Design: We assessed INI1 inactivation in 16 MRTs and 34 other tumors of the brain or kidney by immunohistochemistry using the BAF47/SNF5 antibody. Eleven brain, 3 renal and 2 soft tissue MRTs were examined along with 4 glioblastomas, 4 pilocytic astrocytomas, 4 oligodendrogliomas, 2 ependymomas, 2 choroid plexus papillomas, 5 pituitary adenomas, 4 germinomas, 4 renal cell carcinomas, 2 clear cell sarcomas, 2 Wilms' tumors and 1 medullary carcinoma.
Results: The neoplastic cells of all MRTs and the medullary carcinoma did not express INI1 consistent with inactivation of the gene. The neoplastic cells of all other tumors expressed INI1.
Conclusions: The findings suggest that INI1 inactivation occurs in a small subset of brain and kidney tumors that includes MRTs and, possibly, renal medullary carcinomas and that immunohistochemistry using BAF47 may be useful diagnostically.

1419 Cell Cycle Regulatory Proteins in the Podocyte Cell in Idiopathic Collapsing Glomerulopathy (CGN) in Children

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Background: The podocyte cell is a terminally committed cell in G_1 arrest of cell cycle. The cell cycle regulatory proteins (CCRP) are altered following podocyte injury and it is unable to overcome the G_1/S transition phase in children with minimal change disease (MCD) and classic focal segmental glomerulosclerosis (FSGS), in contrast to the dysregulated phenotype observed in adults with CGN (Kidney Int 2003:1374).
Design: Aim : To study the alterations in expression of cyclin dependent kinase inhibitors p27, p21 and p57, and cyclins D and A, in podocytes of children with CGN. 42 kidney biopsies were studied: MCD (14), FSGS (12), CGN (4) and normal (CON) (12). The sections were examined by dual staining immunohistochemistry. Podocytes were first identified by Wilm's tumor-1 staining and subsequently CCRP expression was analyzed. Statistical analysis was performed for the proportion of podocytes expressing each CCRP. ANOVA followed by Tukey HSD was used to compare the four groups.
Results: The podocyte expression for p27, p21, p57, cyclin D and A are shown in Table (mean ± SD).

	CON	CGN	MCD	FSGS
p27	100±0.0	24.2±19.3	45.8±31.5	16.6±18.8
CON vs		<0.001	<0.001	<0.001
CGN vs			NS	NS
p21	69.8±9.9	15.5±18.4	2.2±4.3	0.6±1.6
CON vs		<0.001	<0.001	<0.001
CGN vs			0.02	0.009
p57	55.7±14.3	55.5±26.6	44.7±18.7	45.0±18.1
CON vs		NS	NS	NS
CGN vs			NS	NS
Cyclin D	7.2±9.4	26.8±13.3	1.6±3.6	0.0±0.0
CON vs		<0.001	NS	NS
CGN vs			<0.001	<0.001
Cyclin A	0.0±0.0	10.3±6.7	0.0±0.0	0.0±0.0
CON vs		<0.001	NS	NS
CGN vs			<0.001	<0.001

p27 and p21 but not p57 was decreased in CGN, as in FSGS, compared to CON. Cyclins D and A were upregulated in CGN. The CCRP expression would suggest that podocyte cell in CGN is able to overcome the G_1/S transition phase. Thus the podocyte cell in CGN has the potential to proliferate in contrast to FSGS.
Conclusions: At the cellular level, changes in CCRP indicate the cell's response to injury. We propose based on the significant contrast observed in the podocyte cell's injury response between CGN (proliferative phenotype) and FSGS (non proliferative phenotype) that CGN should not be considered as a histological variant of FSGS.

1420 Quantitative Study of Topoisomerase Gene and Gene Product in Wilms Tumor Using FISH and IHC with Automated Imaging Analysis

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Background: Long-term survival rates for children with Wilms' tumor (WT) approach 90%, however prognosis for patients who relapse is only 30-50%. The goal of this study was to compare TOP2A gene copy numbers and TopoIIα protein expression by FISH and immunohistochemistry (IHC) with automated imaging analysis in a WT tissue microarray (TMA) to define prognostic parameters and mechanisms of WT drug resistance.
Design: Fifty seven WT cases (39 primary and 18 metastatic/relapsed and 12 normal kidneys) were subjected to TMA followed by IHC and FISH analysis. Nuclear expression of TopoIIα was quantified by using Chromavision Automated Cellular Imaging System (ACIS). To evaluate the TOP2A gene copy LSI TOP2A/CEP17 multi-color FISH was performed. Tumors with TOP2A/CEP17 ≥ 2 were amplified; ratios of 1.5-2.0 indicated gain and ratios ≤0.8 indicated deletion.
Results: TOP2A gene amplification/gain was detected only in 12% of WT. All these tumors belonged to patients with anaplastic tumors who died of disease progression. No deletions were found. Positive TopoIIα staining by IHC was observed in all 57 tumors. TopoIIα protein overexpression in WT was 51-fold as compared to non-cancerous adjacent kidney tissue ($p<0.001$). The average expression level of TopoIIα in primary WT was 17.9, whereas in metastatic and recurrent WT it was 24.1. 73% of patients did not have disease progression at 5 years if TopoIIα nuclear indices were below average (19.9) compared to 36% of those with TopoIIα levels above average. Higher TopoIIα protein expression levels were associated with higher tumor stage, anaplasia, development of metastasis ($p\leq0.05$), whereas decreased Topo IIα levels were associated with actinomycin D pre-op chemotherapy and better prognosis. The correlation between TOP2A/CEP17 ratios and TopoIIα protein expression levels in WT was weak ($r=0.292$). However TopoIIα nuclear indices were higher in all tumors with TOP2A gene amplification/gain: 27.8 vs WT mean value of 19.9.
Conclusions: Strong correlation between TopoIIα protein and mRNA levels, and lack of correlation between TOP2A gene copies and TopoIIα protein levels suggest that the abnormality responsible for elevated TOP2A gene expression is at the transcriptinal level in the majority of WT. TopoIIα protein expression by IHC may have a high prognostic value for predicting the responsiveness to TopoII inhibitors.

Pulmonary

1421 DAX-1 and Androgen Receptor Expression in Diffuse Malignant Mesothelioma: Possible Targets of Hormonal Therapy

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Background: Diffuse malignant mesothelioma (DMM) is an aggressive malignancy of the pleura and peritoneum that invariably results in death of the patient. New approaches to therapy are needed for DMM. The nuclear receptor/ steroidogenesis regulator DAX-1 and androgen receptor (AR) are potential targets of hormonal or other pharmacological manipulations that might modify neoplastic growth. Expression of DAX-1 and AR by DMM has not been previously examined.
Design: A tissue microarray of 45 DMM cases was immunostained for DAX-1 (1:100 Santa Cruz Biotechnology, Santa Cruz, CA) and AR (1:40 BD Pharmingen, San Diego CA) using standard immunohistochemical techniques. Staining results were graded on a scale of 0-3 (0=no nuclear staining; 1=<33% of tumor cells; 2=33-66% of tumor cells; 3=>66% of tumor cells).
Results: All DMM were immunopositive for DAX-1. 13 DMM (30%) showed immunopositivity in 33-66% of cells and 32 (70%) showed expression in >66% of cells. 6 (13%) of DMM were negative for AR, 5 (12%) showed expression in <33% of cells, 10 (23%) showed expression in 33-66% of cells, 23 (52%) showed immunopositivity in >66% of cells.
Conclusions: The high frequency of DAX-1 and AR expression by DMM suggests that these receptors could represent possible targets of hormonal or anti-hormonal therapeutic agents. Additional studies exploring the effects of pharmacologic manipulation of DAX-1 and AR should be considered to more fully evaluate the therapeutic potential of this approach.

1422 Androgen Receptor Expression Correlates with Improved Survival in Early Stage Squamous Cell Lung Carcinomas: A High-Throughput Tissue Microarray Study

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Background: Androgen receptor (AR) immunonegativity has been associated with aggressive features in breast carcinoma, and AR plays a role in the development of prostate carcinoma. AR expression has not been previously investigated in NSCLC.
Design: High-throughput tissue microarrays containing 340 NSCLC with 5 years or more follow-up were immunostained with antisera to AR (1:40, BD Pharmingen, San Diego CA) using standard immunostaining techniques. For each sample, the percentage of NSCLC cells demonstrating nuclear AR staining was scored on a scale of 0-3 (0=no nuclear staining; 1=<33% positive; 2=33-66% positive; 3=>66% positive). Results were correlated with patient survival using Kaplan-Meier analysis.
Results: 299 NSCLC were stages I and II, and 61 were stages III and IV. Results of NSCLC staining for AR were as follows: 44 (13%) immunonegative; 14 (4%) = <33% positive; 31 (9%) = 33-66% positive; 251 (74%) = >66% positive. Nuclear staining with AR was closely associated with better 5-year survival in stage I and II squamous cell carcinomas ($p=0.02$). No statistically significant correlation was identified in other histologic types of NSCLC or in more advanced stage tumors.
Conclusions: AR-positive stage I and II squamous cell carcinomas have a significantly better prognosis than AR-negative stage I and II squamous cell carcinomas. This improved 5-year survival was not observed with other cell types.

1423 Intensity of Estrogen Receptor beta Expression Predicts Prognosis in Early Stage Pulmonary Adenocarcinoma

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Background: Estrogen receptor beta (ERb) has been identified in non-small cell lung cancers (NSCLC) and is a potential target for hormonal or other therapies. A study utilizing lung cancer cell lines showed significantly reduced cell proliferation in response to tamoxifen and slightly increased tumor cell growth with 17 beta-estradiol treatment, suggesting physiologic activity for this receptor. The prognostic significance of ERb in NSCLC, however, has not been investigated.
Design: Tissue microarrays consisting of 340 NSCLC with long-term follow-up of five or more years were immunostained with antisera against ERb (1:300, GeneTex, San Antonio, TX), using standard immunostaining techniques. The percentage of cells with

nuclear staining was graded on a scale of 0-3 (0=no nuclear staining; 1=<33% of tumor cells; 2=33-66% of tumor cells; 3=>66% of tumor cells). Intensity of nuclear staining was scored as negative, weak, moderate, or strong. Results were correlated with patient survival using Kaplan-Meier analysis.
Results: 299 NSCLC were classified as stage I and II; 61 were classified as stage III and IV. The percentage of NSCLC with each grade of nuclear staining cells was as follows: 40 (11%) = grade 0; 44 (13%) = grade 1; 231 (68%) = grade 2; 16 (5%) = grade 3. Nuclear staining was absent in 40 (13%), weak in 62 (18%), moderate in 181 (53%), and strong in 16 (5%). Strong nuclear staining with ERb was associated with improved 5-year survival in 14 stage I and II adenocarcinomas (p=0.04) compared to adenocarcinomas with negative, weak or moderate staining. No statistically significant correlation in intensity or extensiveness of nuclear staining was identified in other histologic types of NSCLC.
Conclusions: 5% of NSCLC showed strong intensity immunostaining with Erb. Early stage adenocarcinomas with strong ERb staining intensity had a statistically significant advantage in 5-year survival as compared to NSCLC showing negative, weak or moderate staining intensity. This finding suggests that increased expression of ERb may predict better prognosis in stage I and II adenocarcinomas.

1424 Leber Hereditary Optic Neuropathy Mutations in Lung Cancer

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Background: Leber Hereditary Optic Neuropathy (LHON) is mitochondrial disease, in which a primary role for mitochondrial dysfunction is confirmed by strict maternal inheritance and association with specific mutations in the mitochondrial DNA. LHON is characterized by early onset of visual loss (younger than 30 years of age), which is exacerbated by use of tobacco and alcohol. The molecular mechanism for LHON is mitochondrial dysfunction, resulting in reduced energy (ATP) production and increased oxidative stress. Previously, we detected the presence of LHON secondary mutations at 4216, and 13708 in multiple head and neck cancer, raising the possibility that these LHON mutations may play a role in head and neck cancer. In this study, we analyze these two LHON mutations in lung carcinoma in order to identify 1) whether these two mutations are associated with increased risk of developing lung cancer; and 2) whether these two mutations affect tumor behavior and survival of patients with lung cancer.
Design: Eighty-six (86) cases of lung cancer and 107 cases of normal skin of age-matched control subjects were obtained from the Department of Pathology at John L. McClellan Memorial Veterans Hospital in Little Rock. DNA samples from these cases were subjected to PCR-based restriction fragment length polymorphism (RFLP) analysis.
Results: T4216C and G13708A mutations were detected in 13/86 (15%) and 21/86 (24%) cases of lung cancer and in 12/107 (11.2%) and 20/103 (19%) cases of normal skin derived from control subjects. There is no statistical significant difference in the prevalence of these two mutations between lung cancer patients and control subjects. Using Kaplan-Miere Survival analysis. Only G13708A mutation was found to be significantly correlated with decreased overall patient survival for early-stage lung cancer (Stage I and II) (p = 0.04). The median survival time is 16 months for the tumors with G13708A mutation and is 65 months for those without G13708 mutation.
Conclusions: 1) G13708A LHON mutation is significantly correlated with decreased survival for patients with early-stage (stage I and II) lung cancer (p = 0.04). 2) The presence of T4216C and G13708A LHON mutations does not seems to increase an individual's risk of developing lung cancer.

