrosis and lymphovascular invasion were present in 39(23%) and 37(22%) cases, respectively.Tumor necrosis, but not lymphovascular invasion, was found to be a major prognostic factor. The 5-year survival rate was 94% in cases without necrosis, and 54% in those with necrosis.



Finally, we performed a multivariant analysis on the histological patterns and found that only tumor necrosis was significantly associated with survival.

Conclusions: Our study confirms that histological patterns of stage I lung adenocarcinoma has a great impact on survival. A scoring system based on the two major predominant patterns may serve as a valuable tool in risk stratification. Finally, tumor necrosis is the most important prognostic factor for stage I lung adenocarcinoma.

2010 Mucin5B (MUC5B) Expression Correlates with High Stage in Lung Adenocarcinoma by Quantitative Proteomics and Immunohistochemistry

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Background: Pathogenesis of lung cancer is characterized by multiple genetic alterations and subsequent abnormal protein expression leading to phenotypic changes. EGFR mutation is well established in the pathogenesis of lung cancer. Recent studies have shown mucin (MUC) proteins (a group of over 20 heavily glycosylated, high molecular weight proteins) to play important roles in cancer differentiation, invasion, and metastasis in a variety of adenocarcinomas. Among them, the expression of MUC5B has been suggested in the EGFR signaling pathways in lung cancers; however, it is not well studied. We quantitatively analyzed protein expression in lung adenocarcinomas by a newly improved proteomic technique to identify potential biomarkers differentially expressed based on pathological stage. The expression of MUC5B by proteomics and immunohistochemistry (IHC) was then correlated with pathological stage.

Design: Formalin fixed paraffin-embedded tissue from 8 cases each of early (I/II) and late (III/IV) stage lung adenocarcinomas, and normal lung were quantized for expression of 370 proteins by liquid chromatography and tandem mass spectrometry (LC-MS/MS). Tissue microarrays containing 51 cases of lung adenocarcinoma were stained for MUC5B by immunohistochemistry (IHC).

Results: Of the 370 proteins quantitatively analyzed by LC-MS/MS, 13 proteins, including MUC5B, were markedly elevated in stage III/IV compared to stage I/II and normal lung tissue (Table 1). By IHC, MUC5B was found to correlate with high stage (Table 2).

Table 1: Proteomics Data

Protein	Normal tissue*	Stage I/II*	Stage III/IV*
Alpha-depensin 1	0.187	0.686	0.867
Annexin A3	0.179	0.446	0.945
Azurocidin 1	0.081	0.508	1.311
G-6-P isomerase	0.528	0.985	3.421
Histone cluster 1	0.354	0.971	1.645
Histone H4	0.588	1.226	1.652
Lactotransferrin	0.217	0.471	1.151
Lysozyme	0.291	0.334	1.120
Mucin 5B	0.515	0.573	1.474
Myeloperoxidase	0.164	0.890	1.059
Plastin-2	0.295	0.562	1.668
S100-A8	0.163	0.382	1.023
Utrophin	0.143	0.429	1.387

*Ratio of relative peak area to internal standard

Table 2: MUC5B Expression by IHC

Tissue	MUC5B
Normal lung	0/6 (0%)
BAC, Stage I/II	34/44 (77.3%)
Stage III/IV	7/7 (100%)

Conclusions: Our data indicate that the expression of MUC5B is higher in stage III/IV than I/II lung adenocarcinomas, suggesting that MUC5B could be a predictor of tumor aggressiveness. Since aggressive cancers frequently present at high stage, future studies will evaluate whether MUC5B is useful for predicting tumor aggressiveness in early stage cancers.

2011 Identification of Protein Signature in the Bronchoalveolar Lavage (BAL) Specimen from Lung Adenocarcinoma by Quantitative Proteomics

Q Li, Y Li, F Askin, E Gabrielson, H Zhang. The Johns Hopkins Medical Institutions and Bayview Medical Center, Baltimore, MD.

Background: Bronchoalveolar lavage (BAL) is a commonly used method to recover lung fluid by endobronchoscopy. Proteins in BAL fluid are derived from secretion or leakage of lung parenchymal cells. Levels of these proteins directly reflect the physiological and pathological status of the lung. Recently, quantitative proteomic analysis of BAL specimen has been used to study a variety of benign lung diseases such as asthma, cystic fibrosis, and interstitial lung disease. However, the protein profile of BAL in lung cancer has not been well studied. In this study, we have used newly improved proteomic techniques and quantitatively analyzed the protein profile in BAL specimen from lung adenocarcinoma patients.

Design: Eight BAL specimens were collected from patients with either early or late stage of lung adenocarcinomas. Paraffin-embedded lung adenocarcinoma tissue from eight patients, including four cases each of early stage (stage I/II) and four cases of late stage (stage III/IV) tumors were studied and tumor-matched normal lung tissues included as controls. Tumor cells were microdissected. Peptides from both BAL and tumor cells were extracted and quantified by LC-MS/MS using an LTQ Orbitrap Velos mass spectrometer.

Results: A total of 107 proteins were identified and quantified. Among them, 59 proteins was differentially expressed between BAL samples and tumor tissues (**Figure 1**). Thirteen proteins were significantly increased and two proteins were significantly decreased in BAL from bronchi with lung adenocarcinoma

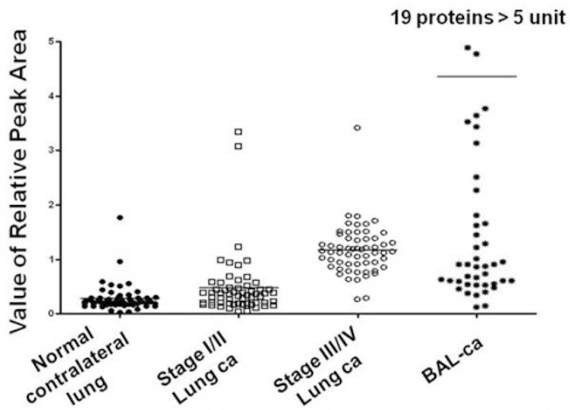

Figure 1. Protein identified among different groups of samples

Conclusions: Using highly sensitive proteomic technique, we were able to quantify protein profile in BAL specimen from lung adenocarcinoma. The proteomic analysis of BAL fluid in lung cancer may lead to the discovery of potential tumor-associated as well as non-tumor associated protein biomarkers. They can be used for the early diagnosis of cancer and monitoring the disease progression in lung cancer patients.

2012 Identification of an Effective Immunohistochemical Panel in Distinction of Breast Carcinoma from Lung Adenocarcinoma

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Background: When working on a tumor of unknown origin, lung adenocarcinoma versus breast carcinoma often presents a diagnostic challenge because of the overlapping morphological features and immunostaining profile. TTF-1, Napsin A and estrogen

receptors (ER) are the recommended panel of markers for this workup. However, these three markers are not entirely sensitive and specific for differentiating a lung primary from a breast primary. In this study, we re-evaluate the expression of an extensive panel of biomarkers, including recently described markers GATA3, Trefoil factor 1 (TFF1) and Trefoil factor 3 (TFF3) using a single immunostaining system (Dako).

Design: We immunohistochemically evaluated the expression of 1) epithelial markers (AE1/3, CAM5.2, CK7, CK20, CK17, CK19, CK903, EMA); 2) mucin gene products (MUC1, MUC2, MUC4, MUC5AC, MUC6); 3) tumor suppressor genes and transcription factors (ER, PR, p53, beta-catenin, WT-1, CDX2, pVHL, ERG); and 4) tumor-associated proteins (TTF-1, napsin A, GATA3 [Santa Cruz; sc-268], TFF1 [Epitomics;AC-0045], TFF3 [AC-0103], FOXA1, HepPar1, glypican 3, SALL4, OCT4, PAX2, PAX8, RCC GCDFP-15, mammaglobin, S100P, IMP3, maspin, MOC31, CEA, CA19-9, CA125, CD10, CD15, villin, and P504S) on 146 cases of breast carcinoma (98 ductal carcinomas and 48 lobular carcinomas) and 111 cases of lung adenocarcinoma on tissue microarray sections. The staining intensity was graded as weak or strong. The distribution was recorded as negative (<5% of tumor cells stained), 1+ (5-25%), 2+ (26-50%), 3+ (51-75%), or 4+ (>75%).

Results: The positive staining results from selected antibodies, which demonstrated diagnostic value, are summarized in Table 1. When combining ductal and lobular carcinomas, the positive staining results for GATA3, ER, TFF1 and TFF3 were 95%, 86%, 77%, and 88%, respectively, with a strong and diffuse staining (3+ or 4+) in 131 cases (90%), 90 cases (72%), 79 cases (56%) and 103 cases (72%), respectively.

Table 1. Summary of immunostaining results on selected antibodies

Antibody	Lung ADC	Breast ADC	Breast LCA
TTF1	87/111 (78%)	0/98	0/48
Napsin A	84/109 (77%)	0/98	0/48
GATA3	0/111	90/98 (92%)	48/48 (100%)
ER	0/111	77/98 (78%)	48/48 (100%)
TFF1	5/111 (5%)	68/95 (72%)	41/47 (87%)
TFF3	24/111 (22%)	81/96 (84%)	45/48 (94%)

Conclusions: These data demonstrate that TTF1, Napsin A, GATA3, ER, TFF1 and TFF3 are the most effective diagnostic panel for distinguishing lung adenocarcinoma from breast carcinoma.

2013 Cribriform Adenocarcinoma of the Lung: Clinicopathologic, Immunohistochemical and Molecular Study of 15 Cases

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Background: Lung adenocarcinoma is characterized by marked heterogeneity and may be composed of an admixture of histologic growth patterns, including acinar, papillary, solid and lepidic (bronchioloalveolar). Tumors displaying a prominent or predominant cribriform architecture are rare and most often confused for metastases from other organs. We report the clinical, histologic, immunohistochemical, *EGFR* and *K-RAS* mutational status and *ALK* rearrangement status in 15 primary lung adenocarcinomas with a predominant cribriform histology.

Design: A retrospective review of lung adenocarcinomas with a predominant cribriform architecture (>70%) and an immunohistochemical profile consistent with a primary lung adenocarcinoma (CK7/TTF1-positive; CK20/CDX2-negative) was performed. EGFR, K-RAS mutation and ALK/EML4 fusion analysis was performed in 6 cases. Clinical data was retrieved from the medical records.

Results: All 15 patients were adults and heavy smokers. The tumors showed histologic features that were closely reminiscent of metastases from prostate, breast, pancreas, ovary, uterus, colon and thyroid carcinoma. The main differential diagnosis was with metastatic adenocarcinoma with a cribriform pattern. The immunohistochemical profile in all cases was consistent with a primary lung adenocarcinoma. None of the cases had an EGFR mutation. One patient had a K-RAS mutation and one patient had an ALK/EML4 fusion.

Conclusions: The cases described here represent a morphologically distinctive group of primary lung adenocarcinomas that can resemble metastatic adenocarcinomas from multiple sites. Thorough clinical studies, coupled with careful histological examination and immunohistochemical stains help in the differential diagnosis. The molecular profile of these cases is heterogeneous. It is important to recognize these tumors in daily clinical practice to distinguish them from metastases.

2014 Acute Exacerbations of Interstitial Pneumonias in Amyopathic Dermatomyositis: Different Pathogenesis in Ordinary Dermatomyositis/ Polymyositis When Compared to Amyopathic Dermatomyositis

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Background: Interstitial pneumonias (IPs) are frequently identified in patients with dermatomyositis (DM) and polymyositis (PM). Amyopathic dermatomyositis (ADM) is a variant of DM that is characterized by the typical skin rash but without the muscle abnormalities. Some patients with ADM develop rapidly progressive IP, which may prompt aggressive therapy. This study compares the cell kinetics of the lung lesions in DM/PM with ADM.

Design: We studied 19 autopsy cases with IP of DM/PM (10 women, mean age 56 yrs., follow-up 2.3 yrs.), including 8 with ADM (4 women, mean age 57 yrs., follow-up 0.8 yrs.) emphasizing pulmonary disease. We compared the clinical features and lung pathology between 8 patients with ADM and 11 patients with ordinary DM/PM. We performed immunohistochmical examination for cell cycle markers, cell proliferation, cell injury, apoptosis, and the tissue reconstruction process using a standard indirect avidin-biotin horseradish peroxidase method with various antigen retrievals.

Results: Of the 11 patients with DM/PM, three had a UIP pattern, three showed a NSIP pattern, and four had widespread organizing pneumonia. All eight patients with ADM demonstrated acute and organizing diffuse alveolar damage (DAD) of whom seven had chronic IP including subpleural or paraseptal distribution of dense fibrosis and tissue remodeling of lung architecture. Immunohistochemically, increased expression of cyclin A2, Ki-67, surfactant proteins, MMP1, MMP7, TIMP-1, TIMP-2 and TGF beta-1 was observed in regenerating pneumocytes, bronchiolar epithelial cells and macrophages and not in fibroblasts in ADM but rarely in ordinary DM/PM. There was widespread positive epithelial apoptotic DNA strand break by nick end-labeling technique (TUNEL) staining in the lungs of ADM, but TUNEL signals were seldom seen in those of ordinary DM/PM.

Conclusions: Acute exacerbation of IPs occurs both in patients with ordinary DM/PM and in ADM. However, the acute exacerbation in the former group is generally an organizing pneumonia, while the acute exacerbation in the latter group is DAD superimposed on chronic IP. Upregulation of cell cycle markers were generally expressed only in the latter group. These results suggest an intrinsic difference in the progression of pulmonary disease between DM/PM and ADM.

2015 Expression of the Transcriptional Regulators BAP1 and Cyclin D1 in Malignant Pleural Mesothelioma

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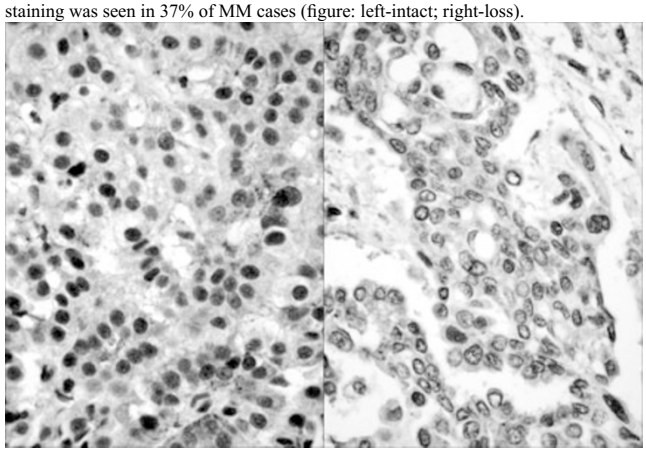
Background: It has recently been shown that some families with germline mutations of the gene encoding breast cancer associated protein-1 (BAP1) are at risk for developing malignant mesotheliomas (MMs) and other cancers. Furthermore, it has been shown that up to 40% of apparently non-familial cases of MM have mutations of the gene or loss of its protein expression. It has also been shown that more than 40% of MMs have inactivation of the *NF2* tumor suppressor gene, which is hypothesized to play a role in the regulation of G1 cell cycle progression through inhibition of Cyclin D1 expression. Here, to help elucidate the molecular changes seen in MM, we investigate the expression of these two regulators of transcription, BAP1 and Cyclin D1, in a large series of MMs.

Design: 53 cases of MM (33 epithelioid, 9 sarcomatoid, and 11 biphasic) were identified and a tissue microarray was constructed. A microarray was constucted of 21 cases of pleuritis. Immunohistochemistry was performed using monoclonal antibodies against Cyclin D1 and BAP1. Tumor cells were assessed for nuclear staining for both antibodies. For Cyclin D1, the percentage of tumor cells showing positive nuclear staining was recorded and the intensity of staining was graded as weak, moderate, or strong. For BAP1, complete absence of nuclear stain was recorded.

Results: 70% of MM showed nuclear staining for Cyclin D1. The majority (29 of 37) were positive in >25% of tumor cells, with 45% (17 of 37) of cases showing staining in in >75% of tumor cells. The staining intensity was strong in most cases. 14% cases of fibrosing pleuritis showed nuclear staining in 10-25% of cells. Loss of BAP1 nuclear staining was seen in 37% of MM cases (figure: left-intact; right-loss).