1425 Pleuropulmonary Desmoid Tumors Versus Solitary Fibrous Tumors: A Multinational Study of Immunohistochemical Phenotype Including Assessment of Beta-Catenin and Cyclin D1

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Background: Desmoid tumors arising in the lung and pleura are extremely rare, and can resemble other more common neoplasms native to these sites. Alterations of the APC/beta-catenin pathway have been detected in sporadic desmoid tumors, and have been associated with nuclear accumulation of beta-catenin and cyclin D1 overexpression. We analyzed the expression of these substances in desmoid tumors and solitary fibrous tumors (SFTs), and compared the utility of these substances for distinguishing between these entities with those of other stains more commonly used to evaluate pleuropulmonary spindle cell neoplasms.
Design: Formalin-fixed paraffin-embedded tissue sections of four desmoid tumors (1 pulmonary, 1 pleural, 2 pleural/chest wall), and five benign and seven malignant SFTs of the pleura were immunostained for beta-catenin (1:400, Becton-Dickinson Transduction), cyclin D1 (1:200, Biocare), Alk1 (1:100, DakoCytomation), CD34 (1:40, Becton-Dickinson), vimentin (1:400, DakoCytomation), desmin (1:150, DakoCytomation), smooth muscle actin (SMA, 1:500, DakoCytomation), muscle-specific actin (1:200, Novocastra), S100 (1:500, DakoCytomation), and pankeratin (cocktail of CAM 5.2 and AE1/3). Staining intensity was graded as 0 (negative), 1+ (weak), 2+ (moderate), or 3+ (strong). The percentage of cells staining was assessed semi-quantitatively as 0=<10%, 1=10-50%, 2=>50%.
Results: Diffuse moderate or strong nuclear staining for beta-catenin was found in all desmoid tumors, 4/5 benign SFTs, and all malignant SFTs. Similarly, all desmoid tumors and benign SFTs, and 5/7 malignant SFTs showed diffuse staining for cyclin D1. The best distinction between desmoid tumors and SFTs was provided by CD34 (desmoid tumors-0/4, SFTs-9/12) and SMA (desmoid tumors-4/4, SFTs-0/12).
Conclusions: Our findings suggest that alterations in the APC/beta-catenin pathway may contribute to the pathogenesis of pleuropulmonary desmoid tumors. The meaning and significance of the high beta-catenin and cyclin D1 expression seen in SFTs need further elucidation. CD34 and SMA stains may be useful for differentiating between pleuropulmonary desmoid tumors and SFTs.

1426 Prognostic Significance of Combined Use of Ki-67 and Angiogenesis (CD-105) in Non-Small Cell Lung Cancer

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Background: Tumor aggressiveness depends on the properties of neoplastic cells and the stromal framework, and proliferative activity of tumor cells can influence the growth of the stromal component and angiogenesis. We studied the impact of combined use of Ki-67 and microvessel density (MVD) in overall survival (OS) in a series of patients with non-small cell lung cancer (NSLC).
Design: Tumor specimens of 100 patients (94 male and 6 females) with NSLCC were studied. All patients were treated with standard resections. Median follow-up was 32 months (range 6-65). The mean age was 62 years (range 35-78); 79 cases were in stage I-II and 21 in stage III-IV. Regarding histological type: 51 were SCC, 44 adenocarcinomas and 5 LCC. Immunohistochemistry (IHC) for CD105 and Ki-67 was performed. For MVD, the average of vessels in 3 high power fields (x400) was calculated, and the results were classified in three levels: low MVD (<50 vessels/mm2), intermediate (50-99) and high (>99). Tumors with ≥30% positive cells for Ki-67 were considered with high expression. We further defined two groups based on the combination of the MVD and Ki-67 results, as follows: group A (low Ki67 and low/intermediate MVD, or high Ki-67/low DMV; n=54) and group B (high Ki-67 and intermediate/high MVD; n=46). Clinico-pathological features (sex, pTNM, histologic type and grade) were evaluated. The univariate analysis was performed by the Kaplan-Meier method, and log-rank test.
Results: The mean MVD was 71.5 vessels/mm^2 (range 20-130, SD 25.0), and for Ki-67 was 28.4% (range 1-80, SD 20.4). No correlation was seen between Ki-67 expression or MVD and clinico-pathological factors (sex, age, pTNM, histology or grade). OS rate, independently of the stage, was 88% when low MVD, 69% if intermediate MVD, and 44% with high MVD (p=0.021). However, Ki-67 had nearly significant value (p=0.078). In contrast, when we analyzed the patients in stage I-II, those with tumors with high Ki-67 had shorter survival (p=0.001) but for MVD, showed only a trend (p=0.091). Analysis of the combined data including all stages showed that, longer survival was observed for patients with tumors in the group A compared to those in the group B (80% versus 54%, respectively; p=0.0045). The differences were also statistically significant when only stage I-II were studied (p=0.0132).
Conclusions: Our results indicate that in NSCLC, combination of MVD (CD105) and Ki-67 data can better define groups of patients who are at different risk of death.

1427 FRAT 2 Expression in Malignant Mesotheliomas: A Tissue Microarray and Immunohistochemical Comparison with Other Lung Malignancies

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Background: FRAT1 and FRAT2 are cancer-associated genes encoding GSK-3beta-binding proteins. The FRAT1 gene was first identified as a proto-oncogene involved in progression of mouse T-cell lymphomas. More recently, FRAT/GBP (GSK-3beta Binding Protein) family members have been recognized as critical components of the WNT signal transduction pathway. Over-expression of FRAT1 or FRAT2 leads to malignant transformation through the activation of the WNT—beta-catenin—TCF signaling pathway. It has been demonstrated by several investigators that some gastric cancers show up-regulation of FRAT 2.
Design: High-density tissue microarrays containing samples from 45 malignant mesotheliomas (32/45 (71%) were epithelial and 13/45 (29%) were sarcomatous), 54 carcinoid tumors, 46 small cell carcinomas and 120 non-small cell carcinomas were immunostained for FRAT 2 (1:50, Santa Cruz Biotechnology) using standard avidin-biotin techniques. Staining intensity was graded as 0, 1+ (weak), 2+ (moderate) or 3+ (strong), and the percentage of tumor cells staining was graded semi-quantitatively as follows: 0=no tumor cells staining, 1= 1-32% staining, 2=33-66% staining, 3=>66% staining. For each tumor, an overall percentage of cells stained was calculated as the mean of the individual percentages recorded for the three samples of each tumor in the microarray.
Results: Of the 45 mesotheliomas examined, 42 were positive and 3 were negative. 15% of the immunoreactive cases showed 1-32% of cells staining, 40% fell between 33 and 66%, and 45% of tumors demonstrated staining in >66% of tumor cells. Strong cytoplasmic staining was seen in all epithelial mesotheliomas whereas the sarcomatous types stained weakly. The negative mesotheliomas were of the sarcomatous type. In contrast, none of the neuroendocrine tumors, including small cell carcinomas, was positive for FRAT 2. Only 12 non-small cell carcinomas were focally positive with strong staining in two adenocarcinomas.
Conclusions: The very frequent presence of FRAT 2 staining in malignant mesotheliomas suggests a role for FRAT2 in the development of these tumors. The lack of staining in neuroendocrine neoplasms and the majority of non-small cell carcinomas suggests that other pathways unrelated to the WNT — beta-catenin pathway are more important in the pathogenesis of these neoplasms.

1428 p63 Expression in Tumors of the Mediastinum: Practical Applications and Review of the Literature

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Background: p63 is a p53-related nuclear protein, whose expression in certain normal and neoplastic human tissues has been previously described. The protein is widely expressed in basal cells of stratified and glandular epithelia, and it appears to play an important role in epithelial development through its effect on basal cell regeneration. p63 is also expressed in various human neoplasms, including those of the mediastinum. Our aim was to evaluate the utility of p63 in the diagnosis of mediastinal tumors.

Design: We retrieved 55 primary and metastatic tumors of the lung and mediastinum, as well as 9 primary germ cell tumors, from our department archives: 10 thymomas, 1 thymic carcinoma, 5 sarcomatoid carcinomas, 15 neuroendocrine carcinomas, 6 malignant mesotheliomas, 11 lymphomas, 6 metastatic carcinomas, and 10 germ cell tumors. After confirming the accuracy of the original diagnosis, we selected one paraffin block with the largest amount of viable tumor tissue from each case for evaluation by immunohistochemistry using a monoclonal antibody against p63. Positive cases showed strong nuclear staining for p63 in at least 10% of tumor cells.

Results: p63 was positive in 10/10 thymomas, 1/1 thymic carcinomas, 3/5 sarcomatoid carcinomas, 1/15 neuroendocrine carcinomas, 0/6 malignant mesotheliomas, 5/11 lymphomas, 2/6 metastatic carcinomas, and 1/10 germ cell tumors.

Conclusions: Our findings confirm previously published results, which showed strong p63 expression in thymoma and thymic carcinoma and rare, if any, p63 expression in malignant mesothelioma. We also found p63 expression in some sarcomatoid carcinomas, lymphomas, and metastatic carcinomas. A thorough review of the current literature is presented with an emphasis on the practical use and limitations of p63 in the differential diagnosis of mediastinal neoplasms.

1429 Epithelial Apoptosis/Proliferation Imbalance in End-Stage Emphysema

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Background: Apoptosis has recently been proposed to contribute to the pathogenesis of emphysema.

Design: In order to establish if cell fate plays a role even in end-stage disease we studied 16 lungs (9 smoking-associated and 7 a1antitrypsin (AAT)-deficiency emphysema) from patients who had undergone lung transplantation. Six unused donor lungs served as controls. Apoptosis was evaluated by TUNEL analysis, DNA gel electrophoresis and electron microscopy while cell proliferation was detected by a immunohistochemical method (MIB1). The role of transforming growth factor (TGF)-β1 was also investigated and correlated both with epithelial cell apoptosis and proliferation.

Results: The apoptotic index (AI) was significantly higher in emphysematous lungs compared to the control group ($p \leq 0.05$), particularly if only lungs with AAT-deficiency emphysema were considered ($p \leq 0.05$ vs $p=0.09$). The proliferation index was similar in patients and controls (1.9±2.2 vs 1.7±1.1). TGF-β1 expression in the alveolar wall was higher in patients with smoking-associated emphysema than in cases with AAT-deficiency emphysema ($p \leq 0.05$). A positive correlation between TGF-βRII and AI was observed only in the control group ($p \leq 0.05$, $r^2 = 0.8$).

Conclusions: Our findings suggest that apoptosis of alveolar epithelial cells plays an important role even in end-stage emphysema particularly in AAT-deficiency disease. The TGF-β1 pathway does not seem to directly influence epithelial turnover in end-stage disease.

1430 CXCR3/CXCL10 Interactions in the Development of Hypersensitivity Pneumonitis

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Background: Hypersensitivity pneumonitis (HP) is an inflammatory interstitial lung disease caused by repeated inhalations of finely dispersed antigens, mainly organic particles or low molecular weight chemicals. The disease is characterized by a high intensity alveolitis sustained by CD8(+) cytotoxic T lymphocytes, granuloma formation, and, if expositions of inhaled particulate antigens continue, fibrosis. Although it is known that the attraction of T lymphocytes into the pulmonary parenchyma represents an essential step in mechanisms ultimately leading to HP reaction, the mechanisms implicated in this process remain undefined.

Design: In this study we evaluated whether IP-10 (CXCL10), a chemokine which is induced by IFN-γ, and its receptor CXCR3, plays a role in regulating the trafficking of CD8(+) T cells in HP lungs. 12 sequentially enrolled HP patients were included in the study. BAL and open lung biopsy were achieved from each patient. Immunohistochemistry for the characterization of inflammatory infiltrate was carried out by use of the following antibodies: CD45, CD43, CD45RO, CD20, CD3, CD68, CD4 and CD8.

Results: Our immunohistochemical data demonstrated that lymphocytes infiltrating lung biopsies were mainly represented by CD8 T cells which strongly stained for CXCR3. However, T cells accumulating in the bronchoalveolar lavage (BAL) of HP were CXCR3(+)/ IFN-γ(+) Tc1 cells exhibiting a strong *in vitro* migratory capability in response to CXCL10. Alveolar macrophages expressed and secreted definite levels of CXCL10 capable of inducing chemotaxis of the CXCR3(+) T-cell line 300-19; the secretory capability of alveolar macrophages was upregulated by preincubation with IFN-γ. Interestingly, striking levels of CXCR3 ligands could be demonstrated in the fluid component of the BAL in individuals with HP.. Interestingly, striking levels of CXCR3 ligands could be demonstrated in the fluid component of the BAL in individuals with HP.

Conclusions: These data indicate that IFN-γ mediates the recruitment of lymphocytes into the lung via production of the chemokine CXCL10, resulting in Tc1 cells alveolitis and granuloma formation.

1431 Ki-67 Expression Differs between Chronic Airway Rejection and Lymphocytic Airway Inflammation in Lung Transplant Biopsies

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Background: In lung transplant patients, the histologic lesion of obliterative bronchiolitis usually reflects chronic airway rejection, a diagnosis that usually implies poor responsiveness to anti-rejection therapies. Lymphocytic airway inflammation shares some histologic features with chronic airway rejection, but is often reversible with treatment of the underlying cause, typically either rejection or infection. We investigated whether analysis of Ki-67 expression by constituent airway cells and inflammatory cells could be a useful test for separating chronic airway rejection and lymphocytic airway inflammation.

Design: We selected 18 transbronchial biopsies diangosed as chronic airway rejection and 18 showing lymphocytic airway inflammation, and performed immunohistochemical staining with monoclonal antibody Ki-67 (MIB-1, 1:50, DakoCytomation). Ki-67-stained nuclei were calculated as a percentage of total nuclei at 40x magnification. Ki-67 staining was evaluated for respiratory epithelial cells, type II pneumocytes, fibroblasts, and peribronchiolar mononuclear inflammatory cells.

Results: Higher percentages of Ki-67-positive respiratory epithelial cells, peribronchial mononuclear inflammatory cells and type II pneumocytes were present in biopsies with chronic airway rejection than in biopsies with lymphocytic airway inflammation ($p<0.02$). Ki-67-positive fibroblasts did not differ significantly between the two groups.