Conclusions: Cyclin D1 overexpression is detectable in the majority of MM cases, but in a larger percentage of cases than those reported to have inactivation of the *NF2* tumor suppressor gene. This suggests that other factors likely play a role in its overexpression. Since Cyclin D1 is rather sensitive and specific for MM, it could be a valuable biomarker in cases with borderline morphology. We confirm a previous report regarding BAP expression in MM and show that nuclear expression of BAP1 is lost in 37% of apparently sporadic cases.

2016 Comparative Immunohistochemical Analysis To Distinguish Malignant Mesothelioma (MM) from Reactive Mesothelial Cells (RMC)

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Background: The differentiation of MM from RMC can occasionally present a diagnostic challenge, especially on a small biopsy specimen. Recently several new biomarkers, such as GLUT-1, CD146, and IMP3, have emerged as good candidates for distinguishing MM from RMC. Each study using each marker has reported its high sensitivity and specificity for discrimination between MM from RMC; however, the markers rarely studied by other groups and have not comparatively analyzed. Moreover, CD146 was studied only in cytologic specimens. We, therefore, performed comparative immunohistochemical analysis in MM and RMC cases with antibodies for GLUT-1, CD146, IMP3, EMA and Desmin.

Design: Paraffin-embedded sections from 31 cases of previously confirmed MM and 40 cases of benign lung tissues with RMC were used. 15/31 (48%) MM were epithelioid, 11/31 (36%) were biphasic, and 5/31 (16%) were sarcomatoid. Antibodies used were GLUT-1 (polyclonal, Abcam), CD146 (N1238, Abcam), IMP3 (L523S, Dako), EMA (E29, Dako) and Desmin (D33, Dako). Staining results were scored by percentage of mesothelial or tumor cells staining. When more than 5% of mesothelial/tumor cells were stained with each antibody, the stain was defined as positive.

Results: As positive markers for MM, GLUT-1, CD146, IMP3, and EMA showed sensitivity of 84%, 61%, 94%, and 74%, respectively, and specificity of 100%, 100%, 88%, and 88%. As a negative marker for MM, Desmin showed sensitivity of 48%, and specificity of 97%. In MMs, proportion of positive tumor cells for GLUT-1 was from 10 to 95% (mean 47%); CD146, 6 to 95% (32%); IMP3, 6 to 100% (61%); EMA, 6 to 100 (60%). In RMCs, proportion of positive mesothelial cells for IMP3 was from 10 to 25% (mean 17%); EMA, 10 to 35% (19%); Desmin, 6 to 30% (12%).

Immunohistochemical results

	GLUT-1	CD146	IMP3	EMA	Desmin
MM	26/31	19/31	29/31	23/31	1/31
RMC	0/40	0/40	5/40	5/40	19/40

Conclusions: If the cutoff of IMP3 was set to be 30% of mesothelial/tumor cells and using the combination of GLUT-1 and IMP3, the sensitivity of detection of MM was 100%, and the specificity for discrimination of between MM and RMC was also 100%. Strong perimembranous staining pattern of EMA was also support the diagnosis of MM. Methods and clones of CD146 stain in paraffin-embedded sections probably remain to be further investigated.

2017 Cell Signaling Pathways and Nuclear PTEN and FOXO3a Dysregulation in Pleural Mesotheliomas

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Background: Pleural mesothelioma (PM) is a malignant, highly aggressive neoplasia with little understanding of its pathogenesis. Several studies have indicated that activation of Protein Kinase pathways (PKP) contributes to PM proliferation and survival, and others have identified over-expression of several factors (EGFR, MET, IGFR and VEGFR). But there are very few reports about downstream mTOR factors and PTEN. The aim of our work was to identify the possible PKP activation in PM and pleuritis and to determine significant differences between them which explain the lack of response to their targeted therapies.

Design: Thirty-two (32) cases of mesothelioma (22 epithelioid, 4 biphasic and 6 sarcomatoid) and nineteen (19) cases of pleuritis were selected from our files from 2003 to 2010. Formaline-fixed, paraffin-embedded block was used for all the immunostainings and a whole complete section, instead of TMA were studied. The following primary antibodies were used: pmTor, 4E-BP1, p4EB-P1, pS6rb, pMapk, eiF4E, peiF4E, PTEN, pAKT and FOXO3A. Staining intensity, percentage of positive cells and localization of the staining were compared for each marker. To reduce the subjectivity we applied the formula Hscore. The comparative study was performed using the statistical test of Mann Whitney.

Results: PM showed nuclear over-expression of 4EBP1 (p=0.017) and FOXO3a (p=0.0007) and decrease nuclear PTEN (p=0.0004). In the cytoplasm there was a decrease of pS6 (p=0,0001), pmTOR (p=0,0344) and pMAPK (p=0.0921) and increase of eIF4E (p=0,0012). Pleuritis cells showed granular cytoplasmic FOXO3a expression (0.0009) with higher expression of pmTOR, pMAPK, pS6 in the cytoplasm and higher levels of nuclear PTEN than PM.

Conclusions: a) mTOR and MAPK pathways are activated in pleuritis. b) In pleural mesotheliomas, interestingly, cytoplasmic eIF4E overexpression may be predictive of poor prognosis. c) In pleural mesotheliomas vs pleuritis there is a significant down-regulation of nuclear suppressor PTEN activity and nuclear up-regulation of FOXO3a. The study of these factors may open new approaches in the treatment of PM.

2018 Assessment of Invasion Degree of Small Sized Lung Cancer Using Intra-Operative Frozen Section

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Background: The number of small-sized lung cancer will be expected to increase under CT screening. Small-sized cancer can include surgically curable cancer which will be synonymous of histologically adenocarcinoma, in situ (AIS) or minimally invasive adenocarcinoma (MIA) of a proposed new IASLC/ATS/ERS classification in 2011. Intra-operative frozen diagnosis (IOFD) might be able to help to decide the area of resection for limited operation of AIS/MIA. The purpose of this study is to evaluate the accuracy of IOFD, especially focused on invasion degree of small-sized lung cancer.

Design: Surgically resected 183 lung tumors which were less than 3cm in size were reviewed on pathologic factors of IOFD and final diagnosis (FD), and calculated concordance rate between them. IOFD was performed on HE stained slides of a small part of tumor. FD was made based on the HE and elastic stained slides of whole tumor. The examined pathologic factors were as following; pathologic diagnosis (PDx), presence or absence of invasion/minimally invasion area, histologic predominant subtype of proposed IASLC-ATS-ERS revision of AD classification and tumor differentiation. In discordant cases, the reasons were examined in detail.

Results: Among 183 lesions, 135 were primary lung cancer and 48 were metastasis from other organs. Primary lesions include 110 adenocarcinomas (AD) and 5 squamous cell carcinomas (SQ). Concordance rate for each factor is as following; 75.3% for overall PDx factors, 99.5% for judgment of benign or malignant, 97.0% for tumor type, 99.0% for tumor differentiation, 100% for predominant subtype of AD, respectively. Concordance rate for invasive degree of AD was as following; 75.0% for overall, 85.0 % for AIS, 41.0% for MIA, 100% for invasive AD. The reasons of discrepancy were based on tumor heterogeneity, sampling error, frozen section artifact, or some difficult rare cases.

Conclusions: IOFD can predict the final diagnosis of lung cancer in most cases (75.3%) and invasion degree of AD (75.0%). To prevent disagreement about PDx, pathologist should pay attention to tumor processing (sampling, sectioning and staining). To make accurate diagnosis of invasion degree of AD, a careful macroscopic observation to submit the proper area of tumor in which the most invasive area will be included is strongly recommended.

2019 Comparison of EGFR and KRAS Mutations between Pre- and Post-Chemotherapy Groups in Primary and Metastatic Lung Adenocarcinomas

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Background: EGFR and KRAS mutations are found in 10-20% and 20-30% of primary lung adenocarcinomas, respectively. It is well known that EGFR and KRAS mutations are associated with a beneficial response to tyrosine kinase inhibitor (TKI) therapy. Several recent studies have shown that treatment-induced changes is related to the acquired resistance to TKI, and pre-treatment with TKI may sensitize EGFR mutations in tumor cells. These data suggest that chemotherapy may affect the phenotype of tumor cells. In this study, we analyzed EGFR and KRAS mutation in both pre- and post-chemotherapy groups of primary and metastatic lung adenocarcinomas.

Design: Using the archives of a major medical institution from January 2006 to December 2010, 314 lung adenocarcinomas with EGFR and KRAS mutations were included. Mutation status was determined by sequencing Exons 18 to 21 of EGFR and Exon 2 of KRAS (codons 12 and 13). Clinical information was also correlated.

Results: Among 314 lung adenocarcinomas (55 cytologic and 259 surgical specimens), 279 tumors (170 primary and 109 metastatic lesions) had mutational studies prior to chemotherapy and 35 tumors (21 primary and 14 metastatic lesions) had mutational studies following chemotherapy. The most commonly used chemotherapy agents were: Carboplatin, Cisplatin and Taxol. There was no significant difference of EGFR and KRAS mutations in the primary lung adenocarcinoma pre- or post-chemotherapy. However, in unrelated metastatic tumors, EGFR and KRAS mutations in the pre-treated group were 13% and 36%, respectively. In contrast, in post chemo-treated group, EGFR and KRAS mutations were 36% and 7%, respectively.

EGFR and KRAS Mutations in Metastatic Lung Adenocarcinoma Pre- and Post-Chemotherapy.

	Pre-Chemotherapy	Post-Chemotherapy
EGFR	14 (13%)	5 (36%)
KRAS	39 (36%)	2 (14%)
Wild type	56 (51%)	7 (50%)
Total	109(100%)	14(100%)

Conclusions: Our findings suggest that previous chemotherapy may potentially affect EGFR and KRAS mutational status in tumors, particularly metastatic tumors. The mechanism of this change is not fully understood. The effect may be multi-leveled in tumor cells, such as alteration of cellular protein expression and/or shift of mutational sites which can't be detected by current methods. Additional studies are needed to further investigate the mechanism and clinical significance of these findings.

2020 Expression of Aldo-Keto Reductase Family 1 Member C3 (AKR1C3) in Normal and Neoplastic Lung

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Background: The polycyclic aromatic hydrocarbons (PAH) in tobacco smoke and air pollution require activation to electrophilic metabolites in order to exert their carcinogenic or mutagenic effects. Human aldo-keto reductase (AKR) isoenzymes, despite detoxifying harmful reactive oxygen species, may catalyze formation of deleterious PAH o-quinones and thus play an important role in PAH induced lung carcinogenesis. AKR1C3, or type 2 3α/type 5 17β-hydroxysteroid dehydrogenase, is a multifunctional isoenzyme of the AKR superfamily. It has been suggested that AKR1C3 modulates the risk of lung cancer arising from exposure to PAH-rich environments. We studied the expression of AKR1C3 in normal and neoplastic lung tissue.

Design: Immunohistochemistry for AKR1C3 was performed on formalin-fixed, paraffin embedded sections of 15 small cell carcinomas, 14 adenocarcinomas and 18 squamous cell carcinomas of the lung. The normal tissue sections were obtained from areas uninvolved by tumor in these cases. Immunoreactivity was scored as negative (<5%), 1+ (6-25%), 2+ (26-75%) and 3+ (76-100%). Appropriate controls were employed.

Results: Normal bronchial epithelium was strongly immunoreactive for AKR1C3. In the alveolar parenchyma, the type II pneumocytes were non-reactive but cytoplasmic immuoreactivity was noted in the alveolar macrophages. Out of the 18 cases of squamous cell carcinoma, 15 showed positive staining (83.3%, 3+:12, 2+:3) while 12 out of 14 adenocarcinomas stained positive (85.7%, 3+:4, 2+:7, 1+:1). In contrast, all 14 cases of small cell carcinoma were completely negative.

Conclusions: The presence of AKR1C3 in normal bronchial epithelium reflects its putative protective role against reactive oxygen species. However, the increased expression in non-small lung carcinomas demonstrates the carcinogenic potential of the enzyme, raising the possibility of a potential therapeutic target. Small cell lung carcinomas, although strongly associated with smoking, do not appear to share the same pathway. AKR1C3 could also be used as an adjunct marker to differentiate small cell from non-small cell lung carcinomas. The association between elevated AKR1C3 expression and carcinogenesis, as well as related progression of non-small cell lung cancers requires further study.

2021 Histologic Multivariate Model for Predicting Presence of *ALK*-Rearrangement in Lung Adenocarcinoma

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Background: The identification of *ALK* rearrangement is emerging as an important pathology analysis of lung cancers. However, it is currently impractical for most pathology laboratories to screen all lung adenocarcinomas for ALK rearrangement using FISH. Thus, recognizing distinctive pathologic features of *ALK*-rearranged (ALK+) tumors may help us enrich for cases most likely to be positive for ALK rearrangement for molecular testing. Our previous study has shown the association of ALK rearrangement with a solid histology with numerous signet ring cells; however, experience of others, especially with lung resections, appears to be different. Thus, we sought to evaluate the histologic characteristics of ALK+ lung adenocarcinomas on various types of specimens from a large cohort of patients and develop a scoring system to predict *ALK* rearrangement.

Design: We examined resection, excision, and small biopsy specimens from 104 patients with ALK+ lung adenocarcinomas. The predominant histologic patterns and distinctive cytomorphologic features were assessed in each case and were compared with 215 lung adenocarcinomas negative for *ALK* rearrangement (ALK-). Based on the analysis, we developed a multivariate model for predicting *ALK* rearrangement in lung adenocarcinomas and tested its performance on a validation cohort (n =78).

Results: Compared to the ALK- tumors, ALK+ primary lung adenocarcinomas were associated with solid (47% vs. 16%) and micropapillary (22% vs. 9%) patterns, any signet ring cells (71% vs. 15%), and oncocytic cells (31% vs. 5%). Multivariate analysis further identified a significant association of papillary-predominant histology with ALK rearrangement. Among metastatic tumors and small tumor samples, the only morphologic feature distinguishing ALK+ from ALK- tumors was the presence of any signet ring cells (70% vs. 21%). Our morphologic scoring system based on signet ring cells, oncocytic cells, predominant histologic patterns and specimen types predicted the presence of *ALK* rearrangement with a sensitivity of 76% in the validation cohort.

Conclusions: The results of the current study confirm the association of ALK+ lung adenocarcinomas with distinctive morphologic features. In particular, the presence of signet ring cells predicts *ALK* rearrangement irrespective of specimen types. Our scoring system appears to be useful in predicting the majority of ALK+ tumors. However, additional screening methodology such as ALK immunohistochemistry scoring is warranted since morphologic analysis alone is not sufficiently sensitive to identify all patients who may be eligible for targeted therapy.

2022 A Study of ΔNp63 (p40) Expression in Non-Small Cell Lung Carcinomas

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Background: A majority of lung carcinomas constitute three major subtypes; small cell carcinoma, adenocarcinoma and squamous cell carcinoma (SCC). Distinguishing between the latter two is becoming important because different treatment regimen is available. While histological subdivision between the two is not generally difficult in differentiated tumors, it can be challenging in poorly differentiated tumors, and may require a panel of immunohistochemistry stains, e.g., p63 and CK5/6 for squamous marker and TTF-1 and Napsin A for adenocarcinoma marker. The typical immunoprofile is TTF-1 + or - /p63- for adenocarcinoma and TTF1 -/p63 + for SCC. p63 gene encodes two different N-termini (TA and ΔN). ΔNp63 is selectively expressed in basal and squamous cells, and is expressed in SCC whereas TAp63 is not restricted only to SCC. 4A4, the most widely used anti-p63 antibody, identifies both isoforms, and is expressed in about 15% of adenocarcinomas, and though generally focal, its expression can be more than focal in a subset of adenocarcinomas. This study investigates the utility of ΔNp63 to distinguish between pulmonary adenocarcinomas and SCC.

Design: A total of 150 primary lung adenocarcinomas (3 BAC/in situ, 35 acinar, 2 papillary, 40 solid, 58 mixed, and 12 mucinous) and 35 SCC were retrieved, and immunostains were performed by using tissue microarrays for p63 (4A4), ΔNp63 (p40), and TTF-1. The extent of staining was graded as 1+, 1-25%; 2+, 25-50%; 3+, 50-75%; 4+, >75%.

Results: 26 adenocarcinomas (17%) contained p63-positive neoplastic cells to a variable extent with diffuse (3+/4+) reaction being seen in 12 tumors (8%). p63 expression was seen in all of the subtypes of adenocarcinomas except for mucinous type. p40 was negative in all adenocarcinomas. All SCC were positive for both p63 and p40 predominantly in diffuse fashion. 116 adenocarcinomas (77%) were positive for TTF-1 to variable extent while all SCC were negative. p63 expression was seen in 23 TTF-1 positive adenocarcinomas (20%) while p40 was completely negative in all of the TTF-1 positive tumors.

Conclusions: p63 is not uncommonly seen in adenocarcinomas while p40 is specific in SCC and as sensitive as p63. Presence of p63-positive cells in poorly differentiated lung adenocarcinoma may be erroneously interpreted as evidence of squamous cell differentiation. p40 can potentially be a more reliable marker for SCC.

2023 PTEN Expression in Progressive (Idiopathic Pulmonary Fibrosis – IPF) Versus Non-Progressive (Sub-Pleural Fibrosis in Recurrent Spontaneous Pneumothorax – RSP) Pulmonary Fibrosis

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Background: Pro-fibrotic cytokines, Transforming Growth Factor-β (TGF-β) and Platelet Derived Growth Factor (PDGF), secreted by the pulmonary epithelium play a major role in the development of IPF by repressing PTEN expression resulting in smooth muscle actin (SMA) positive myofibroblastic proliferation. PTEN is known to have a role in fibrosis of the liver, kidney, and skin. One of the most enigmatic questions in IPF is the pathogenesis of the progressive fibrosis. The objective of this study is to evaluate PTEN expression in IPF versus non progressive subpleural fibrosis with fibroblastic foci (RSP).

Design: 10 IPF, 5 RSP and 5 normal lung cases (open biopsy or explants) were selected. The slides were stained with hematoxylin and eosin, and immunohistochemical stains for PTEN rabbit monoclonal antibody, (Cell Signaling Technology, Danvers, MA), and SMA mouse monoclonal antibody (Dako, Carpinteria, CA) were performed. A MACH 3 Rabbit HRP Polymer Detection system (Biocare Medical, Concord, CA) was used as a detection system.

Results: In the normal lung, SMA highlighted the muscle fibers within vessels and bronchi, as well as rare interstitial cells. PTEN stained the respiratory epithelium, type 1 and 2 pneumocytes and endothelium. In the RSP cases, SMA and PTEN expression were both strongly positive in subpleural areas of fibrosis. In the IPF cases, SMA showed strong positivity in fibroblastic foci and honeycomb areas while PTEN expression was decreased.

Conclusions: We demonstrate that SMA expression increases while PTEN decreases in IPF in areas of fibroblastic foci and honeycombing; however, in non-progressing fibrosis, SMA and PTEN are strongly coexpressed. Based on these results, we hypothesize that PTEN repression contributes to myofibroblastic differentiation and continued matrix deposition in IPF and may be a key factor in progression.

2024 Diffuse Pulmonary Exogenous Lipoid Pneumonitis – An Indicator of Inhalational Exposure to Aerosolized Oils?

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Background: Exogenous lipoid pneumonia (LP) is the result of a foreign body type reaction to the presence of exogenous lipid material in the lungs. Exogenous LP is most commonly seen in the setting of aspiration, where it is typically characterized by patchy lesions corresponding to the distribution of the aspirated material. We report here a series of six unusual cases of exogenous LP in which there was diffuse interstitial deposition of exogenous lipoid material associated with severe emphysema, suggestive of inhalation of aerosolized oils.

Design: Cases were identified through review of pathology specimens from patient undergoing lung volume reduction surgery or lung transplantation for severe emphysema, collected between January 2002 and July 2011.

Results: All 6 cases demonstrated similar histological features, consisting of a background of severe emphysema on which there were numerous macrophages containing abundant variably sized, well-delineated intracytoplasmic vacuoles, suggestive of exogenous lipid material and similar to those typically seen in exogenous LP. The vacuoles were admixed with varying amounts of brown-black pigment. These macrophages were diffusely distributed throughout the lung tissue and predominantly interstitial, although similar macrophages were also present within alveolar spaces. On review of the clinical data, the mean age of these patients was 50±4, and all 6 were past or present smokers. Three patients had known alpha-1 antitrypsin deficiency. Three of the patients had a history of marijuana or other illicit inhalational drug use, while four had histories of occupations in which there may have been exposure to aerosolized oil.

Conclusions: Our findings suggest that diffuse exogenous lipoid pneumonitis may be associated with inhalational exposure to aerosolized oils, whether through illicit inhalational drug use or occupational exposure.

2025 Usual Interstitial Pneumonia with Granuloma. Idiopathic Pulmonary Fibrosis Versus Chronic Hypersensitivity Pneumonitis

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Background: When granulomas are found in usual interstitial pneumonia (UIP), chronic hypersensitivity pneumonitis (CHP) is always in the differential diagnosis, but presence of a few granulomas can be just an incidental finding of idiopathic pulmonary fibrosis (IPF). Clear distinction between IPF and CHP is challenging for sometimes. Here, we compared the clinico-pathological features of UIP with granuloma and those with IPF to see if there are any other differences.

Design: A total of sixteen UIP with granuloma (UIP-G) patients and fifteen IPF patients were collected. Clinical, radiological, and histopathological findings in both groups were observed and compared. Prognosis was evaluated by the pulmonary function tests. The statistical analyses were performed using Mann-Whitney's U-test and Fisher's exact test. *P* values <.05 were considered statistically significant.

Results: Mean age of UIP-G and IPF was 61.2 and 64.5 years, respectively. Both showed male predominance (81% and 73%), and smoking experience was also similar (75% and 80%). Exposure history was positive for twelve in UIP-G and eight in IPF. In computed tomography, IPF showed honeycombing and lung volume loss more frequently than UIP-G (p<.01 in both). Lower and peripheral distribution was most frequent in both groups, though upper lung predominance was seen in two of UIP-G and one of IPF cases. Histopathologically, inflammatory cell infiltration was more frequent in UIP-G (p=.024) whereas honeycombing and vascular wall thickness were significantly severe in IPF (p<.01 and p=.019). There was no significant difference regarding fibroblastic foci, small airway disease, and airway centered change. IPF showed significantly worse outcomes of the forced vital capacity and the diffusing capacity of carbon monoxide than UIP-G (p=.045). Nine cases of UIP-G were suspected as CHP by clinical-radiological-pathological discussion, while six cases were considered as IPF.

Conclusions: Although patient demographics are similar, UIP with granuloma showed several different pathological and radiological features and clinical course from IPF. It is still unclear whether UIP with granuloma is a homogenous condition, but our data indicates that it seems to be distinct from IPF and that granuloma in UIP may not be just an incidental finding of IPF but suggest a different inflammatory process associated with better prognosis, probably of CHP.

2026 FOXP3+ Regulatory T-Cells Are Associated with Acute Rejection in Lung Transplants

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Background: Acute rejection (AR) is the major risk factor for bronchiolitis obliterans syndrome, the clinical manifestation of chronic rejection in lung transplants. AR is thought to be a T-cell mediated process. The balance of Tregulatory (Treg) and Teffector (Teff) cells likely ultimately determines graft acceptance or rejection. The best characterized Treg are CD4+CD25+ T cells that express FoxP3 transcription factor. In heart, kidney and liver transplant, increased FoxP3+ cells and mRNA expression were shown during AR in post-transplant biopsies suggesting that FoxP3+ cells are associated with antidonor immune reactivity rather than with immunological quiescence. Targeting Tregs might become an important treatment tool to prevent chronic rejection. We herein characterize changes of FoxP3+ and total CD4+ cells in post-lung transplant transbronchial biopsies (TTBx).

Design: TTBx were selected from pts transplanted at our institution. Rejection was histologically classified according to the 2007 Revision of the 1996 working formulation for lung rejection. TTBx were stained with FoxP3, and CD4. FoxP3+ cells and CD4+ cells were counted in 10 Hpf by two authors (ACR, JP) independently. % FoxP3+ cells of total CD4+ cells was calculated as (100*FoxP3)/CD4. Distributions were compared between groups using linear regression with generalized estimating equations to adjust standard errors for repeated data within patients. Analyses were conducted in SAS version 9 (Cary, NC).

Results: 61 TTBx from 28 pts were included. In B0C0 cases, numbers of FoxP3+ cells and CD4+ cells in TTBx without (A0) and with (A1-A4) acute cellular rejection (ACR) are shown in Table 1.

Table 1: FoxP3 and CD4 + cells in cases without and with acute cellular rejection

Grade of ACR	A0	A1	A2	A3	A4	p-value (for comparison A0 vs A1-A4)
N (# of biopsies)	32	14	1	2	1	
FoxP3+cells[1]	1.0; 0.0, 3.0	4.0; 1.0, 5.0	3.0	22.0, 106.0[2]	45.0	0.039
CD4+cells[1]	28.0; 10.0, 77.0	53.8; 33.0, 101.0	17.0	347.0, 406.0[2]	352.0	0.044
%FoxP3+/total CD4+ cells[1]	1.5; 0.0, 7.1	6.7; 2.1, 8.9	17.6	6.3, 26.1[2]	12.8	0.84

[1]Median; Q1, Q3; [2] raw datapoints

Interobserver variability was good with correlation of 0.936 (FoxP3) and 0.995 (CD4).

Conclusions: FoxP3+ Tregs and total CD4+ cells are associated with acute graft rejection in patients with lung transplant. Our findings suggest that Tregs might be recruited to the lung during episodes of acute cellular rejection. However, since the relative number of Tregs does not significantly increase, other subsets of CD4+ T cells are probably also part of the immune response during acute cellular rejection in lung transplants.

Enumeration of FoxP3+ and CD4+ cells appears to be easily reproducible.

2027 Fluorescence In Situ Hybridization (FISH)-Assessed Amplification of Anaplastic Lymphoma Kinase (ALK) Gene Is Detectable in a Subset of Pulmonary Sarcomatoid Carcinomas (PSC)

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Background: The prevalence of ALK gene alterations for targeted therapy with specific inhibitors is a largely unexplored issue in pulmonary sarcomatoid carcinoma (PSC), a life-threatening tumor subset whose treatment still is disappointing.

Design: ALK gene status by fluorescence in situ hybridization (FISH) and protein expression by immunohistochemistry (IHC) were performed on formalin-fixed, paraffin-embedded material from 34 PSC, including 30 pleomorphic carcinomas (PLC), two pulmonary blastomas (PB) and two carcinosarcomas (CS). Fifty-one consecutive metastatic lung adenocarcinomas (MELAD) were also assessed in parallel for ALK with both FISH and IHC. The occurrence of ALK gene rearrangement and amplification was evaluated according to widely agreed-upon criteria on at least 100 tumor cells.

Results: While no rearrangements of ALK gene were detected in PSC, relevant amplification was identified in 6/34 (18%) PLC (1 female and 5 males, all smokers, aged 63 to 75 years), but not in PB or CS. The percentage of amplified tumor cells ranged from 11% to 43%, with a mean gene copy gain (GCG) ± standard deviation (SD) of 7.5±1.5. No differences in GCG (±SD) were seen in amplified tumors between the epithelial (7.4±1.5) and the sarcoma-like (7.7±1.6) components, suggesting an early engagement of ALK amplification during tumor progression of a common ancestor lesion. In the remaining 28 non-amplified PSC, the relevant GCG±SD for ALK was 3.4±0.9 with a percentage of involved tumor cells ranging from 19% to 92% (p=0.0002). Out of 51 MELAD used as controls, 10 showed ALK rearrangement (p=0.012) with no differences in gender and age distribution, whereas only one case exhibited amplification (p=0.015). ALK protein IHC was found in rearranged MELAD, but not in amplified PLC.

Conclusions: ALK gene amplification clustered into a significant subset of elderly, smoking, predominantly male PLC patients, revealing significant differences with lung adenocarcinoma. ALK amplification was not associated with protein accumulation when assessed by IHC. Response to therapy of ALK amplification in these tumors is at present unknown and clinical studies are clearly warranted.

2028 Immunoreactivity for DeltaNp63-p40, a Basal-Type Marker, as a Holistic, Single-Shot Diagnostic Adjunct Approach to Morphology for Lung Cancer Subtyping

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Background: DeltaNp63-p40, a basal-type marker corresponding to non-transactivating or truncated isoforms of the *p63* gene family of nuclear transcription factors, is a sensitive detector of squamous cell carcinoma (SQC). Little is know, however, about the diagnostic power of p40 as a single-shot adjunct to morphology when used in a daily routine practice-based, consecutive series of lung carcinomas.

Design: Forty-nine consecutive surgical specimens from 27 adenocarcinomas (AD), 11 SQC, 4 sarcomatoid carcinoma (SC), 3 small cell carcinoma (SCLC), 2 adenosquamous carcinomas (ADSQC), 1 basaloid large cell carcinoma (B-LCC) and 1 large cell neuroendocrine carcinoma (LCNEC) were collected from the routine diagnostic activity over a period of two months and then processed comparatively by immunohistochemistry for p40 (polyclonal antibody), p63 (clone 4A4) and thyroid transcription factor-1 (TTF-1, clone 8G7G3/1). A histologic (H) score was obtained for each antibody multiplying the percentage of positive cells by the immunostaining intensity (dichotomized as low to strong according to internal positive controls).

Results: H-scores for p40, p63 and TTF1 were 0.1±0.4, 25.1±56.7 and 143.2±65.6 for AD; 190.4±11.3, 190.4±11.3 and 0 for SQC; 176.5±32.8, 176.5±32.8 and 20.0±34.6 for biphasic tumors containing SQC lineage (1 SC, 2 ADSQC, 1 B-LCC); 0, 20.0±28.3 and 73.3±53.3 for biphasic tumors containing AD lineage (3 SC); and 1.2±2.2, 20.7±23.3 and 190.0±7.1 for SCLC and LCNEC, respectively (p<0.001). While p40 was always negative in tumors showing AD lineage and in neuroendocrine tumors, p63 was positive (>5% tumor cells) in 7/27 AD, 1/3 gland-differentiated SC and 3 SCLC/LCNEC (p=0.0001). In turn, TTF1 was negative in 3/27 AD and all SQC, but variably positive in all tumor categories.

Conclusions: P40 was a terrific, single-shot marker in lung cancer subtyping, unlike p63 and TTF-1, because excluded by definition SQC or ADSQC when negative and modulated finely morphology to address both TTF1-negative (including some AD, SQC, squamous cell-differentiated SC, ADSQC, B-LCC) and TTF-1 positive (including AD, ADSQC, gland-differentiated SC, SCLC, LCNEC) tumor categories. B-LCC confirmed a basal-type phenotype.

2029 Histopathological Findings of Usual Interstitial Pneumonia (UIP) and Non- Specific Interstitial Pneumonia (NSIP) in 34 Explants

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Background: Idiopathic pulmonary fibrosis (IPF) is the most frequent and serious idiopathic interstitial pneumonia, with a histological pattern of Usual Interstitial Pneumonia (UIP) and typical HRCT findings; its main differential diagnosis is Non-Specific Interstitial Pneumonia (NSIP). Most histological criteria for interstitial pneumonia have been described from biopsies, and little is known on explants' findings with the exception of one previous study (A.-L. A. Katzenstein et al. 2002).

Design: Histological features of 34 UIP or F-NSIP explants were retrospectively analysed blinded to clinico-radiological data, and compared with 20 previous biopsies and HRCT. As patients had underlying diseases, the corresponding specimens were considered separately.

Results: Final consensus diagnoses were 22 IPF/UIP (including 3 exacerbated), 4 idiopathic fibrosing NSIP (F-NSIP), and 8 non-idiopathic fibroses with F-NSIP pattern (n=3) or UIP pattern (n=5). Concordance between radiologists and pathologists was only for IPF diagnosis (19/22 cases versus 18/22 cases, respectively). Temporo-spatial heterogeneity of fibrosis and sub-pleural and para-septal distribution were found in 91% to 95% of IPF/UIP explants and biopsies. High fibroblastic foci mean score and honeycombing predominated in explants, whereas normal lung was rare. NSIP areas were observed in 7/11 of IPF/UIP biopsies and in 9/19 of IPF/UIP explants. Most UIP explants showed an extension of the fibrotic process around bronchioles; large enlargements of restructured airspaces were observed in 13/22 (59%) explants and in 5/11 (45%) biopsies, in association with emphysema in 10 patients with lower lobe fibrosis and upper lobe emphysema on HRCT.