Conclusions: These observations suggest that proliferation of inflammatory cells and airway epithelial cells, as reflected by increased Ki-67 expression, forms a more significant part of the constellation of responses in chronic airway rejection than in lymphocytic airway inflammation. Given these results, prospective evaluation of Ki67 staining should be pursued to assess its predictive value for development of chronic airway rejection.

1432 Histopathologic and Molecular Cytogenetic Characterization of Tumor Xenografts and Transplantable Tumor Lines from a Variety of Human Lung Cancers

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Background: In response to the need to develop clinically relevant models for lung cancer that reflect the histologic diversity of the disease, we have established several tumor xenograft lines in NOD/SCID mice derived from patient surgical samples or biopsies of a variety of tumor subtypes. These included: moderately and poorly differentiated squamous cell carcinoma, moderately and well-differentiated adenocarcinoma, adenosquamous carcinoma, small cell carcinoma, large cell undifferentiated carcinoma and carcinosarcoma.

Design: Fresh normal lung and/or tumor tissue was obtained from 14 consecutive cases of lung cancer resections or bronchial biopsies performed at Vancouver General Hospital and BC Cancer Agency (mean age: 63 years; range 49-73 years; 9 males and 5 females). Tissue fragments were grafted under the renal capsules of 6-8 week old NOD/SCID mice within 24 hours of removal and harvested after 4-12 weeks. Xenograft histology was compared with that of the original tissue by H and E staining. Immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) were used to distinguish human cells from host mouse cells in selected cases. Four tumors showing rapid growth were selected to be re-grafted over several generations (>5) to produce transplantable tumor lines. Spectral Karyotyping (SKY), metaphase comparative genomic hybridization (CGH) and array CGH analyses were carried out for these lines.

Results: Microscopic analysis of xenografts and original tumor samples showed excellent retention of histologic features in the majority of cases. IHC and FISH confirmed the presence of human tumor cells and development of a murine supportive stroma in the xenografts. Distant metastases were found in a case of large cell undifferentiated cancer. Molecular cytogenetic studies on the transplantable tumor lines showed clonal structural abnormalities and genomic imbalances comparable to those previously reported for lung cancer.

Conclusions: This highly successful xenograft approach will provide new opportunities for studying the biology of lung cancer progression and chemoresistance and the development of novel therapeutic regimens with the possibility of patient-tailored therapies.

1433 Molecular and Immunohistochemical Analysis of Nm-23 and Kai-1 Tumor Metastasis Suppressor Genes in Adenocarcinoma of the Lung

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Background: The expression of tumor metastasis suppressor genes, nm-23 and Kai-1, may play a role in the tumor progression, such as prostate and breast carcinomas, but their significance in lung carcinogenesis is controversial. Several studies have shown decreased nm-23 and Kai-1 protein expression in non-small cell lung carcinoma by immunohistochemistry; however, there have not been any studies correlating the protein expression with molecular changes at the DNA level.

Design: 16 stage I and 9 stage II lung adenocarcinoma (ADC) with their paired lymph node metastasis (LNM) were obtained from the paraffin-block archives of the University of Pittsburgh Medical Center. Nm-23 and Kai-1 protein expressions were assessed by immunohistochemistry using polyclonal antibodies with a standard Streptavidin-biotin approach. DNA was extracted from microdissected normal and tumor tissues. Loss of heterozygosity (LOH) was analyzed with fluorescently labeled primers for polymorphic microsatellite makers located at the nm23 locus, 17q21-23, (D17S.1161, D17S.1877, D17S.956) and Kai-1 locus, 11p11.1, (D11S.1344, D11S.1385, D11S.1319) by capillary electrophoresis. Ratios between the tumor and normal samples that were <0.7 were indicative of LOH.
Results: Immunohistochemical expression of nm-23 protein was present in all stage I and stage II ADC and their LNM. LOH of nm-23 gene in informative cases was seen in 70% (7/10) stage I ADC, 67% (6/9) stage II ADC and 67% (6/9) LNM. Immunohistochemical expression of Kai-1 was present in 56% (9/16) stage I ADC, 56% (5/9) stage II ADC and 67% (6/9) LNM. LOH of Kai-1 was seen in 69% (9/13) stage I ADC, 44% (4/9) stage II ADC and 33% (3/9) LNM. There was no significant difference in protein expression and gene allelic imbalances between stage I and stage II ADC. The results of nm-23 and Kai-1 protein expression as determined by immunohistochemistry did not correlate with the status of the gene at the DNA level. Neither protein expression nor LOH correlated with outcome in either gene.
Conclusions: LOH of the nm23 and Kai-1 genes is frequent in primary lung ADC. However, these genes do not appear to be differently expressed or altered at the DNA level in tumors with or without metastases. Discrepancy between protein expression and LOH analysis suggests that some other mechanisms play a role. Our data suggest that nm23 and Kai-1 may be important tumor suppressor genes in lung carcinogenesis, but probably do not function as tumor metastasis suppressor genes.

1434 Microsatellite Instability, Proliferation and Apoptosis in Lymphoepithelioma-Like Carcinoma of the Lung

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Background: Previous studies failed to demonstrate a significant role of tumor suppressor genes such as p53 in tumorigenesis of lymphoepithelioma-like carcinoma of the lung (LELC). These findings together with histologic appearance of LELC, its favorable prognosis, infrequent metastases and frequent lack of smoking history suggest that other genetic mechanisms such as microsatellite instability (MSI) may play a role in pathogenesis of these rare lung tumors. The aim of this study was to compare MSI status, proliferation and apoptosis of LELC with stage I non-small cell lung carcinoma (NSCLC) with marked lymphocytic host response (MLHR).
Design: 7 cases of LELC and 16 cases of stage 1 NSCLC with MLHR were retrieved from the paraffin-block archives of University of Pittsburgh. MSI was analyzed on microdissected normal and tumor tissues by PCR using fluorescently labeled mononucleotide (BAT25 and BAT26) and dinucleotide (D2S.123, D5S.346, D17S.259) markers. Proliferation and apoptosis were determined by standard immunohistochemistry using Ki-67 and "Apoptag Peroxidase Kit" on formalin-fixed paraffin embedded tissue. The proliferative index (PI) and apoptotic index (AI) were defined as percentage of the tumor cells showing nuclear staining with each antibody counted per 20 high-power fields (HPF). AI:PI ratio was calculated in each case.
Results: MSI was detected in 2 cases of LELC (2/7)(29%) with only one marker (D17S.250), and in 3 (3/16)(19%)cases of NSCLC with MLHR with only two markers (1 D2S123 and 2 D17S.250). The mean PI was lower in LELC 65.6 (range 22-148) than in NSCLC (mean 97.4) (range 67-131), but that difference did not reach statistical difference (p=.07). There was no significant difference in AI between the two groups (22.3 in LELC vs. 20 in NSCLC). Although, mean AI:PI ratio was higher in LELC (0.51) than in NSCLC (0.23), that difference was not statistically significant (p=.11).
Conclusions: MSI is very uncommon in LELC indicating that MSI is not an important event in LELC carcinogenesis. In this study we showed that proliferation, but not apoptosis, is different in LELC as compared to conventional stage I NSCLC with MLHR. The wide range of PI and AI reflects marked genetic heterogeneity in both groups of tumors. Additional molecular studies of genes involved in regulation of cell proliferation are warranted to further clarify the tumorigenesis of LELC.

1435
[Abstract Withdrawn]

1436 LOH Mutations in Pleural Mesotheliomas, Metastatic Pulmonary Adenocarcinomas, and Benign Pleural Mesothelium: Preliminary Microsatellite Marker Panel for a Rapid Molecular Assay

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Background: Pleural fluid specimens are common. Cytology is the standard method of evaluating whether malignancy is present in pleural fluid specimens. Benign mesothelial cells, mesothelioma (meso), and pulmonary adenocarcinoma (adenoCA) in pleural fluid specimens are often cytologically similar, resulting in a equivocal cytologic diagnosis. A molecular assay using the presence and unique mutations in meso and adenoCA to assist in this distinction would be useful. We evaluated a preliminary marker panel for this purpose.
Design: Diagnostic tissues from 10 pleural mesos and associated benign mesothelium, 2 peritoneal-primary low-grade, papillary mesos, ten pulmonary adenoCAs that involved the pleural surface, and 10 benign mesothelial fibrous decortications were microdissected from paraffin blocks and DNA was extracted. Following PCR with D13S153, D1S551, D3S1038, D4S171, D4S1586, D9S157, D9S171, D13S1247, D15S1012, D22S446, D22S683, and TP53, results were analyzed on an ABI-310. *LOH* was +/-50% neoplasm allelic ratio compared to normal tissue ratio.
Results: All 10 pleural-mesos had ≥1 LOH in chromosome 4, 13, or 22 markers. Pleural mesos had a mean of 3.8 LOHs/ case and adenoCAs had a mean of 4.4. The highest LOH rates in pleural-mesos were in D22S683 (75%), D4S408 (71%), and D15S1012 (67%), whereas their mutation rates in adenocCAs were 14%, 0%, and 50%. The highest LOH rates in adenoCAs were in TP53 (60%) and D3S1038 (60%), whereas their mutation rates in pleural-mesos were 40% and 22%. The two peritoneal-mesos each had one LOH in D3S1038 and TP53. Two benign mesothelium specimens from pleural-mesos each had a LOH in D4S408 and D22S683 that were also present in the associated pleural-meso. There were no mutations in benign mesothelial decortication specimens.
Conclusions: LOH mutations were detected in 100% of pleural-meso and pulmonary adenocarcinoma cases. Several LOH-microsatellite markers occurred in both pleural-mesos and adenoCAs which could serve as malignancy screening markers. Pleural-mesos had LOH-mutations that were distinct from LOH mutations in pulmonary adenoCAs. Peritoneal-mesos may be genetically distinct from pleural-mesos. Benign-appearing mesothelium associated with pleural-meso had some but not all the mutations seen in the corresponding meso. Benign mesothelium does not harbor background mutations. These results are the initial step towards developing a rapid pleural fluid malignancy screening molecular assay.

1437 Detection of New Primary In Situ and Invasive Squamous Lung Carcinomas in Patients with and without a History of Lung or ENT Squamous Carcinomas

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Background: Non-small cell lung carcinoma survival is driven by stage. Current goals are to identify new primaries at an earlier stage, and to define the natural history of pre-invasive dysplasias, including carcinoma in situ (CIS). Bronchoscopic surveillance may allow earlier detection of new squamous carcinomas and their precursors. At UNC, high-risk patients are being followed using autofluorescence (LIFE) and white light bronchoscopy.
Design: Biopsy results of 47 patients in the UNC LIFE bronchoscopy trial from 1999-2004 were reviewed. Patients whose biopsies showed CIS or invasive carcinoma (CA) were studied regarding past histories of airway squamous CA and the strategies required to obtain diagnostic biopsies.
Results: 47 high-risk patients were screened with endobronchial biopsies. 29 of these 47 had a history of previous lung or ENT (airway) squamous CA, and 18 of these 47 had no such history. 6 (13%) of these 47 were found to have either in situ (n=5) or invasive (n=1) squamous CA. 4 of these 6 had a history of previous airway squamous CA, representing 14% (4/29) of this subset. 2 of these 6 had no previous history of squamous CA, representing 11% (2/18) of this subset. These percentages are not statistically significant (Fisher exact test). 3 of these 6 were diagnosed on the first set of biopsies (1 invasive carcinoma, and 2 CIS), 2 of these 6 on the second set of biopsies from the same sites, and 1 of these 6 on the third set of biopsies from the same sites. In the 3 CIS patients identified on followup biopsies, 2 had prior diagnoses of moderate to severe dysplasia from the same site, while 1 had a prior diagnosis of mild dysplasia from the same site.
Conclusions: In high-risk patients followed with bronchoscopy, CIS or invasive squamous CA was identified in 14% of patients with a history of previous airway squamous CA, and in 11% of patients without this history (p=NS). This prevalence of CIS and CA suggests a potential benefit for bronchoscopy of high risk patients. Differences in the degree of dysplasia seen in same-site serial biopsies may be related to neoplastic progression, or may represent mosaic variation in dysplasia. Bronchoscopic surveillance of high-risk patients may allow earlier detection and treatment of new primary squamous carcinomas and their precursors.

1438 Clinicopathological Study of 23 Malignant Mesotheliomas Showing Osseous and Cartilaginous (Heterologous) Differentiation

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Background: Osseous and cartilaginous differentiation is rare in malignant mesothelioma (MM) being observed in less than 1% of cases. We present a clinicopathological study of 23 cases.
Design: 21 pleural MM, 1 peritoneal and 1 pleuro-peritoneal cases were evaluated for clinical, histopathological and immunohistochemical features.The latter included broad spectrum cytokeratin, CK5/6, calretinin, thrombomodulin, CEA, CD15, Ber EP4 and MOC31.
Results: The age ranged from 50 to 82 years (mean 64.7), 14 were in men and 8 in women.They presented with chest or abdominal pain and serosal effusion. Grossly or radiologically the tumours appeared as diffuse masses. 70% were biphasic and 30% sarcomatoid MM. 5 cases showed cartilagenous differentiation only, whereas the remainder also showed ossification. Combinations of calcification, cartilage, osteoid and bone at different states of maturation were seen in a myxoid and malignant spindle celled stroma. All epithelial immunohistochemical markers were negative. Cytokeratin and the mesothelial markers, CK5/6 and calretinin, were nearly always positive in the epithelioid component of the biphasic types but only occasionally and focally in the sarcomatoid areas. Survival varied from 1 to 17 (mean 7.1) months after diagnosis .
Conclusions: Although very rare, osseous and cartilaginous differentiation can occur in MM. Diagnosis is particularly difficult in the sarcomatoid type because of frequent absence of cytokeratin and mesothelial immunohistochemical markers.Clinical presentation and prognosis appear similar to MM in general.