Conclusions: We confirm that temporo-spatial heterogeneity of fibrosis, its sub-pleural and para-septal distribution, fibroblastic foci and honeycombing are constant findings in IPF/UIP explants; we emphasize the high frequency of NSIP and large cystic airspace enlargements, this end-stage disease lesion being not frequently reported on open lung biopsies.

2030 Usefulness of MicroRNAs as Prognostic Factors in Early Stage Non Small Cell Lung Carcinoma (NSCLC)

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Background: The transcription factor SOX2 is overexpressed in many solid tumours, including NSCLC. miR-145 and the miR-302-367 cluster are involved in stemness through SOX2 regulation. miR-145 plays an important role in SOX2 translation, and SOX2 regulates the expression of the miR-302-367 cluster. We have analyzed the

expression of miR-145 and the miR-302-367 cluster in tumour and paired normal tissue samples from resected NSCLC patients and correlated our findings with time to recurrence (TTR).
Design: We analyzed the expression of miR-145 and miR-302-367 cluster in 70 tumour and 70 paired normal tissue samples from NSCLC patients who had undergone complete surgical resection from 2007 to 2009. We focused in 36 Adenocarcinoma and 28 Squamous Cell Carcinoma. RNA was obtained from fresh frozen tumour and normal tissue using the Trizol method and microRNA expression was detected using TaqMan MicroRNA Assays.
Results: Patients(p) characteristics: stage I, 12 (17.1%) stage II, 14 (20%) stage III; 36 (51.4%). 36 (51.4 %) Adenocarcinoma (ADC), 28 (40%) squamous cell carcinoma (SCC) 6 (8.6 %) NSCLC NOS. With a mean follow-up of 17 months (m), 23 p (32.9%) had relapsed. miR-145 expression was downregulated (P<0.001), and miR-367 expression was upregulated (P<0.001) in tumour compared to normal tissue samples. Mean TTR for p with low miR-145 levels was 18.4 m vs 28.2 m for p with high miR-145 (P=0.015). Mean TTR for p with low miR-367 levels was 29.1 m vs 23.4 m for p with high miR-367 (P=0.048). Concerning the prognostic differences between the main histological types, there was no correlation between the overexpression of both miRNAs in ADC cases. Conversely, there was a significant prognostic value of miR-367 for TTR (p=0.012) in SCC cases, but it was just a trend with miR-145 (p=0.071) for TTR.
Conclusions: In our study we have demonstrated a relationship between expression of miR-145 and miR-367 and TTR in SCC patients. On the other hand these microRNAs have no prognostic significance in ADC patients. Both conclusions support the importance of distinguishing SCC and ADC cases in all the studies with miRNAs.
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2031 Hyaluronan and Its Impact as New Biochemical Marker on Diagnosis and Prognosis of Lung Cancer

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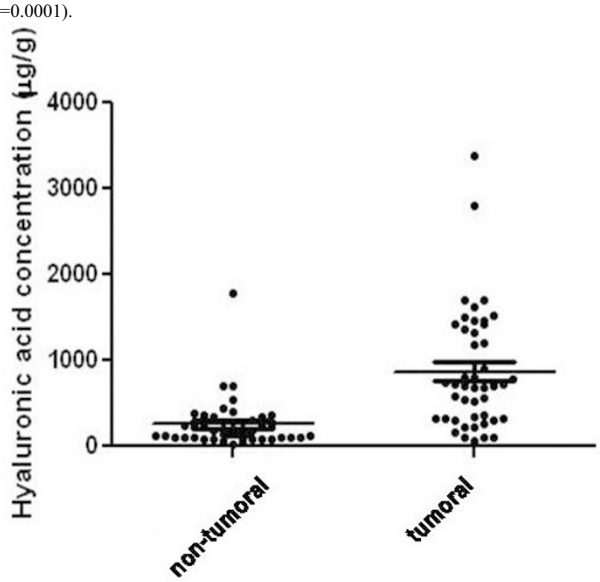
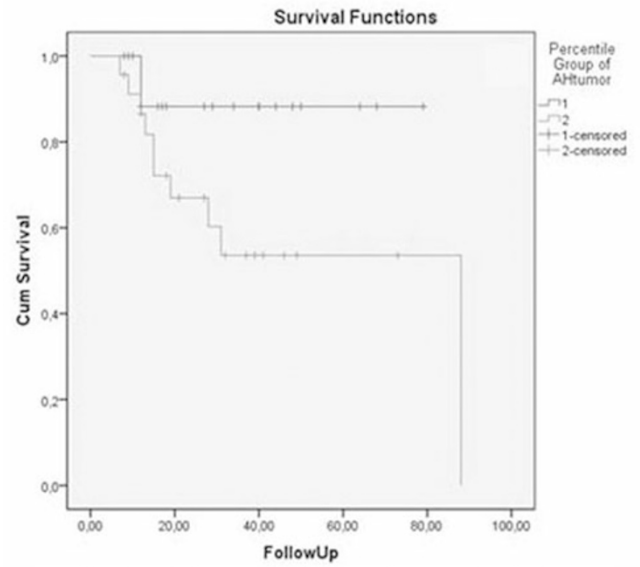
Background: Different markers have been investigated to better discriminate lung cancer evolution. Glycosaminoglycans (GAGs) are important molecules of the extracellular matrix and many have reported that GAGs have different behaviors when in the presence of malignant tissues. In this study, we sought to examine the Hyaluronan (HA) concentration and its impact on diagnosis and/or prognosis of patients with non small cell lung cancer.
Design: HA was examined in tumoral and non-tumoral tissues from 45 patients. Their preoperative clinical stages were $T_{1-3}N_{0-1}M_0$ and the mean follow-up was 19.3 months. Their histologic types were adenocarcinomas (n=23), squamous cells carcinomas (SqCC) (n=16) and large cell carcinoma (n=6). Tissue samples were dehydrated with acetone and incubated with a proteolytic enzyme. The HA chains were diluted (1:100) in blocking buffer [0.05M Tris-HCl, pH 7.4, 1% BSA]. The levels of HA were measured by a noncompetitive enzyme-linked immunosorbent assay (ELISA)-like fluorometric assay.
Results: A distinct profile of HA was observed between non-tumoral and tumoral areas. HA showed significantly higher concentration in tumoral than in normal areas (p=0.0001).



HA showed higher concentrations in tumoral than in non-tumoral areas (p=0.0001)

The impact of HA was tested on follow-up until death from surgery day. In Fig 2, the Kaplan-Meier survival curves shows that tissues with lower concentrations of HA have better long-term survival than those with higher concentrations (Log Rank=3,59; p=0.05).



The Kaplan-Meier survival curves (Fig 2) shows that tissues with lower concentrations of HA have better long-term survival than those with higher concentrations (Log Rank=3,59; p=0.05).

Conclusions: The presence of higher concentrations of HA in patients with lung cancer suggest a possible role of these molecule in this pathological condition and provide a potential biochemical marker for differentiating normal from lung cancer patients as well. However, further studies are needed to determine whether or not these concentrations are able to be diagnostic/prognostic markers of lung cancer.

2032 Resolving the Controversy on *EGFR/KRAS* Mutations in Pulmonary Squamous Cell Carcinoma Via Comprehensive Pathologic Assessment Incorporating Immunohistochemistry

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Background: There is a persistent controversy as to whether *EGFR*/*KRAS* mutations occur in pulmonary squamous cell carcinoma (SQCC). We hypothesized that the reported variability may reflect diagnoses rendered on poorly-sampled tumors and the inherent difficulties in morphologic distinction of poorly differentiated SQCC from adenocarcinoma (ADC). The recent development of a robust immunohistochemical (IHC) approach that aids in this critical distinction provides an opportunity to reassess *EGFR*/*KRAS* and other targetable kinase mutation frequencies in a pathologically homogeneous series of SQCC.
Design: Ninety-five resected SQCC verified by IHC as p63+/TTF-1- were tested for activating mutations in *EGFR*, *KRAS*, *BRAF*, *PIK3CA*, *NRAS*, *AKT1*, *ERBB2/HER2*, and *MAP2K1/MEK1*. In addition, all tissue samples from rare patients with the diagnosis of "SQCC" harboring *EGFR*/*KRAS* mutations encountered during 5 years of routine clinical testing at MSKCC were reassessed pathologically.
Results: The screen of 95 IHC-verified SQCC revealed no *EGFR*/*KRAS* mutations (0%; 95% CI 0-3.8%), but a low rate of *PIK3CA* (4%; 95% CI 1-10%) and *AKT1* (1%; 95% CI 0-5.7%) mutations. Detailed morphologic and IHC reevaluation of *EGFR*/*KRAS*-mutant "SQCC" identified during routine clinical testing (n=16) resulted in reclassification of 10 (63%) cases as adenosquamous carcinoma (AD-SQC) and 5 (31%) cases as poorly-differentiated ADC morphologically mimicking SQCC (i.e. ADC with "squamoid" morphology). One (6%) case had no follow-up.
Conclusions: We conclude that *EGFR*/*KRAS* mutations do not occur in pure pulmonary SQCC, and samples diagnosed as "SQCC" harboring these mutations represent pitfalls in pathologic diagnosis of AD-SQC and ADC, which can largely be resolved by comprehensive pathologic assessment utilizing IHC. Our findings 1) highlight the value of IHC in the diagnosis of SQCC, which clarifies conflicting molecular data, 2) suggest a sharp biological divide in the patterns of oncogenic driver mutations between lung ADC (pure or combined) vs pure SQCC, and 3) establish the rate of several potentially targetable mutations in a pathologically homogeneous set of SQCC.

2033 A Practical Approach To Differentiate between WHO Types A and B3 Thymomas and Thymic Carcinomas

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Background: Interobserver agreements for the WHO classification of thymic epithelial neoplasms (TEN) is only moderate. Common disagreements occur for type B3 vs thymic carcinoma (TCa), type A vs B3, and A vs TCa. However, the prognosis of TCa is worse with 10-yr survival of 20-67% vs 36-83% for B3 and 76-100% for A. No single marker is available to facilitate the histologic assessment. We studied whether a combination of TdT, Glut-1, and CD205 might be used to facilitate the distinction between type A and B3 thymomas and TCa.

Design: Specimens from 50 pts treated with TEN were included. Three pathologists independently classified all cases according to WHO and agreed upon type A, B3 or TCa. Sections were stained for CD205, TdT, and Glut-1. Membranous (CD205, Glut-1) or nuclear (TdT) staining were considered positive. Staining distribution was assessed for CD205 and Glut-1. Number of + cells was expressed as (100 * number + epithelial cells) / number total epithelial cells (mean of 5 Hpf counted).

Results: Expression of TdT, CD205, and Glut-1 and distribution of CD205+ and Glut-1+ cells are shown in Table 1.

Table 1: Expression and staining distribution of TdT, CD205, and Glut-1

WHO Type	TdT	CD205			Glut-1		
	# + cases[1]	# + cases[1]	Diffuse	Patchy	# + cases[1]	Diffuse	Patchy
A (n=16)	11 (68.7)	14 (87.5)	1	13	6 (37.5)	0	6
B3 (n=15)	14 (93.3)	15 (100)	8	7	10 (66.7)	0	10
TCa (n=19)	0	10 (52.3)	3	7	17 (89.5)	10	7

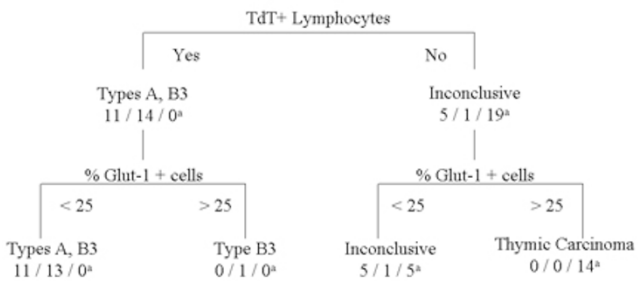
[1]Absolute number of + cases (%)

Counts of Glut-1 and CD205+ cells are in Table 2.

Table 2: Counts of CD205+ and Glut-1+ cells

% + cells	CD205			Glut-1		
	Type A	Type B3	TCa	Type A	Type B3	TCa
<5	2	0	5	3	3	4
5-25	3	4	2	3	6	1
25-50	5	0	2	0	0	3
50-75	2	4	0	0	1	2
75-100	2	7	2	0	0	9

TENs that do not contain TdT+ lymphocytes and show either diffuse expression of Glut-1 and/or Glut-1 in >25% cells (vs. type A) or > 5% (vs. type B3) are TCa.



[a] Number cases per group shown as WHO type A / B3 / Thymic carcinoma.

Some type A thymomas can be differentiated from B3 based on Glut-1 expression (>25% cells, B3). B3 thymomas usually have TdT+ lymphocytes while some A thymomas have not. CD205 staining was patchy to diffuse in most TEN (TCa 52.3%, type A 87.5%, B3 100%).

Conclusions: A panel of Glut-1 and TdT might be used as adjunct to distinguish type A from TCa and type B3 from TCa. In a few cases, these markers might also help to differentiate type A from B3. CD205 appears not useful in this context.

2034 Highly Sensitive Real-Time PCR for the Detection of EGFR Mutations in Lung Adenocarcinoma. Is It Worth It?

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Background: Mutations involving the epidermal growth factor receptor (EGFR) correlate with responsiveness to tyrosine kinase inhibitors (TKIs), which significantly improves patient survival in lung adenocarcinoma (AC). These mutations are described to be more frequent in non-mucinous lepidic adenocarcinomas (nmBAC). The unquestionable importance of determining the presence of these mutations, even in samples with a small tumor representation, has boosted the appearance of highly sensitive methods which would allow the detection of 1-5% mutated DNA. The aim of this study was to test the ability of a real-time PCR kit to detect EGFR mutations in a series of lung AC considered wild-type by the Sanger's sequencing method.

Design: 52 primary lung ACs were revised by two independent pathologists to establish histological type and immunoprofile (CK7, CK5/6, 34βE12, CK20, p63 and TTF1) of every case. DNA was extracted from formalin-fixed paraffin embedded sections of every tumor sample. EGFR mutational status analysis of exons 19, 20 and 21 was performed by Sanger's direct sequencing and by the real-time PCR kit *Therascreen EGFR PCR* (Qiagen). Fluorescent in-situ hybridization (FISH) was performed to assess the copy number status of EGFR gene using *Vysis LSI EGFR SpectrumOrange/CEP7 SpectrumGreen* Probe (Abbot Molecular).

Results: Sanger's direct sequencing method allowed the determination of 7 cases with EGFR mutations (13.5%) and 45 EGFR wild-type tumor samples. *Therascreen EGFR PCR* kit detected 4 new cases with EGFR mutations, three of which showed 100% infiltrating nmBAC pattern and one acinar with 50% nmBAC component, increasing the global number of mutant cases to 11 (21.2 %). The most prevalent histological subtype in the mutated group corresponds to nmBAC pattern, being present in 8 of 11 (72.7%) cases. FISH analysis reveals 9 of 11 (81.8%) EGFR mutated cases being FISH positive, 2 by EGFR amplification and 7 with high polisomy.

Conclusions: The use of real-time PCR in the global series increases the percentage of EGFR mutated samples detected by 7.7%. Considering that all 4 recovered cases have nmBAC component, the use of highly sensitive techniques in this subtype of AC should be recommended despite their substantial cost.

2035 Epidermal Growth Factor Receptor Copy Number Variations, but Not EGFR or KRAS Mutations, Are Frequent in Lung Squamous Cell Carcinomas

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Background: Epidermal growth factor receptor (EGFR) mutations, and to a lesser extent EGFR copy number variations, have been correlated with response to EGFR tyrosine kinase inhibitors (TKIs). In contrast, KRAS mutations have been recently associated with resistance to TKIs. Most of these studies have been carried out on adecarcinomas (ACs) due to the association of this histologic type with the presence of alterations in the EGFR gene pathway. The aim of this study was the molecular characterization of a series of lung squamous cell carcinomas (SCCs) and the possible implications on TKIs therapy.

Design: A series of 47 surgically resected paraffin embedded SCCs were reviewed and their histological classification further confirmed by IHC (CK7, CK5/6, CK903, CK20, p63 and TTF1). The presence of EGFR and KRAS mutations was analyzed by direct sequencing and the incidence of EGFR copy number variations determined by fluorescent in situ hybridization (FISH). These results were then compared to those found in a series of 48 ACs and their statistical significance analyzed using Fisher's exact test.

Results: Despite there were no EGFR or KRAS mutations found in this series of SCCs, there was a high percentage of cases (55%) presenting EGFR copy number variations, which is not statistically different from that found in the series of ACs (p=0.14). These FISH positive SCCs included 6 cases with EGFR amplification and 20 cases with high polysomy.

Conclusions: Our results confirm the absence of EGFR and KRAS mutations in lung SCCs observed in other series. Nonetheless, the significant number of EGFR copy number variations observed, and the possible correlation with TKIs sensitivity cannot be overlooked and should be further analyzed. This study suggests that FISH may be an appropriate methodology to assess the EGFR status of SCCs.

Table 1: Univariate analysis to correlate molecular alterations with histologic type.

	SCCs (n=47)	ACs (n=48)	P value
EGFR FISH			
(+)	26	34	0.14
(-)	21	14	
EGFR mutations			
wild-type	47	42	0.03
mutated	0	6	
KRAS mutations			
wild-type	43	31	0.0008
mutated	0	9	
not assessed	4	8	

SCCs: series of squamous cell carcinomas, ACs: series of adenocarcinomas. P values of Fisher test were considered statistically significant when less than 0.05.

2036 Massively Parallel Sequencing in NSCLC: Comparison to Traditional Hot Spot Analysis for Selection of Approved and Novel Targeted Therapies

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Background: The recent introduction of next generation (NGS) DNA sequencing to clinical samples has enabled the discovery of novel and unanticipated genomic-derived targets of therapy response and resistance for patients with NSCLC.

Design: DNA was extracted from 4 x 10 μm FFPE sections from 45 primary NSCLC (26 female; 19 male; mean age 68 years; 24% Stage I; 13% Stage II; 5% Stage III; 16% Stage IV; 46% Stage unknown). The exons of 145 cancer-related genes were fully sequenced using the Illumina HiSeq 2000 (Illumina, San Diego, CA) to at an average sequencing depth of 253X and evaluated for point mutations, insertions/deletions (indels), specific genomic rearrangements and copy number alterations (CNA). Samples included 5% fluid cellblocks; 5% regional lymph nodes; 3% pericardial biopsy and 87% lung biopsies or resections. There were 42 adenocarcinomas (27acinar, 12 lepidic, 2 mucinous, 1 papillary), 1 large cell carcinoma, and 2 squamous cell carcinomas. In 23 adenocarcinomas, the NGS results were compared with commercial laboratory allele-specific PCR genotyping on the same tissue blocks.

Results: In the comparison study of EGFR status, the NGS result was concordant with commercial laboratory genotyping in 23/23 (100%) cases. In 22 additional NSCLC samples, NGS revealed 53 total genomic alterations, including 14 (64%) base substitutions, 2 (9%) INDELs, 6 (27%) CNA, and 0 (0%) rearrangements. Genomic alterations associated with sensitivity or resistance to targeted therapies for NSCLC were found in 16/22 (73%) of cases including 10 *KRAS*, 4 *STK11*, 3 *JAK2*, 2 *PIK3CA*, 2 *BRAF*, 2 *EGFR*, 1 *NF1*, 1 *TSC1*, 1 *TSC2*, 1 *CCNE1*, 1 *PTCH*, 1 *CDK4*, 1 *CCND1*, 1 *BRCA2*, 1 *CDKN2*A, and 1 *ATM* mutation. In comparison with the COSMIC database, NGS results were similar for most genes except for a lower rate of *EGFR* mutations (9% vs. 21%), a higher rate of *KRAS* mutations (41% vs. 16%) and an unprecedented rate of *JAK2* mutations (14% vs. 0%). 7/22 (32%) of the NSCLC had 2 or more potentially actionable alterations after NGS.

Conclusions: Deep sequencing of clinical NSCLC samples is completely concordant with traditional hot-spot genotyping and also uncovers an unexpected number of genomic alterations that could influence therapy selection for this disease. Broad-based, deep sequencing of cancer-related genes results in sensitive detection of all classes of genomic alterations in NSCLC and can reveal actionable genomic abnormalities that inform treatment decisions.

2037 Prognostic Value of O-GlcNAc Modification and Its Related Enzymes in Lung Adenocarcinoma

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Background: Post-ribosomal protein modification with an N-acetylglucosamine (O-GlcNAc) residue is functionally similar to phosphorylation and is vital to cellular regulation and homeostasis. The enzymes responsible for the addition and removal of O-GlcNAc are O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA). It has been proposed that O-GlcNAcylation may be an important regulator of cancer initiation and progression, based on the O-GlcNAcylation of many oncogenes and tumor suppressors. Here, we investigated the prognostic significance of O-GlcNAc modification components in lung adenocarcinoma.

Design: Immunohistochemical staining for O-GlcNAcylation, OGT, and OGA was performed on 65 primary lung adenocarcinoma and matching metastatic lymph nodes. Scoring was accomplished by evaluating localization (membrane, cytoplasm, peri-nuclear, and nuclear), stain intensity (0: no staining, 1: weak staining, 2: strong staining) and frequency. A score was calculated based on these parameters for statistical comparisons. We then evealuated the following parameters: differential primary tumor, adjacent normal tissue and lymph node staining; recurrence status; recurrence free and overall survival.

Results: The global O-GlcNAcylation levels in cytoplasm were significantly elevated in lung adenocarcinoma tissues (n=40) than that in healthy lung tissues ($p<0.001$). There was no significant difference in status of O-GlcNAcylation level and OGT expression between primary tumors with and without lymph node metastasis, however, cytoplasmic OGA was lower in lymph node positive group ($p=0.033$). Comparing primary tumors with their matching metastatic lymph nodes revealed that cytoplasmic level of OGlcNAcylation ($p<0.001$), OGT ($p=0.003$), and OGA ($p=0.001$) was overexpressed in metastatic lymph nodes. Finally, survival analysis showed that better overall and recurrence free survival was observed in patients with higher cytoplasmic O-GlcNAc modification ($p<0.001$, 0.024 respectively), but high nuclear OGA expression in primary tumors was correlated with lower recurrence free survival and overall survival ($p=0.039$, <0.001 respectively).

Conclusions: Our results suggest that O-GlcNAcylation might play important roles in lung adenocarcinoma initiation and progression and may be a potential prognostic factor to predict patient risk of recurrence after surgery. Also, these findings may provide us with added insights regarding the mechanism of metastasis, although, further investigations are warranted to validate our results.

2038 Expression of Biomarkers of Tumor Cell Plasticity in Lung Adenocarcinoma Isotypes

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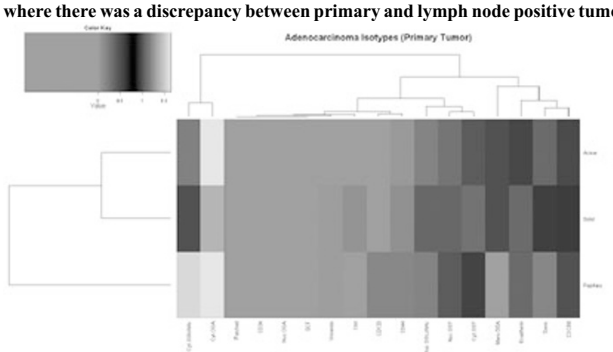
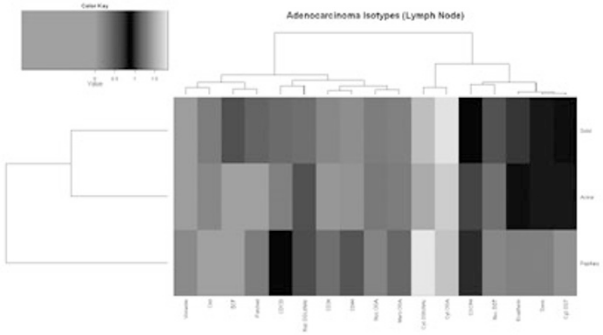
Background: Adenocarcinoma is the most common histologic subtype of lung cancer. The clinical course is different among individuals even in cases of the same histologic subtype, presumably due to heterogeneity within the biologic properties of tumor cells. Therefore, lung adenocarcinoma isotyping has recently been proposed as a means to better prognosticate clinical outcome and is encouraged to assist in better determine patient treatment options.

Design: Immunohistochemical staining for 13 different markers of tumor cell plasticity was performed on 65 primary lung adenocarcinoma and matching metastatic lymph nodes. Histologic isotyping was performed with acinar, papillary, and solid isotypes selected for further analysis based on their frequency.

Results: We observed approximately 21% discrepancy between primary and lymph node in presenting predominant isotype, but with no enrichment of any specific isotype in primary vs lymph node metastatic plaque. Solid type showed lower expression of sonic hedgehog compared to other forms in primary tumors. In lymph node, cytoplasmic OGT and CD133 were significantly lower in solid type. Although there was not an observed significant difference in overall survival and recurrence free survival among different isotypes. **However, we observed a higher rate of recurrence ($p=0.05$), lower overall survival ($p=0.036$), and lower recurrence free survival ($P<0.001$) in cases where there was a discrepancy between primary and lymph node positive tumors.**





Conclusions: This is the first study demonstrating differences in histologic subtype of adenocarcinoma in primary and lymph node metastases that can is correlated to patient outcome. Further validation of these results is currently underway with a larger, external cohort of patients.

2039 Expression of ErbB2 and ErbB3 in Non-Small Cell Lung Cancer

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Background: In non-small cell lung cancer (NSCLC),EGFR has been studied extensively, but less is known about the expression and role of other ErbB receptors. The aim of this study is to determine ErbB2 and ErbB3 expression in NSCLC.

Design: Expression of ErbB2 (Dako) and ErbB3 (Antibody supplied by A. Pandiella) was analyzed inmunohistochemically using tissue microarrays from tumor samples from 126 patients (median age 67 years,81% males,6% never smokers,p-stage 33% I/29% II/30% III/9% IV),diagnosed with NSCLC (51 adenocarcinoma (ADC)/47 squamous-cell carcinoma (SCC)/28 others), who underwent complete resection between October 2007 and September 2010. Cytoplasmic (cyt) and membranous (mb) staining expression was assessed by two observers as follows:0, no staining;1+,weak,incomplete mb staining;2+,weak to moderate,complete mb staining of at least 30% of tumor cells or strong,complete mb staining of 10-30% of tumor cells; and 3+,strong,complete mb staining of at least 30% of tumor cells. Score of 2+/3+ was regarded as positive.ErbB2 by FISH was analyzed in patients with positive ErbB2 expression.EGFR-mutation (mut) was determined in selected patients (23 cases). Statistical and survival study was performed.

Results: Positive mb ErbB2 expression was found in 5 cases (4%):3 ADC/2 SCC (all males,1 never smoker), all cases were erbB2 FISH negative. Positive ErbB3 mbs and cyt expression was found in 27% and 21% of all cases, respectively; 10.3% of cases expressed both. Positive ErbB3 expression was more frequent in females than in males (mb 46% *vs* 22%,p 0.038;cyt 33% *vs* 18%,p 0.099), in ADC than in SCC (mb 36% *vs* 21%, p 0.07; cyt 32% *vs* 13%, p 0.014). No correlation was found between ErbB3 expression and age and p-stage. Five of 23 patients had EGFR-mut (2 del exon 19,3 L858R exon 21): all females with ADC,4 never smokers. No EGFR-mut cases had concomitant positive ErbB2. Four out of 5 EGFR-mut patients had positive cyt ErbB3 expression (80%), 3 had mb expression (60%). Preliminary results (median follow-up, 17 months) showed no association between ErbB3 expression and disease-free or overall survival.

Conclusions: Positive ErbB2 expression is infrequent in NSCLC and no correlation is found with EGFR-mut cases. Positive ErbB3 expression occurs more frequently (25% of cases,females, ADC and EGFR-mut tumors). However, ErbB3 warrants further study in NSCLC to clarify its role and its potential use as a therapeutic target.

2040 New MicroRNA-Based Diagnostic Test for Lung Cancer Classification

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Background: Lung cancer is the leading cause of cancer deaths in the USA. With the advent of therapies showing varying response rates for different lung cancer types, a growing need exists for a standardized and highly accurate diagnostic classification tool. Furthermore, in 20%-30% of pre-operative biopsies significant limitations of tumor quantity and quality prevent full classification of the tumor using traditional diagnostic methods. We have previously described a microRNA-based assay which accurately differentiates between squamous and non-squamous non-small cell lung cancer (NSCLC). Here, we present the development and clinical validation of a new microRNA-based qRT-PCR assay that differentiates primary lung cancers into four types: squamous cell carcinoma, non-squamous NSCLC, carcinoid and small cell carcinoma.

Design: Over 750 primary tumor samples representing different lung cancer histological types were collected. Samples included formalin-fixed, paraffin-embedded (FFPE) blocks from resection or biopsies and cell blocks from cytological procedures. Expression levels of potential microRNA biomarkers were profiled using a microRNA

microarray followed by a sensitive and specific qRT-PCR platform. A classifier that identifies the lung tumor types was developed and a diagnostic assay was defined and validated on an independent, blinded set of 451 samples.
Results: Using the expression levels of eight microRNAs, accurate classification of the lung tumors into the four above-mentioned categories was obtained. The microRNA-based assay that was developed reached an accuracy of 93.7% in an extensive independent, blinded validation set. Cytological samples, which constituted over 50% of the validation set, reached an accuracy of 95%.
Conclusions: We present here a novel microRNA-based assay for the classification of the four main types of lung cancer based on the expression of a small set of microRNAs. This assay displays high levels of accuracy in both pathological and cytological samples. The assay is a standardized, well-tested tool which can assist caregivers in the diagnosis of lung cancer.

2041 Central and Peripheral Squamous Cell Carcinoma of the Lung. Are They Different?

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Background: Lung squamous cell carcinoma (SCC) can be classified into the central type (cSCC) and the peripheral type (pSCC) according to the location. Referring to recent reports, pSCC may be biologically different from cSCC, but the evidence is weak. If they are biologically different, treatment should be considered separately.
Design: 50 cases with SCC resected by lobectomy from 2005 to 2010 in Toyama University hospital in Japan were collected. In histologically, cSCC was defined as a tumor from trachea to segmental bronchi and pSCC as in more peripheral location. Pathologic reports were reviewed for age, sex, location, smoking status, pathologic stages, differentiation, pleural invasion, and vascular invasion. H&E slides were reviewed to score levels of necrosis, keratinization, inflammatory cell infiltration, alveolar filling pattern inside the tumor, emphysema, and fibrosis outside the tumor. Tissue Microarray containing triplicated 0.6mm cores from each case were composed. Immunohistochemical staining for CK7, TTF1, p63, CK14, Napsin A, CK34βE12, CK5/6, and p53 were performed. Levels of entrapped pneumocytes inside the core were also scored by observing CK7.
Results: The numbers of cSCC and pSCC were 15 and 35, respectively. Strong male predominance (48:2) and association to smoking history (46/48) were observed. Most of the histological findings did not separate cSCC and pSCC, whereas presence of severe emphysema only showed statistically predominance in pSCC (p=0.02). Immunohistochemical patterns were also identical, and none showed statistically significant difference between cSCC and pSCC. Observed difference was a presence of entrapped pneumocytes highlighted by CK7 predominantly in pSCC (p=0.04). The-5-year survival showed no difference in the prognosis between cSCC and pSCC.
Conclusions: Immunohistochemical patterns and survival are not different between cSCC and pSCC. The central and peripheral SCC may not be biologically different except the ways of proliferation. Emphysema may increase the risk of carcinogenesis possibly due to its localized poor clearance of carcinogenic agents.