1439 Establishing 'Control' Standards to Aid the Diagnosis of Asbestosis; Asbestos Fibre Burden and Fibrosis in the Lungs of Non-Occupationally Exposed Persons

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Background: Diagnosis of asbestosis is problematic, particularly in early stage disease, requiring the presence of sufficient multifocal interstitial fibrosis in association with adequate numbers of asbestos bodies and/or suitably elevated lung asbestos fibre burden. The aim of this study was to determine the range of fibrosis and asbestos fibre burden in the lungs of non-occupationally exposed subjects.

Design: Lung tissues were examined histologically and fibre burdens determined by TEM with EDXA in 254 subjects coming to autopsy from 11 urban (heavy and light industrial) and rural locations. All had died from non-asbestos related disease and had no occupational history of asbestos exposure.

Results: The group comprised 114 men, 21 women and 119 not specified.Age varied from 26 to 87 years. Asbestos fibres were detected in 98.4%. One asbestos body was identified in two cases (1.1%). Interstitial fibrosis (all grades) was common (41%) and showed no zonal predilection. Unsuspected usual interstitial pneumonia was observed in 2.2%. Commercial amphibole asbestos was detected in 57%, chrysotile in 98% and tremolite in 13% (see table).

Conclusions: Diagnosis of early stage asbestosis should be considered only when there is a good history of asbestos exposure, latency is appropiate, there is a lower zone predominance of fibrosis, and either asbestos bodies are present or mineral analysis shows a significantly elevated retained amphibole asbestos fibre burden.

Fibrosis and fibre burden (median and (5-95%) in 254 control subjects given in millions of fibres per gram dry lung

fibrosis grade	%	amosite	crocidolite	tremolite	chrysotile
0	59	ND (ND - 0.6)	ND (ND-0.78)	ND (ND - 0.29)	4 (0.31-19.63)
1	23	ND (ND - 1.12)	ND (ND - 1.71)	ND (ND - 0.22)	3.4 (0.59-17.56)
2	12	0.23 (ND - 1.4)	0.3 (ND - 1.6)	ND (ND - 0.3)	5.23 (0.7-11.4)
3	4	ND (ND - 1.29)	ND (ND - 0.87)	ND (ND - 0.63)	1.7 (0.21-20.61)
4	2	ND (ND-ND)	0.05 (ND - 0.44)	ND (ND - ND)	0.5 (0.13-3.08)

ND = not detected

1440 Percutaneous Fine Needle Aspiration and Concurrent Core Needle Biopsy of Pulmonary Lesions: A Retrospective Comparison of 362 Patients

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Background: We retrospectively compared the diagnostic accuracy of percutaneous fine needle aspiration biopsy (FNAB) and concurrent core needle biopsy (CNB) in the diagnosis of lung nodules at a cancer center.

Design: The results of computed tomography-guided FNAB and concurrent CNB of pulmonary nodules from 362 patients were compared with the final diagnoses of the lesions. According to the final diagnoses, which were based on the combined information of definitive biopsy diagnoses, diagnoses of resected tumors, microbiologic findings, clinical and radiologic follow-up data and therapeutic response, the study cases were grouped into 188 malignant, 161 benign, and 13 inconclusive lesions.

Results: Complete agreement between the results of the FNAB and CNB was found in 285 (78.7%) cases, which included 150 (79.8%) malignant, 132 (82.0%) benign, and 3 (23.1%) inconclusive lesions. Of the 188 malignant tumors, 34 (18.1%) were diagnosed by only one of the modalities, 16 by FNAB only and 18 by CNB only. The diagnostic accuracy of each modality and the combination of both was as follows:

	Malignant (n=188) (%)			Benign (n=161) (%)	
	Malignant	A/S	Benign/nonDX	Benign lesion[a]	Others[b]
FNAB	160 (85.1)	12 (6.4)	16 (8.5)	21 (13.0)	140 (87.0)
CNB	162 (86.2)	9 (4.9)	17 (9.0)	49 (30.4)	112 (69.6)
FNAB+CNB	178 (94.7)			52 (32.3)	

A/S: atypical/suspicious; nonDX: nondiagnostic; [a] Including granuloma/granulomatous inflammation, fungal infection, pulmonary hamartoma, solitary fibrous tumor, neurofibroma, and thymoma; [b] Including normal parenchyma, histiocytes, necrotic debris, nonspecific inflammation, and nondiagnostic specimens.

Conclusions: For the malignant lesions, FNAB and concurrent CNB resulted in similar diagnostic accuracy. For the benign lesions, FNAB had less diagnostic yield than did the CNB. The combination of FNAB and concurrent CNB markedly increased the diagnostic accuracy for malignant lesions. Because a substantial number of the malignant cases were diagnosed by only one of the modalities, using both is recommended when clinically/radiologically indicated.

1441 Glutathione (GSH2) Expression in 201 Resected Non-Small Cell Lung Cancers (NSCLC): Correlation with Survival

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Background: Oxidative stress from tobacco smoke components and other agents is believed to have a crucial role in the development of lung cancer. Gamma-1-glutamyl-1-cysteinylglycine (GSH) is an antioxidant that is likely involved in carcinogenesis. We investigated the relationship of GSH2 expression, the second enzyme in the pathway of GSH synthesis, to patient survival in NSCLC.

Design: Formalin-fixed, paraffin-embedded sections of 201 resected NSCLC from patients with more than 5 years of post-surgical follow up were immunostained with GSH2 antibody (1:100, Santa Cruz) using manufacturer procedures, and were graded using the following scale: 0 = 0-9% cells positive; 1 = 10-50%; 2 = 51-80%; 3 = 81-100%. Intensity was graded as weak or strong. Statistical examination was performed using Kaplan-Meier analysis.

Results: Cytoplasmic staining of greater than 80% of tumor cells with GSH2 demonstrated similar trends of improved survival for stage I squamous cell carcinoma (p=0.15) and stage I and II adenocarcinoma (p=0.09).

Conclusions: Our findings suggest that increased GSH2 expression may indicate better survival in early stage squamous cell and adenocarcinoma. Manipulation of GSH expression may be a potential basis for future therapy of NSCLC.

1442 Glutathione-S-Transferase pi (GST-pi) Expression Correlates with Survival in Stage 1 and 2 Squamous Cell Lung Carcinoma

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Background: The GST family of genes encode for detoxification enzymes that protect against reactive oxygen species and influence host susceptibility to carcinogens, including tobacco smoke. We evaluated isoenzyme GST-pi expression in relation to survival in lung cancer.

Design: 201 lung cancers with long-term follow-up were immunostained with GST-pi antibody (1:100, DAKO) using standard immunostaining techniques. Results were graded on a scale of 0-3 (0 = < 10% tumor cells staining; 1 = 10-50%; 2 = 51-80%; 3 = > 80%) for both nuclear and cytoplasmic staining. Results were correlated with patient survival using Kaplan-Meier analysis.

Results: Nuclear staining with GST-pi in greater than 10% of the cells was closely associated with improved survival (p=0.02) in stage 1 and 2 squamous cell carcinomas (N=56). Cytoplasmic staining showed a similar trend that did not reach statistical significance. No statistically significant correlation in either cytoplasmic or nuclear staining was observed in other histologic types of NSCLC.

Conclusions: GST-pi nuclear expression predicts prognosis in stage 1 and 2 squamous cell lung carcinoma. It does not appear to predict prognosis in other histologic types of NSCLC.

1443 Interdigitating and Follicular Reticulum Cell Sarcomas Arising in the Lung and Comparison to Those in Other Sites - A Histological and Immunohistochemical Comparison

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Background: Interdigitating and follicular reticulum cell sarcomas (IRS, FRS) are rare malignant tumors of the antigen presenting cell system. They have not been described in the lung or thoracic cavity to the best of our knowledge. Although histologically similar they can be differentiated by their immunohistochemical profile.

Design: Two cases arising in the lung and thoracic cavity, respectively, were seen in our institutions in the last year. Other primary sites were excluded by radiological and clinical investigations. For comparison three further cases of IRS arising in the skin, prostate, and mammary gland/axillary lymph node were selected as well as a few cases of FRS. They were investigated for antibody reactions using CD21, CD35, CD83, fascin, as well as S100 protein, CD68, cytokeratin, CD31, CD34, smooth muscle actin, and lysozyme antibodies.

Results: Morphologically IRS and FRS of the lung show two cell types, a spindle and an epitheloid cell type. In FRS some cells look like immature epitheloid cells. There was focal vascular invasion in both, focally simulating an angiosarcoma. IRS stained positively for CD68, CD83, S100 protein, and focally for CD35 (can also be negative), but was negative for CD21. FRS was positive for CD83, CD21, CD35, fascin, S100, and CD68.

Conclusions: IRS and FRS are rare pulmonary tumors. In H&E stained sections many epitheloid and spindle cell neoplasms enter the differential diagnosis. They can be differentiated by their immunohistochemical profile of strong staining for S100 protein, positivity for CD68 and lysozyme, and finally by their reaction for specific markers CD83, CD35 in IRS and additionally CD21 in FRS. If there is a difference in the biological behavior between the two entities, as proposed in the literature, cannot be answered yet, because of our short observation period.

1444 Promoter Hypermethylation of the p16 (CDNK2a/INK4a) Gene in Lung Cancer

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Background: The p16 (CDNK2a/INK4a) protein is an important tumor suppressor gene, which inhibits cyclin-dependant kinase 4 and 6 that function as a negative regulator within the G1 to S phase of the cell cycle. Loss of p16 gene function can cause decreased apoptosis and increased cell proliferation, leading to eventual tumor formation. Promoter hypermethylation is one important mechanism of gene inactivation. P16 promoter hypermethylation has been described as a contributing factor in a variety of human cancers, including lung cancer. In this study, we examine p16 promoter methylation status of non-small cell lung cancer in 110 cases and of histologically negative bronchial resection margin in 34 cases in order to identify 1) the prevalence of p16 promoter hypermethylation in lung cancer. 2) To identify whether p16 promoter hypermethylation is present in histologically negative bronchial resection margin.

Design: 110 non small cell lung cancer and 34 histologically negative bronchial resection margin were collected from the Department of Pathology of the John L. McClellan Memorial Veterans Hospital in Little Rock, Arkansas from 1999 to 2003. DNA Samples were subjected to methylation-specfic PCR, using p16 gene specific primers.

Results: Promoter hypermethylation of the p16 gene was detected in 41/110 (37.2%) cases of non-small cell lung cancer. In 34 lung cancer cases where the matched bronchial resection margins were available for analysis, 17 were positive for p16 promoter hypermethylation. Seven bronchial resection margins derived from these 17 (41.1%) tumors were also positive for p16 promoter hypermethylation. Among 17 lung cancer specimen that were negative for p16 promoter methylation, 15 (88.2%) matched bronchial resection margins were also negative for p16 methylation with 2 bronchial margins (11.8%) being weakly positive for p16 promoter methylation.
Conclusions: 1) p16 promoter hypermethylation is frequently detected in non-small cell lung cancer (37.2%), supporting an important roles p16 gene in the development of lung cancer. 2) 41% (7/17) of histologically negative bronchial resection margins display p16 promoter hypermethylation, raising the possibility that these histologically negative margins may not be truly negative at the molecular levels.

1445 c-Met Receptor Tyrosine Kinase Is Expressed and Activated in Lung Carcinoma and Carcinoid Tumors

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Background: c-Met is involved in cellular proliferation, angiogenesis, branching morphogenesis, and signal transduction. In tumors, disregulation of c-Met signaling can be seen with overexpression or activating mutations of the receptor.
Design: We examined the expression of c-Met in cell lines and archival paraffin embedded lung cancers, using immunoblotting and immunohistochemistry (IHC) techniques, respectively. Activated Met was examined using two phospho-Met antibodies by IHC in tumor sections. In the A549 cell line, we also studied activation of c-Met receptor by its ligand hepatocyte growth factor (HGF) using immunofluorescence.
Results: There was moderate to strong expression of c-Met in 6 of 10 small cell lung cancer (SCLC) cell lines; and strong expression in 8 of 9 non-SCLC (NSCLC) cell lines. In archival tumor sections, 61% (14/23) of NSCLC and 25% (1/4) SCLC strongly expressed c-Met. Strong c-Met expression was evident in 6/9 adenocarcinomas, 4/7 large cell carcinomas, 4/7 squamous cell carcinomas (SCC), and 3/5 pulmonary carcinoid tumors. Activated Met expression was also demonstrated in all of the tumor types, except SCC, at varying degrees using the phospho-specific antibodies against pY1003 (juxtamembrane) and pY1230/1234/1235 (auto-phosphorylation site). There was preferential expression of the activated phospho-Met in the tumor cells located in the invasive front of the tumor tissues. HGF induced pY1230/1234/1235-Met expression dramatically in A549 (NSCLC) cells.
Conclusions: c-Met is widely expressed and activated in 25 to 67% of most major types of lung cancers, thus it may potentially serve as an important anti-tumor molecular therapeutic target in selected patients.

1446 Expression and Activation of c-Met Receptor Tyrosine Kinase in Malignant Mesothelioma

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Background: c-Met is a potential therapeutic target with its disregulated signal transduction in solid tumors. Novel therapies such as small molecule inhibitors against c-Met (SU11274 and PHA665752) are being tested in vitro.
Design: We examined c-Met expression in 3 mesothelial cell lines and 12 archival paraffin-embedded malignant mesotheliomas by immunoblotting and immunohistochemistry (IHC), respectively. In addition, activated Met was examined using two phospho-Met antibodies, recognizing the pY1003 (juxtamembrane) and pY1230/1234/1235 (auto-phosphorylation site) epitopes, by IHC in tumor sections. Activation of c-Met by its ligand hepatocyte growth factor (HGF) was performed in all 3 mesothelioma cell lines and was evaluated by immunoblotting.
Results: All 3 cell lines, 5 of 10 epithelioid and 1 of 2 sarcomatoid mesotheliomas were positive for c-Met expression, with all cell lines and 2 of the epithelioid mesotheliomas showing strong expression. Activated Met was positive or inducible by HGF in all cell lines, while it was negative in tumor tissue sections except for 2 epithelioid mesotheliomas.
Conclusions: c-Met expression is present in a significant proportion of mesotheliomas, which may allow molecularly targeted therapy in selected patients. Further evaluation of activated Met in fresh frozen tissues would help to overcome the limitation of using paraffin-embedded archival tissue for IHC staining.

1447 EGFR Mutation Analysis in Atypical Adenomatous Hyperplasia of the Lung

S Hussain, Q Pan, M Ladanyi, MF Zakowski. Memorial Sloan-Kettering Cancer Center, New York, NY.
Background: Atypical adenomatous hyperplasia (AAH) had long been recognized as a precursor lesion for bronchioloalveolar carcinoma (BAC) and possibly invasive adenocarcinoma. However, molecular studies have provided few conclusive findings supporting the multistep progression from AAH to BAC and invasive adenocarcinoma. Recently, epidermal growth factor receptor (EGFR) kinase domain mutations have been reported in a subset of adenocarcinomas, especially those with BAC features, but it remains unclear whether these are early or late genetic lesions.
Design: We studied a total of 21 samples, consisting of 14 cases of AAH, including 7 that were studied along with the adenocarcinoma or BAC from the same patient and 7 that were not. In the latter group, the associated cancers in the lung were BAC (1), adenocarcinoma (2), squamous cell carcinoma (3), and metastatic colorectal adenocarcinoma (1). Areas of AAH, BAC, and adenocarcinoma were dissected and re-embedded separately. DNA was extracted from the resulting paraffin blocks. PCR-based mutation analysis was then performed for exon 19 deletions and the exon 21 L858R mutation, which together account for 85-90% of EGFR mutations.
Results: Satisfactory results were obtained in all cases. None of the 14 cases of AAH showed EGFR mutations, and neither did the 7 matching BACs or adenocarcinomas.
Conclusions: Our results suggest that EGFR mutations are rare or absent in unselected samples of AAH. Additional studies need to be done to determine the mutational status of AAH in cases where the concomitant tumor shows EGFR mutations.

1448 Analysis of EGFR Amplification in Pulmonary Adenocarcinomas by Chromogenic In Situ Hybridization

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Background: Mutations in the kinase domain of the epidermal growth factor receptor (EGFR) gene have recently been described in lung adenocarcinomas that respond to the EGFR inhibitors gefitinib (Iressa) and erlotinib (Tarceva). EGFR overexpression (by immunohistochemistry) and amplification [by fluorescent in situ hybridization (FISH)] have also been previously reported in lung adenocarcinomas but their relationship to EGFR mutations remains to be determined. We have used EGFR chromogenic in situ hybridization (CISH) to examine EGFR amplification in relation to EGFR mutation status and response to EGFR inhibitors.
Design: We studied 15 lung adenocarcinomas from 13 patients who had received gefitinib or erlotinib (9 responders and 4 non-responders) and from 2 untreated patients. DNA was extracted from formalin-fixed paraffin-embedded tumors. PCR-based mutation analysis was performed for exon 19 deletions and the exon 21 L858R mutation, which together account for 85-90% of EGFR mutations. Exons 18 to 24 of EGFR were also subjected to direct sequencing to identify less common mutations. EGFR CISH (Zymed) was performed. At least 30 tumor nuclei were counted and the averaged count interpreted as follows: non-amplified if 1-5 signals/ nucleus, low level amplification if 6-10 signals/ nucleus, and high level amplification if > 10 signals/ nucleus.
Results: Three cases (20%) showed high level EGFR amplification and 4 cases showed low level amplification. There was no clear relationship of EGFR amplification to EGFR mutation status or to gefitinib/erlotinib response.
Conclusions: Analysis of EGFR copy number by CISH detected a higher prevalence of EGFR amplification in lung adenocarcinomas than previous studies based on FISH. High level EGFR amplification can occur in EGFR mutated / gefitinib/erlotinib responsive cases as well as EGFR non-mutated / gefitinib/erlotinib non-responsive cases.

1449 C4d Deposition in Lung Allografts with Antibody Mediated Rejection

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Background: Antibody-mediated rejection (AMR) is well established for renal transplantation but remains controversial for lung allograft. Donor specific antibodies to human leukocyte antigen (HLA) and evidence for their complement-dependent action, demonstrated by C4d deposition, are currently used in renal allografts to diagnose AMR allowing for a more targeted therapy.
Design: Lung allograft biopsies from 29 patients with mild (A2), moderate (A3) and severe (A4) cellular rejection were selected from the paraffin block archives of the University of Pittsburgh Medical Center. Two groups were identified based on the presence or absence of circulating antibodies (Ab) to donor HLA by ELISA and matched to post transplant date (POD) and rejection grade. Local complement activation was demonstrated by evaluation of linear, continuous subendothelial C4d deposition by immunohistochemistry. B cell dynamics was evaluated by IHC stains for CD20, CD79 and CD138 and compared with CD3, as well as correlated with the grade of rejection and peripheral blood serology, performed at the time of the interpreted biopsy.
Results: Initial presence of circulating Ab was associated with ACR (35.7%A2, 57.1%A3, 5.7%A4) and occurred on average on POD 139 (range 7-670). C4d deposition was seen in 21.42% of 14 patients with documented circulating anti-HLA AB, and was absent in 15 POD and ACR grade matched patients without Ab ($p = 0.058$). T cell profiles were similar in the two sets of patients. B cell profiles from pre-B cell to plasma cell were not associated with the presence of anti-HLA Ab, but correlated with high grade rejection.
Conclusions: The presence of HLA Ab and infiltrating B cell aggregates were associated with high-grade ACR. In the setting of ACR, coexistent AMR maybe supported by C4d deposition in a minority of cases and requires further investigation.

1450 Recurrent Sarcoidosis in Lung Transplant Allograft- Are Granulomas of Recipient Origin?

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Background: Sarcoidosis accounts for only 2.8% of lung transplants in the United States. It is, however, the most commonly reported disease to recur after lung transplantation. In most cases, recurrence is diagnosed as an incidental finding in transbronchial lung allograft biopsy (TBLAB) and is unrelated to clinical or radiologic abnormalities. Identity of the histiocytes composing noncaseating epithelioid granulomas in the allograft lung in patients with recurrent sarcoidosis (RS) was analyzed using DNA identity testing in two cases.
Design: Native lung resections and corresponding TBLAB from patients who underwent lung transplantation for sarcoidosis between 1990 and 2004 and who developed RS were gathered from the paraffin block archives of University of Pittsburgh Medical Center. Clinical parameters including age, sex, grade of rejection, number of episodes of RS and follow-up were recorded. Native lungs (NL) and corresponding TBLAB showing granulomas consistent with RS were microdissected in cases where adequate material was available. DNA was extracted and an ABI AmpflSTR

commercial kit was used that simultaneously amplify 15 STR loci as well as the XY chromosomes. The informative STR loci in native lung (pure recipient), non-granulomatous lung from TBLAB (on donor lung) and granulomas on donor lung were analyzed in three patients. The relative proportion of donor and recipient cells in the chimera was quantified using the fluorescent intensity of each peak on an electropherogram.

Results: 8 patients with RS were identified, 5 females and 3 men. Two had bilateral lung transplantation and 6 had single lung transplantation. The age at transplantation ranged between 39 and 53. Recurrent disease was diagnosed in 1-11 biopsies/patient, and occurred in the first 6 months following transplantation in 25% of cases, between 6 months and one year in 25% and between 1 and 2 years in 50% of cases. In two patients sufficient material allowed for DNA analysis. TBLAB from patient one showed no ACR and granulomatous inflammation of RS. Donor (D) to recipient (R) profile changed from "normal" donor lung (37% D, 63% R) to 15%D and 85%R DNA in the granuloma. In patient 2, the TBLAB showed minimal ACR and granulomatous inflammation. D to R profile changed from 75%D and 25%R in the "normal" D lung to 54%D and 46%R in the granuloma.

Conclusions: Preliminary DNA analysis of two cases of RS suggests that the presence of recurrent granulomas in the graft is associated with an increase in the percentage of recipient DNA in the epithelioid cell clusters.

1451 Association of HHV8 (Human Herpesvirus 8) in Primary and Secondary Pulmonary Hypertension: An Immunohistochemical and PCR Study on Microdissected Tissue

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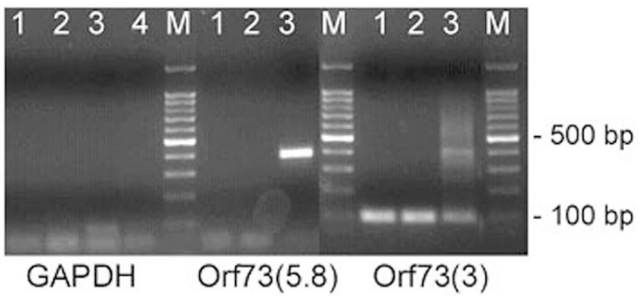
Background: Pulmonary hypertension may be primary (PPH) with a genetic suceptibility or secondary (SPH) and is characterized by proliferating plexiform vascular lesions.The pathogenesis of PPH in unclear. In HIV+ patients plexiform lesions may develop without demonstrable HIV virus in the lesions. HHV8 is frequently seen in association with HIV and is known to facilitate vascular proliferation.We hypothesize that HHV8 may play a role in pathogenesis of plexiform vascular lesions of the lung.

Design: We examined 12 archival lung-tissue samples from patients with PPH and 21 patients with SPH for evidence of infection with HHV8. HHV8 infection was assessed *immunohistochemically* with an antibody directed against LANA-1 (1:1000 dil, ABI, Columbia, MD), and by a *nested polymerase-chain-reaction (PCR*) assay performed on 7 cases (3 PPH and 4 SPH) with and without microdissection. Two sets of Primers: Orf73(5.8, 408 bp) and Orf73(3, 100bp) were used.

Results: All 33 cases were negative on immunohistochemistry as all 7 cases for HHV8 on PCR without microdissection. **Two of the 7**, 1 PPH (Fig 1) and 1 SPH (Pt with Eisenmenger's complex) were positve for HHV8 using the Orf 73(3) primer pair only on *microdissected* plexiform lesions.

Gel electrophoresis of amplified product from microdissected specimen The lanes are designated as follows: 1. PPH + case. 2. BCBL-1 cDNA (+ control).3. HHV8-BAC36 genomic DNA, (+ control). 4. H_2O: Negative control. Note that lane 1 with the test PPH case using the Orf 73(3) probe is positive like adjacent lanes 2 and 3.



Conclusions: HHV8 is present in microdissected plexiform lesions of PPH as reported recently by Cool et al (NEJM 2003;349:1112-1122) using a different primer pair. In addition, contrary to the above report we found 1 of 4 microdissected SPH cases to be positive by PCR only. While the presence of HHV8 in the pulmonary plexiform lesions is intriguing, its role in the pathogenesis remains to be proven.

1452 Telomere Length in Stage I Non-Small Cell Carcinoma of Lung (NSCL) Is Associated with Smoking History

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Background: Shortening of telomeres with age has been proposed to be the signal for replicative senescence in normal cells. In contrast, the majority of immortal cells, such as stem cells and carcinoma cells, do not demonstrate a net loss of telomere length (TL) suggesting that maintenance of telomeres is essential for long- term cell proliferation. Previous studies have indicated that carcinomas with elevated levels of telomerase are associated with poor prognosis. We hypothesized that increased TL may similarly be associated with aggressive biologic behavior. We also examined the association between TL, smoking history, histologic subtype and age of patient.

Design: Touch imprints of non-small cell carcinoma of lung from twenty-nine patients with stage I disease treated only by surgery were evaluated for telomere length (TL) by pan-telomeric nucleic acid fluorescence-in-situ hybridization (FISH) staining. Quantitative FISH analysis on 50 interphase nuclei for TL was performed with image analysis software that measured the average integrated intensity of nuclear telomeres as a measure of TL. TL was compared to histologic subtype, clinical outcome, age and smoking history. Variables were analyzed via Fisher's exact test, logistic regression and Spearman's rank correlation coefficient.

Results: The average age of patients was 68 years and median pack years was 50. Five patients were non-smokers. The median TL was 4.86; of 12 patients with TL > 4.8, 7 had adenocarcinomas (AD) and 5 squamous carcinomas (SqC). Seventeen patients had TL< 4.8 of which 8 had AD, 7 SqC, 1 NSC and 1 adenosquamous carcinoma. Relapse occurred in 8 patients (mean time to relapse 11 months) of which 5 had TL >4.8. There was a significant negative correlation between smoking history and TL, with shorter telomeres being associated with increased pack years. (p=.02). TL was not correlated with age.

Conclusions: This pilot study indicated that NSCL patients with increased TL may be at increased risk for relapse and that TL was inversely correlated with smoking history. These findings are of prognostic significance and should be validated in a larger prospective study.

1453 Gene Silencing of PCNA in Mesothelioma Cell Lines Causes Deregulation of Other Genes

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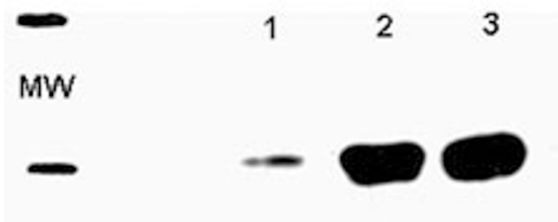
Background: Small interfering RNA (siRNA) can be introduced into living tumor cells and specifically downregulate targeted genes. Cooperating genes might be expressed normal, downregulated, or even overexpressed, depending on pathway organization and feedback loops. Proliferating cell nuclear antigen (PCNA) is conserved in eukaryotic cells, and plays an essential role in nucleic acid metabolism and cell cycle regulation. Targeting PCNA by siRNA can be used to look for expression patterns in different cell cycle genes, such as cyclin D1, and p21.