2042 Diagnostic Certainty of a Mesothelioma Diagnosis Based on Immunophenotype

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Background: A diagnosis of mesothelioma is usually made only after establishing the immunophenotype of the neoplastic cells. The International Mesothelioma Interest Group suggested using two antibodies each for mesothelioma and the diagnoses in the differential diagnosis. We present here a Bayesian model to generate post-test probabilities (PTP) for various stain combinations using epithelioid mesothelioma (EpM) as an example.
Design: Pre-test probabilities for 42 different neoplasms were determined by counting their relative occurrence in 300 random pleural biopsies and resections during an 8-year period (32 different tumors) and by adding to that list other tumors less often seen in the pleura. A model was created using SMILE and GeNIe, an inference engine and development environment for probabilistic models (Decision Systems Laboratory, Pittsburgh, PA), considering the immunophenotype of the various differential diagnoses. Under the assumption that a cytokeratin AE1/3 stain is positive, the PTP of EpM was calculated for combinations of commonly used stains.
Results: The four most common pleural tumors were carcinomas of breast, lung, ovary and EpM (pre-test probabilities 37%, 24%, 11%, 5%, respectively) in women, and carcinomas of lung, esophagus, EpM and biphasic mesothelioma (pre-test probabilities 26%, 6%, 19%, 6%, respectively) in men. PTPs varied slightly based on patient gender. A combination of positive mesothelioma markers Calretinin and CK5/6 with negative adenocarcinoma markers TTF-1 and either MOC-31, BerEp4 or B72.3 (4-stain panel) provided a PTP of 90%. Using CEA instead of the latter three markers reduced the PTP to 70%. When a 6-stain panel was employed by adding D2-40 or WT-1 and using two of the adenocarcinoma makers in addition to TTF-1, the PTP increased to 98% or greater. In the four stain panel, having one stain show an unexpected result decreases the PTP to below 20%, but PTPs can be restored to >80% by adding a both another mesothelioma and adenocarcinoma marker.
Conclusions: A combination of two mesothelial markers and two adenocarcinoma markers provides sufficient diagnostic certainty for EpM, especially since in that situation the PTPs for the differential diagnoses are far below 10%. A panel of at least three stains each is useful if one of the markers show unexpected staining. Calculation of the PTP can be performed for any stain combination and result, and could be used as marker of diagnostic certainty in the clinical and medico-legal setting.

2043 SOX2 Amplification in Bronchial Squamous Dysplasia

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Background: SOX2 is a transcription factor hypothesized to represent a lineage-specific oncogene in squamous cell carcinoma (SCC) of the lung. It has recently been shown to be amplified in high grade squamous dysplasia found in patients without malignancy. Here we present our experience with SOX2 amplification in squamous dysplasias.
Design: Amplification of SOX2 was evaluated in paraffin sections from 14 cases containing dysplastic squamous epithelium by dual-color SOX2 fluorescence in-situ hybridization using a Spectrum Green-labeled probe RP11-286G5 (CHORI, Oakland, CA) and a Spectrum Orange-labeled, probe RP11-43F17 (CHORI). Between 15 and 65 cells were scored for each case. A SOX2 to centromer of chromosome 3 ratio of 2.0 or greater was considered amplified. There were 7 cases with mild, 1 with moderate and 4 with severe dysplasia. Two cases showed squamous metaplasia and basal cell hyperplasia, respectively, but no dysplasia. Specimens included bronchoscopic biopsies (BBx), surgical margins of cancer resections and pneumonectomies for transplant.
Results: SOX2 amplification (ratio 3.6-8.8) was detected in two surgical margins close (< 0.2 cm) to invasive SCC and one repeat BBx of a previously biopsy-proven SCC, all three showing severe dysplasia. No amplification was identified in (a) mild dysplasia found in two surgical margins far (> 1 cm) from invasive SCC (n=1) and adenocarcinoma (n=1); (b) mild dysplasia found in three BBxs from patients without accompanying lung mass; (c) mild, moderate and severe dysplasia, respectively, found in three transplant pneumonectomies; (d) mild dysplasia found in a BBx of a squamous papilloma; (e) squamous metaplasia without dysplasia found in a surgical margin 1 cm from an invasive SCC; and (f) basal cell hyperplasia found in a BBx of a patient with a distant adenocarcinoma.
Conclusions: SOX2 appears to be amplified in high grade dysplasias closely associated with invasive SCC but not in a severe dysplasia involving metaplastic epithelium in a lung explant or in low grade dysplasias. This could indicate that SOX2 amplification is a late event in squamous carcinogenesis. Its assessment in surgical margins containing dysplasia may be helpful in assessing risk for recurrence at the bronchial stump.

2044 *HER2* Mutated Lung Adenocarcinoma Is a Distinct Molecular and Clinicopathologic Entity

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Background: Lung adenocarcinomas (ACA) often contain identifiable "driver" mutations. Recognition of mutation-specific clinicopathologic features has important implications for patient selection for targeted therapy. *HER2* is mutated in ~3% of lung ACA, but little is known about the clinicopathologic or molecular features of these tumors.
Design: 70 lung ACA with known mutations were selected from a patient cohort that had undergone clinical tumor genotyping; 19 of these contained *HER2* exon 20 insertions, and the other 51 contained non-*HER2* mutations in *EGFR* (n=18), *KRAS* (n=15), *BRAF* (n=12), and/or *PIK3CA* (n=13). *HER2* FISH and immunohistochemistry (IHC) using Herceptest were performed on each *HER2* mutated case and interpreted according to ASCO/CAP criteria for breast cancer. Non-parametric analyses were used to test the association between *HER2* mutation status and other characteristics. A 2-tailed α=0.10 criterion was used, given the small sample size. Kaplan-Meier survival analysis was used to evaluate overall survival, adjusted for clinical stage.
Results: *HER2* mutation status was independent of gender, race, and age, but associated with never or limited-smoker status (p=0.07) and higher clinical stage (p=0.05). *HER2* mutated tumors were predominantly acinar, papillary, or solid-subtype, but not significantly associated with any pattern. The tumor cells tended to exhibit coarse chromatin (p=0.07) and columnar shape (p=0.04), and frequently contained eosinophilic hyaline mucin droplets (so-called "targetoid cells") (OR=12.65, p<0.0001). Of the *HER2* mutant tumors, 8 (42%) showed copy number (CN) gain, of which 4 (21%) were amplified. 16 *HER2*-mutated cases had material available for IHC; 7 (44%) showed 1+ and 4 (25%) showed 2+ staining. Only 2+ staining correlated with CN gain (p=0.02). There was no significant difference in survival time between patients with *HER2* mutations and those with non-*HER2* mutations (p=0.80).
Conclusions: Compared with other mutated lung tumors, *HER2* mutated ACA represents a distinctive subset of tumors that tends to present at higher stage in nonsmokers, has unique morphologic features, including "targetoid cells", and shows frequent *HER2* CN gain in the absence of strong protein expression. These findings lend insight into the biology of *HER2* mutation in lung cancer and begin to define a subset of patients who may be amenable to therapy with *HER2* inhibitors.

2045 Selection of Samples for Epidermal Growth Factor Receptor (EGFR) Mutation Analysis in Non-Squamous Non-Small Cell Lung Carcinoma

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Background: Tyrosine kinase (TK) domain mutations in the EGFR gene are well documented in non-small cell lung cancer, with molecular testing established as a critical factor to initiate first-line treatment with EGFR kinase inhibitors. We reviewed data from the first 18 months of EGFR mutation testing in a province-wide population to identify best practice principles for the selection of tumor samples.
Design: Data from EGFR mutation analysis were collected from 1853 consecutive cases starting from March 2010. Samples were reviewed by pathologists to evaluate material for testing, including tumor classification, sample type, tumor cellularity, and immunohistochemical profile. Mutation analysis was performed by PCR fragment

analysis for exon 19 deletions and Sau961 restriction enzyme digest for exon 21 L858R mutation. The significance of rates of mutation and test success were analyzed by chi-squared comparison testing.
Results: 1305 histology and 368 cytology samples were evaluated for testing. 176 cases (9.0%) were excluded at the time of review, most frequently due to inadequate cellular material (n=136). Analysis performed on 1677 samples revealed an EGFR mutation rate of 21.2% with approximately equal prevalence for exon 19 and L858R mutation (11.0% and 10.2%). EGFR mutation rate was higher in cytology (26.7%) versus histology (19.6%) samples, with the greatest difference in mediastinal lymph node samples (40.0% in 43 cytology, 10.1% in 125 histology, p=0.0015). The overall rate of inconclusive test results was 6.5% (n=109) with no significant difference between histology and cytology samples. The most significant factor for test success in histology samples is high percentage of tumour cellularity, regardless of sample type. TTF-1 status was noted in 1156 histology samples (929 positive, 227 negative). 13 of 212 (6.1%) of EGFR mutatant cases were TTF-1 negative, with a negative predictive value of 93.7%.
Conclusions: Histo/cytological review of samples prior to testing will remove cases with inadequate cellular material for testing. Ideal tumor cellularity should be over 30% to improve test success rates. Tumors negative for TTF-1 by immunohistochemistry have a low likelihood of EGFR mutation. Cytology samples are equivalent to histology samples for test success rate and may have advantages for detecting EGFR mutation.

2046 Met Activation Is Associated with Unique Clinicopathologic and Molecular Features in Lung Adenocarcinoma

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Background: *MET* oncogene activation by gene amplification and/or autocrine HGF-Met signaling is associated with an invasive and metastatic phenotype in numerous tumor types. In *EGFR*-mutated lung adenocarcinoma (ACA), Met activation is associated with treatment failure in patients receiving tyrosine kinase inhibitors. Identification of Met-activated tumors has important therapeutic implications, however the clinicopathologic features of Met activation in lung tumors are not well established. We examined a cohort of lung ACA enriched for *EGFR* mutations and correlated Met phosphorylation and *MET* copy number (CN) with other clinicopathologic and molecular features.
Design: Met activation in 44 lung ACA resected from East Asian, never-smoking women was assayed by immunohistochemistry (IHC) using a Met Y1234/35 phospho-antibody (Cell Signaling Technology) (pMet). Tumors with ≥5% cells with moderate membrane staining were scored as positive. *MET* CN as assessed by FISH was defined as: relative gain (MET:CEP7 1-3) or amplified (MET:CEP7 >3). pMet and *MET* CN were correlated with each other and with *EGFR* mutation, *EGFR* CN, and clinicopathologic characteristics.
Results: pMet was expressed in 13 of 44 (30%) tumors, commonly at the tumor edge and in foci of pleural invasion. 68% of tumors had *MET* CN gain; one case was amplified. As compared to pMet negative tumors, pMet positivity correlated with *MET* CN gain (Fisher's exact, p=0.008), micropapillary features (62% *v* 23%, p=0.02), and younger age (mean 53 *v* 62 years; p=0.02). 92% of pMet-expressing tumors had *EGFR* mutations. Both pMet positive tumors and those with *MET* CN gain were more likely to present with distant metastases. *MET* CN gain failed to correlate with other clinical, histologic, or molecular features.
Conclusions: In a cohort of lung ACA enriched for *EGFR* mutations, pMet expression is seen in a third of cases and defines a clinically, pathologically, and molecularly distinct tumor subtype. In contrast, *MET* copy number gain is found in a heterogeneous group of tumors, less than half of which show pMet expression. These findings suggest that pMet IHC is better than *MET* copy number as a marker of Met activation and have important implications for selection of patients who may benefit from Met inhibitor therapy.

2047 The Receptor Tyrosine Kinase ROR2 Is a Novel Marker for TSC-Associated Lesions and a Potential Therapeutic Target Independent of the TSC/mTOR Pathway

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Background: The expression of ROR2, a receptor tyrosine kinase, has been correlated with invasiveness of cancer cell lines as well as with poor clinical outcome for patients with gastrointestinal stromal tumor (GIST) and leiomyosarcoma (LMS). ROR2 is not expressed in the majority of adult normal tissues. Lymphangioleiomyomatosis (LAM) and renal angiomyolipomas (AML) are lesions that occur in the setting of tuberous sclerosis complex (TSC) and are characterized by disregulation of the mTOR signaling pathway. LAM and AML are treated clinically using mTOR inhibitors.
Design: We evaluated ROR2 protein expression by immunohistochemistry in LAM and AML samples and compared its diagnostic utility with current LAM and AML markers, HMB-45 and B-catenin; 12 cases of AML and 9 cases of LAM from patients with unknown TSC status and 15 cases of AML and 3 cases of LAM from TSC patients were studied. *In vitro*, we assayed drug responses to mTOR inhibitors in isogenic cell lines either over-expressing ROR2 or with reduced ROR2 expression. We also examined the transcriptional variation of TSC genes as a function of ROR2 over-expression or knockdown in these cell lines.
Results: By immunohistochemistry, ROR2 outperforms both HMB45 and B-catenin as a diagnostic marker for LAM with low background staining. Of the LAM cases, ROR2 stained nine strongly, two moderately strong, and one weakly. ROR2 staining was positive in 10/12 AML from patients of unknown TSC status and in 14/15 AML from patients with confirmed TSC mutations. *In vitro* studies on AML, GIST and LMS cell lines revealed that ROR2 expression did not mediate differential response to mTOR inhibitors, nor did it have a significant effect on the transcriptional control of TSC genes.
Conclusions: ROR2 is a novel diagnostic marker for LAM and AML and is a potential therapeutic target that is likely independent of the TSC/mTOR pathway.

2048 mTOR Expression in Pulmonary Neuroendocrine Tumors

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Background: The mammalian target of rapamycin (mTOR) is a Ser/Thr protein kinase that functions as an ATP and amino acid sensor to balance nutrient availability and cell growth. mTOR plays a pivotal role in the proliferation and progression of several neoplasms; nevertheless, its role in neuroendocrine (NE) tumors of the lung has not been extensively evaluated. Therefore, the aim of our study was to analize the mTOR activation status in a series of neuroendocrine lung tumors (NELT).
Design: A total of 62 cases of NELT were retrieved from the Surgical Pathology files (43 typical carcinoid-TC-, 8 atypical carcinoid –AC-, 8 large cell neuroendocrine carcinomas –LCNEC-, and 3 small cell carcinomas –SCC-). After identifying tumor area for each case from formalin-fixed paraffin-embedded tissue blocks, 0.4-mm-thick cores were punched and embedded in the donor paraffin block. Tissue microarrays were stained with antibodies against phospho-mTOR (Ser2448, Cell Signaling) and Ki-67 (MIB1, Dako), as a marker of proliferation activity. mTOR tumor cell positivity (diffuse cytoplasmic) (percentage and intensity) was semi-quantitatively scored (range 0-300). Cases with score ≥10 were defined as positive. Inmunohistochemical results were correlated as well as histologic subtype. A p value < 0.05 was considered significant.
Results: Age of patients ranged from 16 to 74 (mean 52); 52% were men and 48% women. mTOR overexpresión was predominantly seen in TC and AC (84% and 87%, respectively) whereas the expression in LCNEC and SCC was lower (12% and 33%, respectively) (p < 0.000). Tumors with mTOR overexpression had lower proliferative rates, as valorated by Ki-67 (p=0.006).
Conclusions: Our findings suggest that the level of expression of mTOR is associated with better differentiated NELT and decreases with more aggressive histologic subtypes. Due to the availability of therapeutic targets that modulate this molecular pathway, these results could be relevant for the selection of patients treatments.
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2049 Molecular Histologic Correlations in the Cancer Genome Atlas (TCGA) Study of Lung Squamous Cell Carcinoma (SQC)

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Background: Most recent advances in molecular understanding of lung cancer are with adenocarcinoma (ADC). Little is known about the genetics of SQC. We report a major genetic analysis of SQC by the TCGA with histologic correlations.
Design: 213 tumors were submitted as squamous cell carcinomas to the TCGA for analysis. All of these were studied for copy number, LOH analysis, expression profiling, miRNA sequencing & methylation analysis. An expert pathology committee was formed to review the tumors to determine if they were SQC or other histologies. SQC & probable SQC cases were subclassified into keratinizing (K), nonkeratinizing (NK) & basaloid (B) subtypes. Histology was correlated with molecular findings. Immunostaining (IHC) for TTF-1, p63 & neuroendocrine markers helped reclassify a subset of problem cases.
Results: 177 SQC (157 definite, 20 probable) were separated from ADC (3), non-small cell carcinoma NOS (28), large cell neuroendocrine carcinoma (3), large cell carcinoma (1), & carcinoid (1). 29 of the 177 SQC were keratinizing (16%), 122 were nonkeratinizing (69%) & 26 were basaloid (15%). All cases found to have canonical EGFR and KRAS mutations were excluded by pathology review & none were present in SQC. Possible molecular therapeutic targets were identified in over 60% of cases including cyclin dependent kinases, fibroblast growth factor receptor mutations & amplifications & PI3 kinase pathway alterations. According to previously published mRNA expression categories the histologic subtypes (K:NK:B) were: 9 (8%) primitive (K:78%; B:22%); 48 (44%) classical (K:13%,NK:75%, B:12%); 17 (16%) secretory (K:18%, NK:59%, B:23%) and 34 (32%) basal (K:24%, NK:67%, B:9%).
Conclusions: As seen with the 17% reclassified tumors, the distinction between SQC & ADC has evolved substantially in recent years & it will become increasingly important as molecular targeted therapies become available for SQC as well as ADC. Even in resected tumors, there are problem cases that require IHC to make an accurate diagnosis.