Design: Two commercially available mesothelioma cell lines were cultivated. In both cell lines comparative genomic hybridization (CGH) was done to define target regions for siRNA investigation. siRNA for PCNA was transfected into these cell lines. PCNA, Cyclin D1, and p21 were evaluated by Western blotting. In addition a cDNA array was constructed and used to monitor additional related genes deregulated by targeting PCNA. This cDNA array contains oncogenes, tumor suppressor genes, and IMAGE clones associated with cell proliferation and apoptosis.

Results: CGH analysis revealed losses on chromosomes 1, 4q, 6, 7, 8p, and gains on 2p, 5p, 8q, 11, 13q, 17q, 20; PCNA located on chromosome 20, and Cyclin D1 on chromosome 11 were overrepresented in one cell line, but not in the other. When PCNA was downregulated by siRNA, cyclin D1 also showed a similar downregulation (fig 1: 1 downregulation by PCNA-siRNA, 2+3 normal controls; WM weight marker), whereas p21 was unaffected.

Conclusions: PCNA and Cyclin D1 cooperate in signaling progression of cell cycle. Both genes are regulated independently from each other. A downregulation of PCNA should normally not affect the regulation of cyclin D1. Therefore a siRNA mediated inhibition of PCNA which causes a downregulation of cyclin D1 might point to a regulatory loop of both genes, whereas the normal expression of p21 can be interpreted, that the p21 pathway is not connected to the PCNA-Cyclin D1 axis. To further elucidate this pathway, mRNA from the mesothelioma cell lines will be hybridized onto a cDNA array and altered gene expression will be studied on a larger scale.



1454 Hypoxia-Inducible Factor 1 (HIF-1) Expression in Non-Small Cell Lung Carcinoma

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Background: Hypoxia-Inducible factor 1 (HIF-1) is a transcription factor and a key regulator of oxygen homeostasis. HIF-1 expression supports tumor growth by aiding in the recruitment of various factors including vascular endothelial growth factor (VEGF) that enable tumor angiogenesis. HIF-1 expression has been shown as a poor prognostic marker in other solid neoplasms including gastrointestinal stromal tumor of the stomach, and T1/T2 breast cancers. To date, no one has examined HIF-1 staining in non-small cell lung carcinomas.

Design: Formalin fixed, paraffin-embedded tissue sections from 100 consecutive cases of lung carcinoma with known follow-up data were immunostained with antibody specific for HIF-1 alpha (dilution 1:25;Novus Biologicals, Littleton, CO) utilizing standard immunohistochemical technique and the Envision (DakoCytomation) detection system. Nuclear staining was considered positive. Slides were interpreted as positive or negative with no arbitrary percent threshold for positivity. Positive controls (glioblastoma multiforme) and negative controls stained appropriately.

Results: Out of one hundred cases examined, 74% were immunoreactive with anti-HIF-1 (squamous cell carcinoma 35/42 (83%), adenocarcinoma 29/41 (71%), adenosquamous carcinoma 3/4 (75%), bronchoalveolar carcinoma 0/4 (0%), large cell carcinoma 4/4

(100%), carcinoid 1/2 (50%) and non-small cell carcinoma not otherwise specified 2/3 (67%)). When correlated with recurrence and/or survival, there was a trend toward HIF-1 expression equating to poor outcome. The strongest correlation was with adenocarcinoma, where HIF-1 expression was correlated with a 67% chance of dying from disease while cases that lacked expression yielded only a 13% chance of dying from disease.

Conclusions: When correlated with clinical outcome HIF-1 expression appears to predict poor clinical outcome as it has been previously shown in other malignancies. Lack of HIF-1 immunostaining in adenocarcinoma appears to be a very good prognostic indicator. HIF-1 stain may be helpful in making treatment decisions in selected cases of pulmonary epithelial malignancy.

1455 MUC4 Expression in High-Throughput Tissue Microarray of 343 Non-Small Cell Lung Carcinomas with Long-Term Follow-Up: Relationship to Survival

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Background: Mucin (MUC) 4 is a high molecular weight membrane-bound glycoprotein that is expressed in the foregut prior to epithelial differentiation and is found in normal adult airway epithelium, non-small lung cancers (NSCLC) and in other human malignancies independent of mucus secretion. Although its tissue distribution has been studied, its utility in predicting prognosis in NSCLC is unknown.

Design: Immunohistochemistry for MUC4 (1:750, Zymed Laboratories Inc., San Francisco, CA) was performed on formalin-fixed, paraffin-embedded tissue from 343 NSCLC arranged in a high-throughput tissue microarray. Information about five-year survival and tumor stage was collected on all of the patients. MUC4-stained slides were evaluated for cytoplasmic and membranous staining, and recorded on a semi-quantitative scale from 0 to 3+ as follows: 0 = no immunoreactivity in tumor cells, 1+ = <33% with immunoreactivity, 2+ = 33-66% with immunoreactivity, 3+ = >66% with immunoreactivity. Kaplan-Meier analysis was used to assess the relationship between patient survival and MUC4 expression.

Results: MUC4 was frequently expressed in adenocarcinomas (152/187, 84%), squamous cell carcinomas (69/89, 78%), adenosquamous carcinomas (6/8, 75%) and large cell carcinomas (32/59, 54%). In patients with stage I and II adenocarcinoma, there was a trend towards longer patient survival with greater MUC4 immunoreactivity (2+/3+, n =111) compared to patients with lesser MUC4 immunoreactivity (0/1+, n = 47) ($p = 0.11$). No significant correlation was observed for patients with other cell types and/or stages of disease.

Conclusions: These findings suggest that in stage I and II adenocarcinomas, more extensive tumor expression of MUC4 may indicate a more favorable prognosis.

1456 Expression of Inducible Nitric Oxide Synthase in Airway Epithelial Cells Adjacent to Non-Small Cell Lung Cancers (NSCLC) Versus in Pulmonary Emphysema without NSCLC

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Background: Inflammation is believed to participate in the development of both non-small cell lung cancers (NSCLC) and emphysema (EMPH) caused by tobacco smoke. Data derived from human NSCLC and animal models suggests that iNOS may be a key intermediary in the linkage between inflammation and carcinogenesis. Increased expression of iNOS has been noted in precancerous lesions, further suggesting that iNOS inhibitors may be potentially useful in NSCLC prevention or therapy (Cancer Res 2002; 62:6850; Acta Pol Pharm 2002; 59:473). However, not all patients exposed to tobacco smoke develop NSCLC. This study evaluated for a potential relationship between iNOS expression and susceptibility to NSCLC by comparing the expression of iNOS in airway epithelium adjacent to NSCLC with that of airway epithelium from smokers with EMPH but without NSCLC.

Design: Formalin-fixed paraffin-embedded tissue sections from 118 resected NSCLC and 25 volume reduction specimens with EMPH and without NSCLC were immunostained with iNOS polyclonal antibody (1:40, Neomarkers, Fremont, CA). All of the patients had a history of tobacco smoking. Extent of iNOS expression in airway epithelial cells was recorded as 0 = <5% cells +; 1 = 5-24% +; 2 = 25-50% +; 3 = 51-75% +; 4 = >75% +. Intensity of expression was graded as 1 = weak; 2 = moderate; 3 = strong; 4 =very strong. Extent score and intensity score were added for each case to give a final combined score designated as: 0 = negative, 1-2 = weak, 3-4 = moderate, 5-6 = strong, 7-8 = very strong.

Results: 72/79 (91%) bronchioles and 12/16 (75%) bronchi adjacent to NSCLC showed a combined score of strong or very strong iNOS expression in epithelial cells. 19/25 (76%) bronchioles in EMPH without NSCLC showed a combined score of weak or moderate iNOS expression, but 6/8 (75%) bronchi in EMPH without NSCLC showed a combined score of strong or very strong epithelial iNOS expression.

Conclusions: In bronchioles adjacent to NSCLC, iNOS expression is increased relative to bronchioles in EMPH without NSCLC. This observation is particularly interesting, given the growth in the numbers of peripheral adenocarcinomas among NSCLC. Whether increased bronchiolar epithelial iNOS expression is causatively linked to development of NSCLC, or represents an epiphenomenon, warrants further study.

1457 Pulmonary Veno-Occlusive Disease (PVOD) and Pulmonary Capillary Haemangiomatosis (PCH): A Study of 28 Cases

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Background: PVOD and PCH are rare conditions grouped separately in the Evian classification of pulmonary hypertension. However, they present with overlapping clinical and histological features and share similar poor prognoses.

Design: To assess their inter-relationship, autopsies (n=12), explants (n=2) and surgical biopsies (n=17) from 28 patients (PVOD n=23, PCH n=5) were reviewed, two with both biopsy and autopsy, one with sequential biopsies. Staining for CD34 was performed in 10 cases.

Results: Of 23 cases initially diagnosed as PVOD, (av. age = 31 yrs (4-68 yrs, 16M:7F), veins and venules showed fibrous stenosis and/or occlusion, with venous recanalisation and medial changes as less frequent features. PCH was present in 83% of cases, progressing from perivenular foci to diffuse parenchymal involvement. Other features were arterial medial hypertrophy (83%) and arterial intimal fibrosis (74%), hemosiderosis (74%), venulitis (13%) and infarction (13%). No plexiform lesions were seen. Of cases initially diagnosed as PCH (av. age = 42 yrs (9 mo-60 yrs, 3M:2F), 80% showed evidence of PVOD, with venous and arterial changes similar to those described above, other than no venulitis or infarction. One surgical biopsy from a neonate with PCH had unexplained pulmonary hypertension. One case of PCH on biopsy was reclassified as PVOD at autopsy. One case of PVOD on initial biopsy showed PCH in a subsequent biopsy. PCH was highlighted by CD34 staining, with capillary proliferation predominantly within alveolar walls but also involving bronchi and venous lumens. PCH also was associated with a mild lymphocytic infiltrate.

Conclusions: Our data suggest that PCH is a reactive angioproliferative process, which occurs most frequently as a result of post-capillary obstruction in PVOD, rather than a separate disease.

1458 MUC4 Expression Differentiates Diffuse Malignant Mesothelioma from Non-Small Cell Carcinoma of the Lung: A High-Density Tissue Microarray Study

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Background: Poorly differentiated epithelioid neoplasms diffusely involving the pleura can sometimes represent differential diagnostic quandaries, yet distinction between diffuse malignant mesothelioma (DMM) and metastatic carcinoma has important clinical consequences. Differentiation of adenocarcinoma (ADC) of the lung from mesothelioma has been the subject of considerable investigation using immunohistochemical markers. Squamous cell carcinoma (SQCC), however, can also metastasize to the pleura and mimic epithelial DMM on routine histopathology. SQCC is not infrequently immunopositive for so-called "mesothelial" markers that differentiate ADC from DMM, including CK 5/6, thrombomodulin, calretinin and mesothelin. Even occasional ADC are reported positive for these DMM markers. Therefore, investigators continue to search for markers that differentiate between DMM and NSCLC. MUC4 has been proposed to be a potential marker to differentiate between ADC and DMM (*Mod Path* 2004; 17: 150-157). To confirm and expand on the previous observations, we examined MUC4 expression in high-density tissue microarrays of NSCLC and DMM.

Design: High-density tissue microarrays were prepared using triplicate punches of formalin-fixed, paraffin-embedded tissue for 343 NSCLC and 43 DMM. Immunostains for MUC4 (1:750, Zymed Laboratories) were performed on microarray sections using Evision + Labelled Polymer Universal Kit (DakoCytomation). Immunostaining for MUC4 was evaluated for intensity as negative, weak, moderate and strong.

Results: All DMM were negative for MUC4 including 30 epithelial and 13 sarcomatous DMM. MUC4 expression was observed in 259 NSCLC (76%) including most ADC (84%) and SQCC (78%) with 47/89 (53%) SQCC showing moderate to strong expression.

Conclusions: MUC4 not only differentiates between ADC and DMM but also between SQCC and DMM with high sensitivity and 100% specificity in this study.

1459 Immunostaining for RSV Antigen in BAL Cell Blocks: A Sensitive Method for Diagnosis of Significant RSV Infection in Lung Transplant Recipients

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Background: Respiratory syncytial virus (RSV) is a common cause of upper respiratory infections in adults; in immunocompromised patients, RSV is a major cause of lower respiratory infection with significant morbidity and mortality. Current methods of diagnosis include ELISA and culture of bronchoalveolar lavage fluid (BAL) or nasopharyngeal swabs (NP). This study evaluates the utility and significance of staining BAL cell blocks (CB) and transbronchial biopsies (TBB) for RSV antigen using immunohistochemistry, as compared with ELISA assay and culture of BAL fluid and NP swabs.

Design: BAL CB and transbronchial biopsies from 12 lung transplant recipients diagnosed with RSV infection (1997-2000) using ELISA and culture were stained using an anti-RSV antibody (Biodesign International). Clinical and laboratory features of immuno-positive patients were compared to those of immuno-negative patients.

Results: 5/12 patients were antigen-positive by immunostaining: 5/5 BAL CB+, and 1/4 TBB+. CB staining compared favorably with other methods of diagnosis (table 1). Clinical features and BAL differentials also differed (table 2). No differences were noted in patient age or % decrease in FEV_1.

Conclusions: In lung-transplant recipients, BAL CB is more often RSV antigen-positive than is TBB using IHC. IHC for RSV antigen performed on a CB is more often positive than is any single culture or ELISA modality (5/5 positive, vs 2/4 NP-ELISA, 0/4 NP-culture, 9/11 BAL-E, 3/7 BAL-culture). Immuno-positive patients had higher mean BAL % lymphocytes and % PMN's, a difference not accounted for by differences in duration of clinical illness or patient age.

Table 1

IHC status	BAL-E	BAL-culture	NP-E	NP-culture
Immuno-positive	5/5	1/2	N/A not done	0/1
Immuno-negative	4/6	2/5	2/4	0/3

Table 2

IHC status	Mean duration of sx	Mean time since tx	Mean BAL % PMN's	Mean BAL % lymphs
Immuno-positive	8.2 days	540 days	21.8	31.4
Immuno-negative	10.0 days	319 days	4.5	9.2

1460 Diagnostic Value of Transcriptional Factors and Differential Cytokeratins in Separating Primary Pulmonary from Metastatic Mucinous Cystadenocarcinoma

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Background: Primary mucinous cystadenocarcinoma (MCA) of the lung is a rare malignancy that is histologically similar to ovarian and pancreatic MCAs. Although the diagnosis of pulmonary MCAs is usually not difficult based on histology, it can occasionally be challenging to exclude a metastatic mucinous cystadenocarcinoma from the pancreas and ovary. Previous studies have indicated that organ specific transcription factors of TTF-1 and CDX-2 and differential cytokeratins can be very useful in identifying an adenocarcinoma of unknown origin. However, the diagnostic value of TTF-1, CDX-2, PDX-1 (a pancreatic transcription factor) and differential cytokeratins in separating MCAs of various sites has not been studied.

Design: A total of 37 MCAs from the lung (3), pancreas (12) and ovary (22) were retrieved from the hospital computer system. Immunostaining for PDX-1, TTF-1, CDX-2, CA-125, CK7 and CK20 were performed on an automated immunostainer with appropriate positive and negative controls. The statistical analysis was performed with Chi-square method.

Results: The immunohistochemical findings are summarized in the table below.

Primary	No.	CDX-2	PDX-1	TTF-1	CA-125	CK7	CK20
Lung	3	100.0%*	0.0%	0.0%	0.0%	100.0%	100.0%
Pancreas	12	66.7%*	100.0%*	0.0%	0.0%	83.8%	100.0%
Ovary	22	9.1%	54.5%	0.0%	45.5%	100.0%	54.5%

*: $P < 0.01$

Conclusions: 1. Pulmonary MCA is an immunophenotypically unique pulmonary adenocarcinoma (positive for CDX-2, CK7 and CK20, but negative for TTF-1) that is different from conventional pulmonary adenocarcinoma (positive for TTF-1 and CK7, but negative for CDX-2 and CK20).

2. Pulmonary MCA does not express PDX-1.

3. PDX-1 is a sensitive but not a specific marker for pancreatic MCAs, since it is also observed in 54.4% ovarian MCAs.

4. A IHC panel of CDX-2, PDX-1, CA-125, CK7 and CK20 is useful to confirm a primary pulmonary MCAs or diagnose a metastatic MCA in the lung.

1461 Global Gene Expression Profiling of Epithelioid Mesotheliomas and Lung Adenocarcinomas for the Identification of New Differential Diagnostic Markers and the Development of a Microarray-Based Prediction Model

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Background: The differential diagnosis of epithelioid pleural tumors can be difficult and includes epithelioid mesothelioma, lung adenocarcinoma, and other carcinomas metastatic to pleura. Considerable progress has been made in defining immunohistochemistry (IHC) panels useful in making this distinction, but there is a continuing need for more robust markers. Our aim is to use global gene expression profiling to identify genes whose products could be utilized as new differential diagnostic IHC markers in this setting and to provide a dataset for the development of a microarray-based diagnostic prediction model.

Design: High molecular weight RNAs from 69 pleural epithelioid mesotheliomas and 107 lung adenocarcinomas were submitted for Affymetrix U133A microarray analysis to profile the expression of approximately 18500 gene transcripts. We performed unsupervised hierarchical clustering analysis and differentially expressed genes were identified by two-sample t-tests. Several tissue microarrays have been constructed to validate the results by IHC.

Results: Epithelioid mesotheliomas and lung adenocarcinomas were perfectly separated by unsupervised hierarchical cluster analysis. Among the top genes significantly differentially expressed between mesothelioma and adenocarcinoma were many established IHC markers, validating the overall approach. These included calretinin, WT1, TTF-1, CEA (CEACAM5), and neuronal cadherin. In addition, many previously unrecognized genes were strongly differentially overexpressed in epithelioid mesothelioma (uroplakins 1B and 3B, hyaluronan synthase 1) or in lung adenocarcinoma (CEACAM6, aquaporin 3, claudin 3, CD24, ELF3).

Conclusions: Our data represent the most robust comparison of the global gene expression profiles of lung adenocarcinomas and epithelioid mesotheliomas obtained to date, and highlight novel differentially expressed genes as candidates for IHC validation as new differential diagnostic markers. The dataset is also well-suited for the development and validation of a microarray-based diagnostic prediction model.

1462 New Lung Carcinoma Antibody ES1: An Immunohistochemical Study of the Sensitivity and Specificity

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Background: A monomeric heavy chain antibody, AFAI, was produced by panning a naïve phage display library of single domain antibodies (derived from the heavy chain antibody repertoire of a llama) against the non-small cell lung carcinoma cell line A549. The AFAI phages were isolated and the AFAI gene was then sequenced. The new antibody ES1, a pentameric form of AFAI was produced by fusing AFAI and the B subunit of verotoxin, a self-pentamerizing domain. The AFAI antigen was identified as a variant of CEACAM6 known to be present in a wide range of normal tissue and involved in neoplastic progression of colonic adenomas and carcinomas.

Design: Various normal tissues and 119 neoplastic lesions from lung colon, breast and other organs were immunostained for ES1 using the ABC technique

Results: ES1 showed positive immunostaining ranging from slight to moderate in 23 and strong immunoreactivity in 10 of 35 non-squamous large cell carcinomas. The two tumors which were not immunoreactive were a large cell undifferentiated carcinoma with some squamous features and a non-mucinous bronchiolo-alveolar carcinoma. Colonic adenocarcinomas and invasive duct carcinomas of the breast showed a weak to moderate immunoreactivity in 9 and strong immunoreactivity in one of 23 tumors. The other carcinomas and all normal tissues with or without an adjacent carcinoma were not immunoreactive

Conclusions: Unlike other CEACAM6 antibodies, ES1 can be used as a marker for lung adenocarcinoma due to the negative immunoreactivity for normal tissues. ES1 tend to be more immunoreactive with the poorly differentiated than the well differentiated adenocarcinoma. The antibody showed some cross-immunoreactivity with colonic and breast carcinomas. For these cancers, the immunoreactivity was more focal and weaker than for lung adenocarcinoma.

1463 Roles of Wnt/beta-Catenin Pathway Signaling and TGF-beta 1 in Repair and Remodeling of the Lung after Acute Lung Injury

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Background: Diffuse alveolar damage (DAD) and advanced pulmonary fibrosis (APF) represent lung injury with repair and remodeling. DAD progresses over time from exudative through organizing to fibrotic stages. Wnt/beta-catenin signaling pathway has a proven role in lung development and also seems to be related to the repair process after lung injury. Transforming growth factor (TGF)-beta 1, by comparison, promotes production of extracellular matrix. It acts by both transcriptional and post-transcriptional mechanisms in the modulation of pulmonary fibrosis. The underlying molecular mechanisms are not well understood.

Design: Immunohistochemical expression patterns of beta-catenin, TGF-beta 1 and alpha-smooth muscle actin were examined in paraffin embedded sections of lung from 25 autopsies with DAD (10 exudative, 11 organizing, 4 fibrotic stages) and from 10 autopsies with APF. After microwave antigen retrieval, all cases were immunostained with an anti-human beta-catenin (Neomaker, Lab Vision) and with anti-human TGF-beta 1 (Santa Cruz Biotechnology) polyclonal antibodies using a standard indirect avidin-biotin horseradish peroxidase method.

Results: Intranuclear beta-catenin immunoreactivity was seldom observed in bronchial and bronchiolar epithelium in control and exudative stage of DAD. There was increased expression in bronchial and bronchiolar epithelium as well as in hyperplastic type 2 pneumocytes and occasional fibroblasts and smooth muscle cells in the organizing and fibrotic stages of DAD and prominent expression in the same sites in APF. Interestingly the expression was also seen along cellular membranes in addition to the intranuclear expression. Strong intranuclear beta-catenin accumulation was partially seen in various cells such as basal hyperplasia, squamous metaplasia and bronchiolar adenomatosis. TGF-beta 1 was rarely seen in control tissue. TGF-beta 1 was seen in the exudative stages of DAD in macrophages, hyperplastic type 2 pneumocytes and bronchiolar epithelium. There was less expression in the organizing and fibrotic stages of DAD. TGF-beta 1 was only minimally expressed in APF.

Conclusions: Activation of Wnt/beta-catenin pathway signaling and TGF-beta 1 both may have an important role in the repair and remodeling of the lung after acute lung injury. But, TGF-beta 1 is less evident when pulmonary fibrosis is far advanced.

1464 Expression of Smad4 in Non-Small Cell Lung Cancer

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Background: Smad4, a putative tumor suppressor gene localized to chromosome 18q21.1, is a key intracellular mediator for the transforming growth factor-beta (TGF-beta) superfamily of growth factors. Smad4 expression has recently been studied in a variety of tumors. However, the expression of Smad4 in non-small cell lung cancer (NSCLC) and its impact on overall survival has not been widely studied.

Design: One hundred and twenty six cases of NSCLC including 42 squamous cell carcinomas (SCC), 44 adenocarcinomas (AC), and 34 bronchioalveolar carcinomas (BAC) were retrieved from pathology archives. Tumors were graded as high grade and low grade. The tumors were immunostained stained by automated methods (Ventana Medical Systems, Inc, Tucson, AZ) with a monoclonal antibody against Smad4 (sc-7966 Santa Cruz Biotechnology, Santa Cruz, CA). Separate immunostaining for nuclear and cytoplasmic reactivity was scored on a scale based on the percentage of positive cells. (negative = 0-10% positive cells and positive = 20-100% positive cells). The staining pattern was correlated with standard histologic and prognostic variables.

Results: Smad4 nuclear expression was lost in 95/126 (75%) on NSCLC. The difference in Smad4 nuclear expression loss in SCC (88%) and AC (84%) vs BAC (44%) was significant (p<0.0001). Smad4 nuclear expression loss in high grade tumors (85%) versus low grade tumors (68%) reached near significance (p<0.06). Loss of SMAD4 nuclear expression correlated with a worse overall survival in SCC (p=0.03) but not for AC and BAC. Smad4 cytoplasmic expression was lost in 39/126 (31%) of NSCLC. The difference in Smad4 cytoplasmic expression loss in SCC (36%) and AC (43%) vs BAC (6%) was significant (p=0.001). Cytoplasmic Smad4 expression did not correlate with tumor grade. Loss of SMAD4 cytoplasmic expression correlated with a worse overall survival in SCC (p = 0.02) but not for AC or BAC. There was no correlation between SMAD4 nuclear or cytoplasmic expression and tumor stage or lymph node status.

Conclusions: Loss of both nuclear and cytoplasmic Smad4 protein expression occurs frequently in NSCLC. Smad4 expression loss is different in BAC vs other types of NSCLC. Loss of nuclear and cytoplasmic SMAD4 may be a useful predictor of overall survival in SCC, but not in AC or BAC. Further study of Smad4 expression in NSCLC appears warranted.

1465 Comparative Telomere Analysis of Pulmonary Benign Metastasizing Leiomyoma and Uterine Leiomyoma

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Background: Benign metastasizing leiomyoma (BML) is a rare tumor arising in perimenopausal women characterized by multiple benign-looking smooth muscle tumors usually involving the lungs; however, its nature remains unknown. Since benign tumors tend to maintain long telomeres and malignant tumors lose telomere length, we used telomere-specific fluroscence in situ hybridization (FISH) probes to analyze telomere length in three pulmonary BML and associated uterine leiomyomas from two patients.

Design: One case had two pulmonary tumors and one uterine tumor; the other case had one pulmonary tumor and one uterine tumor available for analysis. Telomere length was detected in each tumor using Cy3-labelled FISH probes (NH_3-CCCTAACCCTAACCCTAA-COOH) specific for the mammalian telomere repeat sequence and was measured as previously established. Routine histology and immunohistochemistry (including Ki-67, ER and PR) were performed for confirmation.

Results: Both pulmonary BML cases displayed similar features to uterine leiomyomas (bland histology, no mitosis, very low Ki-67 index, ER and PR nuclear positivity). The two pulmonary tumors of the first case demonstrated long telomere lengths similar to the uterine leiomyoma of the same patient. In the second case, one single pulmonary tumor demonstrated very long telomere lengths also similar to the uterine tumor of the same patient.

Conclusions: Malignancy is usually associated with shortened telomeres. In this study, we demonstrated that the long or very long telomere lengths in both cases of pulmonary BML are similar to the uterine counterparts, characteristic of a benign tumor. Our study supports the notion that benign metastasizing leiomyoma and benign uterine leiomyoma are derived from the same origin.

1466 Expression of STAT3 and STAT5 in Non-Small Cell Lung Cancers

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Background: The signal transducers and activators of transcription (STAT) proteins belong to a family of transcription factors that regulate cellular proliferation, differentiation, signaling and survival. The expression of specific STATs has been linked to oncogenesis. The expression of STAT3 and STAT5 in non-small cell lung cancers (NSCLC) has not been extensively studied and its prognostic significance is currently not known.

Design: Formalin-fixed, paraffin-embedded sections from 120 NSCLC including 42 squamous cell carcinomas (SCC), 44 adenocarcinomas (AC), and 34 bronchioloalveolar carcinomas (BAC), were immunostained by automated methods (Ventana Medical Systems, Inc, Tucson, AZ) using monoclonal antibodies to STAT3 and STAT5 (sc8019 and sc-836 Santa Cruz Biotechnology, Santa Cruz, CA). Staining was semiquantitatively assessed. STAT3 and 5 nuclear and STAT3 cytoplasmic loss of expression was defined as: 0 to 10% expression. STAT5 cytoplasmic loss of expression was defined as 0 to 80% expression. STAT3 and 5 nuclear and cytoplasmic expression loss was correlated with standard pathologic and clinical prognostic factors and disease outcome.