2050 Presence of Epidermal Growth Factor Receptor (EGFR) Mutation Predicts a Lower Grade Morphology and Lower AJCC Stage in Patients with Lung Adenocarcinoma

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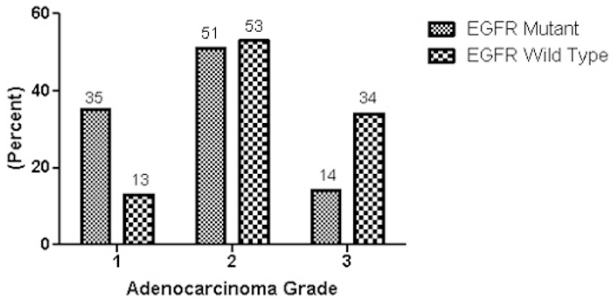
Background: Epidermal growth factor receptor (EGFR) mutations predict favorable response to EGFR tyrosine kinase inhibitors (TKIs) in non-small cell lung cancer but the histopathologic features associated with this mutation have not been well described. We performed this study to determine the relationship between EGFR mutations, clinicopathologic features and tumor stage according to current American Joint Committee on Cancer (AJCC) staging.
Design: EGFR mutation in exons 18, 19, 20 and 21 were screened in 281 patients with lung adenocarcinoma diagnosed between 2008 and 2011 using PCR and *Qiagen*

pyromark 24 sequencer. Histopathologic characteristics were reviewed and correlated with mutation status using appropriate parametric and non-parametric tests for significance.

Results: EGFR mutations occurred in 20% (57/281) of all lung carcinomas. The EGFR mutant group had a male to female ratio of 1:3.4 and mean age of 67 years (range 39 to 90). Mutations seen were L858R, exon 21 (n=18); E746, exon 19 (n=16); L747, exon 19 (n=9); exon 19 deletions (n=4); G719A, exon 19 (n=3); S7681, exon 20 (n=2); L861Q, exon 21(n=2); T790M, exon 20 (n=1); P848L, exon 21 (n=1) and E709, exon 18 (n=1). EGFR mutant patients were more likely to have lower grade morphology, compared to patients with wild type gene.



Grade 1 morphology was seen in 35% of EGFR mutants, compared to 13% of wild types (p<0.0001). Minimally invasive adenocarcinoma was seen in 12% of EGFR mutants, compared to 6% of wild-types. The predominant pattern seen in EGFR mutants were acinar (42%) and lepidic (37%), compared to wild types (acinar in 62% and lepidic in 10%, p<0.0001). 50% of EGFR mutants had N0 nodal status and 7% had pleural invasion. 61% of EGFR mutants had Stage 1 disease, compared to 39% in wild types (p= 0.001).

Conclusions: L858R in exon 21 and E746 in exon 19 mutations were the most frequent mutations. Patients with EGFR mutation were similar to patients with wild type EGFR with respect to age, sex, tumor size, minimally invasive adenocarcinoma, nodal status and pleural invasion. The presence of EGFR mutation predicts lower grade morphology and a lower AJCC stage in patients with adenocarcinoma of lung.

2051 Peripheral Lung Adenocarcinomas with Pleural Invasion Are More Likely To Harbor KRAS Mutation

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Background: Kirsten-RAS (KRAS) mutations are thought to play an important role in the pathogenesis of lung adenocarcinoma but the clinicopathologic features associated with this mutation have not been well described. We performed this study to determine the relationship between the KRAS mutation and demographics, tumor size, tumor grade, predominant architectural pattern, nodal status, pleural invasion and stage of cancer.

Design: KRAS mutations (all known mutations in codon 12 and codon 13 in exon 2 of the KRAS gene) were screened in 251 patients diagnosed with lung adenocarcinoma between 2008 and 2011. Mutational analysis was performed on DNA samples extracted from surgically resected/biopsied/cytologic specimens of lung adenocarcinomas by PCR and *TrimGens's* Shifted termination Assay. Relationships between gene mutation status and clinicopathological features were analyzed using appropriate parametric and non-parametric tests for significance.

Results: Of 251 tumors the frequency of KRAS mutations and no mutations (wild type) was 31% and 69% respectively. Mean age in the KRAS mutant group was 68 years (range 48 to 86 years) and male to female ratio 1:2.2. Seventy-three patient's samples were positive for KRAS codon 12 mutation (30 G12C, 15 G12A, 12 G12V, 9 G12D, 5 G12S and 2 G12T) and only 4 patient samples harbored codon 13 mutations (3 G13C and 1G13A). The tumor size in the mutant group was 2.8cm, compared to 2.4cm in non-mutant group. KRAS mutant patients had grade 2 adenocarcinoma in 61% and grade 1 and 3 in 12 and 27% patients respectively. Minimally invasive adenocarcinoma was seen in 7% of the patients with the mutant gene. The predominant pattern seen in the mutant group was acinar pattern (65% cases). Pleural invasion was seen in 20% of patients with KRAS mutant gene, compared to 6% in non-mutant patients(p=0.014). Nodal status was N0 in 56%, N1 in 8%, N2 in 8% and N3 in 2% cases of the mutant group. 41% of patients had Stage 1A and 1B disease, compared to Stage 2, 3 and 4 which was seen in 26%, 16% and 17% patients respectively in the mutant group.

Conclusions: KRAS mutation is more common in codon 12 in exon 2 of the KRAS gene in lung adenocarcinoma. Patients with KRAS mutation were similar to patients with wild type KRAS with respect to age, sex, predominant architectural pattern of adenocarcinoma, minimally invasive adenocarcinoma, tumor size, nodal status, tumor grade and stage of lung cancer. However patients with KRAS mutation were more likely to have pleural invasion when compared to patients with wild type KRAS gene.

2052 Gene Expression Profiling of Lung Neuroendocrine (NE) Tumors Reveals Gene Clusters Correlated with Central Versus Peripheral Location for Carcinoids

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Background: Primary lung NE tumors include a spectrum of neoplasms from low grade typical carcinoid (TC), intermediate grade atypical carcinoid (AC) to high grade small cell carcinoma (SCLC) & large cell NE carcinoma (LCNEC). This study uses gene expression profiling to provide molecular signatures of the tumor subtypes and their correlation to clinical features.

Design: Gene expression profiling was performed with the Affymetrix U133 Plus 2.0 array in 120 primary lung NE tumors including 64 TC, 13 AC, 20 SCLC & 23 LCNEC. Hierarchical clustering analysis was performed based on the gene expression of the most variable genes among 1286 genes that were analyzed. Carcinoids were divided into central & peripheral locations. The association of the clusters with clinical and histological characteristics were tested using Fisher's exact test or the Wilcoxon rank-sum test. Differential gene expression (limma package in R) and gene set enrichment analyses were performed. Survival was evaluated using competing risk analysis.

Results: Cluster analysis identified three clusters (1, 2 & 3). Cluster 1 included all of the high grade NE neoplasms and 4 TC (6.2%). Cluster 2 included both TC (37.5%) & AC (38.5%). However, the majority of TC (56.3%) & AC (61.5%) grouped into cluster 3. For carcinoids, cluster 2 was associated with a central location (82.8%) while cluster 3 was associated with peripheral (70.5%) location (P<0.01). Supervised clustering analysis revealed many genes in cluster 1 that discriminate SCLC (i.e. ZIC2, ID4) from LCNEC (P<0.001). Cluster 1 was highly associated with smoking history and pack/years compared with clusters 2 and 3. Patients with cluster 2 tumors were much younger than patients in cluster 1 & cluster 3 (mean age 55 vs 67 vs 63 yrs, P<0.01). The 30-month cumulative incidence of death due to disease was much higher for cluster 1 (27%) than cluster 2 (0%) & cluster 3 (4%) (P=0.012).

Conclusions: Gene expression profiling reveals three clusters of genes in lung NE tumors. The high grade NE neoplasms, SCLC and LCNEC share the same cluster while TC and AC are divided between two clusters that correlate with central vs peripheral location. Although SCLC and LCNEC share the same cluster, these tumors are distinguished by expression profiles of many genes. Further study on these genes such as ZIC2 and ID4 may provide important information about these tumors.

2053 Protective Effects of Prostaglandin E_2 on Pulmonary Vascular Remodeling in Allergic Airway Inflammation

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Background: Nonselective inhibition of prostaglandin synthesis augments inflammation in mouse models of airway disease, but the roles of individual prostaglandin are not completely clarified. To investigate the role of PGE_2 in a mouse model of airway inflammation induced by a natural allergen, we used mice lacking the critical terminal synthetic enzyme, microsomal PGE_2 synthase (mPGES)-1.

Design: Mice lacking mPGES-1 (*ptges*$^{-/-}$ mice) and wild-type C57BL/6 controls were challenged intranasally with low doses of an extract derived from the house dust mite *Dermatophagoides farinae* (Der f). Pulmonary inflammation was evaluated by analysis of bronchoalveolar lavage fluid and histology.

Results: The levels of PGE_2 in the bronchoalveolar lavage fluids of Der f-treated ptges$^{-/-}$ mice were ~ 80% lower than the levels in wild-type controls. Both the extent of inflammation and goblet cell metaplasia were modestly increased in the Der f-treated ptges$^{-/-}$ mice compared with the WT controls. Unexpectedly, the numbers of vascular smooth muscle cells and the thickness of intrapulmonary vessels were both markedly increased in the Der f-treated ptges$^{-/-}$ mice. Immunofluorescent staining revealed ICAM-1 expression in the alveolar capillary endothelium was upregulated in the ptges-/- mice. Additionally, these vascular changes were suppressed by the administration of the stable PGE_2 analog 16, 16-dimethyl PGE_2.

Conclusions: PGE_2 is known to be a direct vasodilator by relaxing smooth muscles. Our findings revealed that mPGES-1-derived PGE_2 is essential to protect the lung vasculature from remodeling with inflammation. Upregulation of ICAM-1 expression in endothelium plays an important role in vascular remodeling by potentiating pulmonary inflammation in *ptges*$^{-/-}$ mice.

2054 Pathological Findings in Lung Biopsies in Patients with Clinical Suspicion of Pulmonary Graft Versus Host Disease

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Background: The histopathological features of pulmonary graft versus host disease (GVHD) are not well described. Pulmonary GVHD may lead to bronchiolitis obliterans syndrome (BOS), which manifests pathologically as obliterative bronchiolitis (OB) and has a poor prognosis. We present a series of pulmonary GVHD that had not progressed to OB in order to facilitate early diagnosis and treatment.

Design: We retrospectively reviewed lung biopsies from patients with clinically suspected GVHD status post hematopoietic stem cell transplant (HSCT). Cases were retained for study if infections were excluded by culture, special stains, and immunohistochemistry and showed significant T-cell infiltrates with apoptosis in either the alveolar septa, bronchiolar mucosa, or both. Apoptosis, inflammation, and atypical reactive pneumocytes and bronchiolar epithelial cells were semiquantitated for each case. Alveolar changes were classified as acute lung injury (intra-alveolar fibrin); subacute lung injury (organizing pneumonia), or chronic interstitial pneumonia (CIP).

Results: There were 17 biopsies from 12 men (55 ± 3 years) and 5 women (54 ± 5 years) with 16 allogeneic and 1 autologous HSCT. Mean duration of transplant was 19 months (range 4-82 months). Ten patients had pathologic diagnosis of extrapulmonary GVHD. There was representative bronchiolar mucosa in 14 and alveolar parenchyma in 16 biopsies. Mean duration of transplant to time of biopsy was 6 months for patients with the acute lung injury pattern, 14 months for the subacute pattern, and 33 months for CIP. Diagnostic changes of OB were not identified in any specimens. Lymphocytic vasculitis was present in 11 biopsies. Intraepithelial bronchiolar T-cells averaged 54 ± 11 in acute and subacute alveolar injury, and 55 ± 14 in CIP. Reactive pneumocytes were present in 10 biopsies, with marked atypia in 2. Reactive bronchiolar cells showed mild atypia in 5, and marked atypia in 1, which mimics viral cytopathic effect. Alveolar eosinophils were present in 7 of 9 acute and subacute lung injury pattern, and 2 of 7 CIP. Eosinophils were present in 7 bronchiolar epithelium and prominent in 3.
Conclusions: GVHD is characterized by inflammation and apoptosis of both alveolar and bronchiolar mucosal compartments. In the former, may be associated with acute or subacute lung injury. Vasculitis is a common and helpful diagnostic feature. Recognition of patterns is essential for early diagnosis and prevention of OB.

2055 Expression of Thymoproteasome Subunit ß5t in Type AB Thymoma

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Background: Among the subtypes of thymoma, type AB thymoma, which is composed of a lymphocyte-poor type A component mixed with a lymphocyte-rich type B component, shows a broad range of morphology despite being a single entity. However, the functional characteristics of the neoplastic cells responsible for this morphological variety remain to be clarified. β5t, which is expressed specifically by thymic cortical cells, and involved in T-cell maturation, was discovered recently. We have already reported that most cases of type B thymoma and some cases of type AB thymoma express β5t, whereas all cases of type A thymoma are β5t-negative. More detailed study of β5t expression in type AB thymomas would provide a better understanding of the natural history of thymomas.
Design: A total of 20 cases of type AB thymoma were retrieved. In specimens stained with hematoxylin and eosin, we divided the tumor area into the type A component (a) and type B component (b), the latter being further subdivided into 3 components: those with spindle neoplastic cells (b-1), those with polygonal neoplastic cells (b-2), and those with polygonal neoplastic cells and medullary differentiation (b-3). We then performed immunohistochemistry using anti-β5t and anti-TdT antibodies, and evaluated the following parameters for each of these 4 components: the ratio of β5t-positive neoplastic cells among total neoplastic cells (0-100%), the predominant staining intensity (0 = negative, 1 = weak, 2 = intermediate, and 3 = strong), and the ratio of TdT-positive lymphocytes in infiltrating lymphocytes. For β5t, immunostaining was interpreted as positive when >20% of the neoplastic cells exhibited an intensity of 2 or 3.
Results: Among the 20 cases, 19 cases contained the a component, 15 cases the b-1 component, 12 cases the b-2 component, and 4 cases the b-3 component. In the a, b-1, b-2, and b-3 components, the β5t-positivity ratio was 5%, 20%, 42%, and 75%, respectively. These positivity ratios were significantly different ($P = 0.001$, χ^2 test). The corresponding mean ratios of TdT-positive lymphocytes were 9%, 59%, 75%, and 80%, respectively. These mean ratios were significantly different ($P<0.001$, Wilcoxon-test).
Conclusions: Considering the distribution of a functional molecule of thymic cortical epithelial cells, β5t, as well as morphology, it is suggested that type AB thymoma is not an entity distinct from type A thymoma and type B thymoma, but forms a spectrum and shares its natural history with these subtypes.

2056 PAX8 Is Useful in Discriminating Metastatic Endometrioid Carcinoma with Infrequent TTF-1 and Napsin A Positivity in the Lung

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Background: TTF-1 and napsin A have been considered as reliable biomarkers for primary lung adenocarcinoma. However, studies have shown TTF-1 is expressed in extrapulmonary carcinoma including endometrial endometrioid carcinoma (EEC) in which napsin A expression remains largely unknown. PAX8 has been reported to be positive in gynecological epithelial neoplasms including EEC but it is negative in lung adenocarcinoma. This study was to determine the expression pattern and the diagnostic value of TTF-1, napsin A and PAX8 in metastatic EEC in the lung.
Design: Fifty-one resected endometrial cancers (47 EECs, 3 serous carcinomas and 1 carcinosarcoma), as well as 4 resected and 2 biopsied metastatic EECs in the lung were retrieved. Twenty-six endometrial caners were used to construct a tissue microarray with 3 representative cores from each case. Tissue microarray as well as whole tissue sections were immunohistochemically stained with anitbodies for evaluating TTF-1 and PAX8 nuclear and napsin A cytoplasmic staining. A p value of <0.05, as determined by Fisher's exact test, was considered statistically significant.
Results: Two (33.3%) of 6 metastatic EEC were positive for TTF1 and one of 2 also expressed napsin A. Both TTF-1 and napsin A immunostains were strong and diffuse. All 6 cases showed moderate or strong and diffuse PAX8 positivity. Primary EEC of this TTF1 and Napsin A double positive metastasis was also strongly and diffusely positive for these three markers. Three of 51 primary endometrial cancers expressed TTF-1, one carcinosaroma was strongly positive with >80 positive tumor cells stained in both carcinoma and sarcoma components, and 2 ECCs exhibited 50% of tumor cells with weak to moderate TTF-1 staining. Five (9.8%) of 51 primary endometrial cancers exhibited moderate to strong napsin A staining with >80% stained tumor cells and two of 5 napsin A postive cases showed TTF-1 positivity. All these 5 cases were psotive for PAX8. Forty-eight (94.1%) of 51 primary endometrial cancers were moderately to strongly and diffusely positive for PAX8. The frequency of PAX8 positivity was significantly higher ($p<0.01$) than that of TTF-1 and napsin A expression in endometrial caners.
Conclusions: A small portion of metastatic EECs in the lung were TTF-1 or both TTF1 and napsin A positive and a large portion of endometrial carcinoma expressed PAX8. Infrequent TTF1 and napsin A detection in metastatic EEC could mislead to the diagnosis of lung adenocarcinoma. Combination of PAX8, TTF-1 and napsin A is powerful in separating primary lung cancer from metastatic EEC.

2057 Accuracy of Frozen Sections (FS) in Predicting Predominant Histologic Subtype and Presence/Absence of Micropapillary and Solid Patterns in Lung Adenocarcinoma (ADC) ≤ 3 cm

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Background: In lung ADC ≤ 3 cm, the choice between limited resection vs anatomical resection is an ongoing evaluation. The predominant histologic subtype in the IASLC/ATS/ERS ADC classification can provide prognostic stratification, but currently this classification is available only after surgical resection. If predominant histologic subtype and the poor prognostic micropapillary and solid patterns can be detected in FS, it can help intra-operative decisions for the extent of resection. The aim of this study is to evaluate the accuracy of FS to predict histology in final diagnosis, as well as interobserver agreement.
Design: 378 surgically resected stage I lung ADC were included in the study. All tumors are ≤ 3 cm. FS slides were examined for predominant histologic subtype and presence/absence of lepidic, acinar, papillary, micropapillary and solid patterns. The results were compared with final diagnosis in permanent sections. To test interobserver agreement, FS slides of 50 randomly selected cases were reviewed by two pathologists and 15 were reviewed by three pathologists independently. Kappa statistic was used to measure the degree of agreement.
Results: The concordance rate of predominant histologic subtype between FS and final diagnosis is 68.7% (Kappa=0.581). The sensitivity and specificity of FS to detect five major histologic patterns were shown in Table 1.

Table 1. Sensitivity and specificity to detect histologic patterns in frozen sections

Histologic pattern	Sensitivity(%)	Specificity(%)
Lepidic	74.8	87.5
Acinar	89.9	50.0
Papillary	70.1	71.8
Micropapillary	36.6	90.7
Solid	66.9	92.3

There were substantial agreement on predominant histologic subtype between different pathologists (Kappa=0.729), and moderate to substantial agreement on presence or absence of five major histologic patterns (Kappa=0.648 for lepidic, 0.434 for acinar, 0.672 for papillary, 0.643 for micropapillary, and 0.610 for solid pattern).
Conclusions: There is moderate agreement on predominant ADC histologic subtype between FS and final diagnosis. The interobserver agreement is satisfactory. Because FS have a high specificity in identifying micropapillary and solid patterns, recognition of one of these poor prognostic patterns may help a surgeon to consider anatomic rather than limited resection. However, the value is limited by low sensitivity, especially for micropapillary pattern.

2058 Is "Idiopathic" Pulmonary Fibrosis Overdiagnosed? "Idiopathic" Interstitial Lung Disease Associated with Inorganic Particulate Exposures — Evidence from Scanning Electron Microscopy/Energy Dispersive X-Ray Spectroscopic Analyses

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Background: Often pathologists diagnose interstitial pulmonary disease based solely upon routine light microscopic (LM) examination. Because epidemiologic studies show associations between environmental exposures and idiopathic pulmonary fibrosis (IPF), exclusion of such exposures prior to rendering a diagnosis of "idiopathic" pulmonary fibrosis is mandated. Brightfield and polarized LM only reveal a portion of inorganic particles. Scanning electron microscopy/energy dispersive x-ray spectroscopy (SEM/EDS) facilitates detection, characterization and quantification of inorganic particles, including many too small to be detected by LM. Such identification of potential causes of interstitial lung disease is important for proper diagnosis and prevention of disease.
Design: From 35 cases indexed as pulmonary fibrosis in consultation files (JLA) from 2005-2011, we extracted 21 cases having an initial diagnosis of idiopathic interstitial lung disease. We excluded cases with malignancy, overt silicosis, and those lacking adequate history and/or SEM/EDS analysis. We reviewed medical and occupational/environmental exposure histories, lung histopathology, and SEM/EDS particle analyses results, including the relative abundance of major particle types.
Results: Sixteen cases met the inclusion criteria. Most (13/16) were male. All had documented occupational/environmental exposures. The histopathology varied, including mild peribronchiolar fibrosis, usual interstitial pneumonia (UIP), giant cell interstitial pneumonia (GIP) and honeycombing. The predominant particle types (number of cases) found above background with SEM/EDS analyses were: silica (2), aluminum silicates (4), metals (including steel, Al, Sn, W) (5), talc (2), and asbestos fibers (2). One case revealed no increase in particles above background.
Conclusions: Light microscopic examination alone is insufficient to identify many particulate exposures. Also, even though 11/16 cases showed increased dust particles by brightfield and/or polarized LM, this was not considered by pathologists, and diagnoses of "idiopathic" were rendered. All of these cases, upon investigation, had histories of occupational/environmental exposures known to be capable of causing lung

injury/fibrosis. Therefore, it is important that the diagnosis of "idiopathic" be reserved for cases in which such exposures have been excluded. We believe that public health would be enhanced by making pathologists and clinicians more aware of such cases.

2059 Aberrant and Overexpression of DNA Methyltransferase in KRAS Mutant Pulmonary Adenocarcinomas

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Background: Correlation between KRAS mutations and pulmonary adenocarcinomas (PA) has been well documented. We have previously shown that majority of the KRAS G12/G13 mutations is transversion (G>T and G>C) in PA compared to colorectal adenocarcinomas (p= 0.011). The aim of this study was to evaluate for possible relationships between transverse mutations and expressions of enzymes associated with DNA methylation and oxidative stress in KRAS mutant PA.

Design: A total of 109 PA patients without EGFR TKD mutations (Exon19 and 21) were enrolled in this study. Genomic DNA was used for KRAS G12/G13 codon mutation testing by direct sequencing. A tissue microarray consisting of 62 PA samples including 26 PA with KRAS mutation was evaluated for the expression of DNMT1, DNMT3a, and NQO1 by immunohistochemistry. The correlations between markers expression and clinicopathologic variables were examined by Kruskal-Wallis ranks test and Spearman Rank Order test.

Results: Among 26 mutant KRAS gene, 84.6% were transverse mutation. Moreover, 21 of 22 transverse mutation are G>T (>95%). No correlation was present between patients' KRAS mutation status and age or sex. The presence of KRAS mutations, however, was associated with increased expression of DNMT1 (r=0.582, p<0.0001), NQO1 (r=0.436, p=0.0004), and DNMT3A (r=0.35, p=0.0053) in the tumor cells. Nuclear expression of DNMT1 was seen in 25 of tumors (25/62), and majority (77.3%) of them had KRAS mutation (p=0.0054).

Conclusions: In this study, we further confirmed that most KRAS mutations in PA were C>T transverse mutation. The association between KRAS mutation and upregulation of nuclear DNA methyltransferase (DNMT1, DNMT3a) expressions in the tumor cells suggest aberrant activation of DNMT might involve in the KRAS G12/G13 mutation in PA.

2060 Comparison of Napsin A Expression in Tumors with Polyclonal and Monoclonal Antibodies

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Background: Napsin A is a useful marker in identifying adenocarcinoma of the lung in a tumor of unknown origin. Our preliminary data and literature using a polyclonal antibody to napsin A demonstrated that it was a highly sensitive marker for pulmonary adenocarcinomas. However, expression of napsin A was also observed in a significant percentage of other tumors, including renal cell carcinomas, thyroid papillary carcinomas and esophageal adenocarcinomas. With the availability of a monoclonal antibody to napsin A, we compared expression of the polyclonal and the monoclonal antibodies in tumors from various organs using a single immunostaining system (Dako).

Design: Immunohistochemical evaluation of napsin A (1. Cat No. 760-4446, rabbit polyclonal, prediluted, Ventana; 2. Cat No. CM 338CK, mouse monoclonal, BioCare Medical) expression was performed on 1058 cases of tumors on tissue microarray sections. The staining intensity and distribution were recorded.

Results: The immunostaining results are summarized in Table 1. The sensitivity and specificity for the polyclonal and monoclonal antibody were 83.3% and 95.6%, and 72.6% and 97.9%, respectively.

Table 1. Summary of Immunostaining Results

Tumor	Monoclonal antibody	Polyclonal antibody
Lung ADC	72.6% (61/84)	83.3% (70/84)
Papillary RCC	50% (8/16)	75% (12/16)
Papillary thyroid CA	15.2% (7/46)	22.7% (10/44)
Clear cell RCC	2.5% (1/40)	12.5% (5/40)
Esophageal ADC	0% (0/29)	11.5% (3/29)
Ovarian tumors	1.4% (1/72)	6.9% (5/72)
Endocervical CA	6.7% (1/15)	6.7% (1/15)
Pancreatic CA	0% (0/47)	6.4% (3/44)
Lung neuroendocrine tumors	7.3% (3/41)	4.9% (2/41)
Lung squamous cell CA	2% (1/49)	2% (1/49)
Breast lobular CA	0% (0/49)	2% (1/49)
Germ cell tumors	0% (0/79)	1.25% (1/80)
Pancreatic endocrine tumors	0% (0/16)	0% (0/16)
Thyroid follicular CA	0% (0/34)	0% (0/34)
Colon ADC	0% (0/36)	0% (0/29)
Cholangiocarcinoma	0% (0/11)	0% (0/11)
Hepatocellular CA	0% (0/18)	0% (0/18)
Prostatic ADC	0% (0/133)	0% (0/133)
Breast ductal ADC	0% (0/118)	0% (0/118)
Urothelial CA	0% (0/31)	0% (0/31)
Gastric ADC	0% (0/17)	0% (0/17)
Melanoma	0% (0/77)	0% (0/77)

RCC-renal cell carcinoma; ADC-adenocarcinoma; CA-carcinoma

Conclusions: The polyclonal antibody to napsin A is more sensitive but less specific than the monoclonal antibody in identifying lung adenocarcinoma. A monoclonal antibody is the better choice for a tumor of unknown origin; whereas a polyclonal antibody is preferred for the distinction of primary lung ADC from squamous cell CA.

Quality Assurance

2061 Impact of General Versus Sub-Specialization Pathology Practice Models on Immunohistochemistry Utilization

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Background: Immunohistochemistry (IHC) plays an important role in pathology practice, particularly in the sub-specialties of oncologic pathology, neuropathology, and hematopathology. There is a limited knowledge on the impact of various pathology practice models (i.e., general vs. specialty) on IHC utilization rate.

Design: We performed a cross-sectional analysis of aggregate surgical pathology specimen case data collected during a 7-month period (January to July 2011) encompassing pre- and post-general versus specialization sign-out practice models. In the general practice model 16 pathologists signed out the majority of all cases and in the subspecialty model, 2-4 pathologists signed out each major subspecialty. We compared IHC utilization metrics (e.g., slides and antibodies per month) for the two models. We specifically evaluated the use of specific IHC protocols (e.g., melanoma protocol in patients who had pigmented skin lesions) and individual IHC stains.

Results: During the study period, 707,736 glass slides were produced (mean number of 228 slides per day and 10,105 slides per month) and 24,097 IHC slides were produced (mean number of 115 IHC slides per day and 3,442 slides per month). The IHC utilization rate was higher in general practice (29.5%) compared to subspecialty practice (25.0%) (P < .0001). The use of IHC protocols differed in the two practice models; for example, the IHC melanoma protocol was utilized more in the general practice model compared to sub-specialty practice model (P = .001). Individual stain utilization differed in the two practice models; for example, a pan-keratin stain was the most common IHC stain utilized in subspecialty practice (P < .001), while 34βE12 stain was the most frequent stain used in the general model.

Conclusions: In our institution, subspecialty practice had a lower IHC utilization frequency compared to general practice. We hypothesize that subspecialty practice results in a higher level of standardization in IHC ordering, which may be secondary to diagnostic certainty, knowledge of established IHC protocols, and experience with common and uncommon subspecialty diagnostic dilemmas.

2062 Improving Quality in the Laboratory by Implementing a Novel System of Ownership, Chain of Custody and Verification of Process and Patient

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Background: Approximately 250,000 new cases of prostate cancer are diagnosed annually in USA. This number translates into over one million biopsies and each biopsy encompasses multiple sites. The process of collecting, handling, analyzing, diagnosing, reporting and acting upon tissue biopsies is complex and involves many steps. Despite the utilization of labeling systems, the opportunity for diagnostic mistakes due to occult specimen provenance complications persists. Our aim is to evaluate our novel system in order to identify the number of errors and minimize specimen provenance complications.

Design: Our unique process of specimen ownership involves the following steps: The Patient participates in self-identification via introduction to the Know Error identification process and DNA buccal swab. The Urologist participates via actively placing prostate cores directly into a pre-bar-coded, site-specific cassette after ordering the test electronically in the EMR. The courier participates by scanning each specimen both at the urology office and upon delivery to the pathology lab. The Pathology lab personnel participate by positively identifying each specimen via 2D barcode, verification of the office-based order and registration into the Pathology LIS. Each specimen is handled one at a time using the Ventana Vantage protocol. The Pathologist participates by scanning each case before reading it. Quality of reads is ensured by a second read of all abnormal findings, and 50% of random blind reads. The ultimate step of the chain of custody designed in this lab occurs when the positive cores are verified against the patient's self-identified buccal DNA sample.

Results: In a nine month period, 89 Urologists swabbed 3,754 patients. Of those, 1,282 patients had adenocarcinoma involving 5,198 cores collectively. Although initially there were 8 cores reported as 'mis-match', these were resolved with re-submission of adequate samples. In addition, 2 patient name errors and 8 DOB errors were identified prior to testing. There were no provenance errors in any of the 5,198 positive tissue cores processed.

Conclusions: Implementation of the "IMP Pathology Laboratory Quality System" led to no provenance errors, verifying the effectiveness of the system. The system is LEAN, removing any superfluous steps, it provides an absolute chain of custody of patient tissue samples from the time of biopsy to the verification of positive patient cores.

2063 Analysis of Addendum Reports in Anatomic Pathology as a Quality Improvement Initiative

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Background: An addendum report is commonly defined as a report that provides supplementary information to the original report. On the other hand, an amended report replaces the original report in cases where the initially contained information needs to be significantly changed. There are key differences in how these reports are issued and presented in the electronic record which have implications for patient safety. The purpose of our study was to audit addendum reports and identify opportunities for quality improvement.

Design: All Anatomic Pathology addendum reports in a subspecialized academic department that were issued over a 30 month period were retrieved. These were classified