Results: For all 120 NSCLC, loss of staining for nuclear STAT3, cytoplasmic STAT3, and nuclear STAT5 and cytoplasmic STAT5 were 73%, 36%, 28%, and 52%, respectively. The difference in STAT3 nuclear expression loss in SCC (80%) and AC (84%) vs BAC (44%) was significant (p<0.001). The difference in STAT5 nuclear expression loss in SCC (33%) and AC (34%) vs BAC (6%) was significant (p=0.007). Loss of expression of nuclear STAT3 and STAT5 and cytoplasmic STAT3 and STAT5 did not correlate with tumor grade, stage of disease, and lymph node status. Loss of expression of cytoplasmic STAT5, but not nuclear STAT3 or STAT 5 or cytoplasmic STAT3 was associated with a significantly worse overall survival (19% vs. 40%, p=0.01).

Conclusions: The loss of STAT3 and STAT5 nuclear expression appears to differentiate BAC from SCC and AC. STAT5 cytoplasmic expression loss may be an adverse prognostic factor for NSCLC. These results suggest that the expression of STAT proteins appears to play a role in NSCLC disease biology clinical progression and is worthy of further study.

1467 Different Alternative Splicing Expression and Plasma Detection of hTERT mRNA Transcripts in Non-Small Cell Lung Cancer Patients

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Background: Human telomerase reverse transcriptase (hTERT), the catalytic subunity of telomerase - a marker of cell immortalization - is upregulated in up to 80-90% of tumors, including non-small cell lung cancer (NSCLC). Little is known, however, on hTERT gene regulation in NSCLC, as well as on the role of assessing cell-free plasma circulating hTERT mRNA for tracing these tumors.

Design: We investigated by both RT-PCR and *real-time* quantitative PCR the prevalence and functional implications of several alternative mRNA splicing isoforms, including total hTERT mRNA, full-length $\alpha+\beta$ transcript and α, β, $\alpha\beta$ and γ deletions, in primary tumors of 60 (47 males, 13 females) stage I-III NSCLC patients (33 adenocarcinomas and 27 squamous cell carcinomas). Thirty-four plasma samples from the same series of patients were also evaluated for the presence of total hTERT mRNA.

Results: We detected total hTERT mRNA in 45/60 (75%) tumors, with full-length ($\alpha+\beta$) transcript and α, β, $\alpha\beta$ and γ deletions occurring in 27/60 (45%), 12/60 (20%), 34/60 (57%), 16/60 (27%) and 20/60 (33%), respectively. Normal lung tissue was virtually devoid of any hTERT mRNA transcript. Alpha (44%) and especially γ (22%) deletions were associated with reduced amounts of full-length ($\alpha+\beta$) transcript, a marker of enzyme activity, whereas the opposite was for total hTERT mRNA (93%) and β (96%) and $\alpha\beta$ (56%) deletions. Total hTERT mRNA was detected in the plasma of 4/34 (12%) tumor-positive patients, but in none of 10 plasma samples of healthy volunteers. No association was found between any type of hTERT mRNA and clinicopathological variables of the patients' population for either primary tumors or plasma samples.

Conclusions: The hTERT gene is transcriptionally and post-transcriptionally regulated in NSCLC. Different alternative splicing variants occur in NSCLC, probably related to the fine tuning of telomerase activity in cancer cells. Cell-free circulating hTERT mRNA is detectable in a minority of patients owing to severe degradation, but is likely to be released from tumor cells because it is consistently absent in healthy volunteers.

1468 Gene Expression Analysis in Sarcoidosis – Pro-Survival and Anti-Apoptotic Signaling

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Background: Sarcoidosis is a systemic autoimmune disease based on a T-helper and epitheloid cell granulomatous reaction against unknown antigen(s). A genetic predisposition for sarcoidosis is supposed by studies in twins, and by geographical and racial distribution studies.

Design: Total RNA was isolated from lung lavage cells of at least five slow onset sarcoidosis patients as well as five acute sarcoidosis patients (loefgren syndrome), pooled, transcribed into cDNA, and hybridized on UNI-Gene II filter sets (76000 cDNAs) to analyze gene expression. The results from the cDNA array were confirmed for selected genes by RT-PCR and quantitative Taqman-PCR. To investigate the gene expression pattern of single sarcoidosis patients a small cDNA microarray was prepared consisting of 300 IMAGE-clones and about 2000 cDNA clones obtained from a suppression subtractive hybridization experiment (SSH-library) using mRNA of affected lymph nodes of sarcoidosis patients and inconspicuous active lymph nodes.

Results: We found an upregulation of genes involved in different proliferation pathways (PI3K–Akt2, FABP-PPARD-Akt2, STAT3) mediating pro-survival signals. The expression of genes of the extrinsic and intrinsic apoptosis pathways point to anti-apoptotic signaling mechanism. Differences in gene expression levels between slow onset sarcoidosis and loefgren syndrome (acute sarcoidosis) were found mainly within the HLA-system, whereas the expression pattern otherwise was similar.

Conclusions: Enhanced proliferation and inhibited apoptosis results in increase, accumulation, and prolonged survival of antigen-primed T-lymphocytes and alveolar macrophages in slow onset sarcoidosis as well as loefgren syndrome. This most probably results in disease activity. The mechanisms of counter regulation in cases of spontaneous resolution will be elucidated by small cDNA-array. The different courses of this disease (acute, slow-onset, fibrosis, resolution) might be due to differences in antigen presentation and processing.

1469 Complex Chromosomal Aberrations in Pulmonary Adenocarcinomas Detected by Array-CGH

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Background: Recently we were able to prove that chromosomal aberration are very early events in tumorigenesis of lung adenocarcinomas. Even small preneoplastic lesions like low grade atypical adenomatous hyperplasia and columnar bronchiolar cell dysplasia, comprising no more than a few hundred cells, were characterised by numerical as well as structural chromosomal changes. In this combined study by chromosomal as well as arrayCGH we have tried to gain additional insights into the structure of chromosomal aberrations.

Design: DNA was isolated from frozen tissue by standard proteinase K digestion and phenolization. Chromosomal aberrations were detected by conventional chromosomal CGH and array CGH, performed on a 12k BAC (Bacterial Artificial Chromosome) array representing a 1Mb resolution for the whole genome and a tiling path, that means a series of overlapping clones providing a resolution of about 76kb, for 9 chromosomes.

Results: Up to now nine adenocarcinomas have been successfully analysed by CGH and arrayCGH. In general arrayCGH confirmed the results of chromosomal CGH, however, additional complexity was detected by array CGH. While CGH suggested clear loss of 9p, arrayCGH revealed that these lost regions were interrupted by retained sequences. The same accounts for loss of 3p- and gains of 17q, two other early aberrations in the development of adenocarcinomas.
Conclusions: In this study we have shown that the picture that emerges from classical karyotypes and chromosomal CGH analysis is oversimplifiying the nature of chromosomal aberrations. Our data demonstrate, that losses of whole chromosome arms or chromosomes are repeatedly interrupted by retained or even gained sequences, indicating that loss of this region might be preceded by inversions or other rearrangements. This is even true for those chromosomal aberrations which are the most early events in adenocarcinomas already found in the adenocarcinoma precursor lesions.

1470 Chondromas in Carney Triad Are Distinct from Pulmonary Hamartomas

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Background: Carney triad is a syndrome of epithelioid gastric stromal tumors, pulmonary chondromas, and extra-adrenal paragangliomas that usually affects young women. This study characterized the pathologic features of pulmonary chondromas in the syndrome and compared them with those of pulmonary hamartomas.
Design: Forty-one patients with pulmonary chondroma(s) as a component of Carney triad were identified. For comparison, each chondroma was temporally matched to three pulmonary hamartomas surgically excised at the Mayo Clinic within the same five-year interval. Clinical and pathologic records were reviewed to determine demographics, size, number and location of tumors. H&E-stained sections were evaluated in a blinded fashion on a scale from 0-4 + for the presence of the following characteristics: cartilage, fat, smooth muscle, fibromyxoid stroma, bone, calcification, entrapped epithelium, goblet cells, chronic inflammation and the interface of the tumors with the adjacent parenchyma. Characteristics of chondromas and hamartomas were compared using the chi-square test for categorical factors and the Wilcoxon rank-sum test for ordinal factors. All tests were two-sided, with p-values less than 0.05 considered statistically significant.
Results: Patients with chondromas were mostly women (M:F = 5:36), mean age of 24.8 years (SD ± 9.27 and range 12-44). Hamartomas occurred mostly in men (M:F = 74:49), mean age 59.0 years (SD 11.3, range 11-84). Chondromas were larger than hamartomas (mean 2.8 cm vs 1.7 cm, p 0.0001) and more often multiple (mean 2.1 vs 1.0, p 0.0001). They had a higher mean score for cartilage (3.3 vs 2.8, p0.0171), bone (0.9 vs 0.1, p <0.0001), and calcification (0.7 vs 0.1 p <0.0001). Chondromas had lower mean scores for fat (0.1 vs 1.1, p<0.0001), smooth muscle (0 vs 0.7, p <0.0001), fibromyxoid stroma (1.0 vs 1.7, p<0.0001), entrapped epithelium (0.2 vs 1.4, p<0.0001), goblet cells (0 vs 0.2, p<0.0001), and inflammation (0.2 vs 1.1, p<0.0001). Chondromas had a fibrous capsule with a sharp interface with pulmonary parenchyma in 37 (90%), interface was not evaluable in three (7%), and absent in one (2%). A fibrous capsule was suggested in only one hamartoma.
Conclusions: Chondromas in Carney triad were morphologically distinct from pulmonary hamartomas. Chondromas were sharply circumscribed, encapsulated tumors composed predominantly of benign hyaline cartilage and bone, with foci of calcification. They lacked internal epithelium and other mesenchymal elements.

1471 Immunoexpression of FRAP Is Associated with Poor Survival in Non-Small Cell Lung Cancer

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Background: FRAP (FKBP rampamycin-associated protein) is a 289-kilodalton protein member of the new kinase family that participate in the chromosome maintenance and repair, cell cycle progression and cell cycle checkpoints. Inactivation of FRAP reduces DNA synthesis, tumor proliferation, tumor size and p70/S6 kinase activity in pancreatic cell lines. Overexpression of FRAP increases cell survival, suppressing cell death in apoptosis-susceptible cells. The goal of this study is to determine if FRAP expression correlates with prognosis in non-small cell lung cancer (NSCLC).
Design: Microarray paraffin-embedded sections from 108 cases of lung carcinoma were immunostained with polyclonal antibody against a recombinant protein corresponding to amino acid 1920-2185 mapping an internal region of FKBP-rampamycin associated protein (FRAP) (1:400, Santa Cruz Biotechnology, Inc antibodies H-266) using an enhanced sensitivity avidin-biotin peroxidase technique. Staining was graded: 0=0-5%, 1=6-25%, 2=25-50%, 3=50-75%, 4=>75%. Results were correlated with survival log-rank test and Kaplan-Meier survival plots.
Results: Mean age of group was 60.3 years; 41% were females (mean age: 59 years) and 59% were males (mean age: 61years). Twenty-four of 44 female patients had adenocarcinoma (55%), 14 had squamous cell carcinoma (32%), and 6 had undifferentiated carcinoma (13%). Twenty-five of 64 males had adenocarcinoma (39%), 33 had squamous cell carcinoma (52%) and 6 had undifferentiated carcinoma (9%). Fifty-two percent had Stage I disease, 23% Stage II, 20% Stage III, and 5% Stage IV disease. Mean survival of females was 118 weeks, and of males was 107 weeks; survival with adenocarcinoma was 102 weeks and with squamous cell carcinoma was 155 weeks. Immunoexpression of FRAP was seen in 20% of tumors. Fifty-one percent of patients with no expression of FRAP were alive compared to 23% with FRAP expression (p=<0.010).
Conclusions: Overexpression of FRAP in NSCLC is associated with significantly reduced survival. FRAP is an upstream regulator of S6 kinase and implicated in regulation of p27 and p21; it may affect tumor progression and survival through these cell cycle regulators, by increasing cell survival and suppressing apoptosis. Anti-FRAP therapeutic modalities may be beneficial in these patients.

1472 Molecular Markers of Prognosis in Stage I and II Non-Small-Cell Lung Cancer. A Study Using Tissue-Arrays

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Background: The aim of our study was to investigate early stages of non-small-cell-lung cancer to determine molecular markers that may distinguish groups of tumors with different biological behaviour. Cell-cycle checkpoints, growth factors and angiogenesis, gene repair and apoptosis are complex molecular pathways that play a role in tumor progression.
Design: Surgically resected specimens from 112 patients (107 male and 5 female) with mean age 66 were obtained from our files. 95 cases were stage I NSCLC and 17 were stage II tumors.3 tissue microarrays (TMA) with two 0.6 mm cores from each tumor were constructed.TMA sections were immunostained using antibodies against CDK1, CDK2, Cyclins A, E, D1 and D3, pRb, p16, p21, p53, MIB1, MLH1, MSH2, Topoisomerase IIa, survivin, caspase3a, VEGF, Her2/neu, EGFr, E-Cadherin, Cathepsin D, gamma-catenin, and hybridized with APOPTAG (TM).Univariant and multivariant analyses (Cox proportional hazards) were performed.
Results: Mean overall survivall in our series was 51 months. Multivariate statistical analysis showed that overexpression of cyclin E and CDK2 were associated (p<0.05) with patients overall survival, with age-adjusted risk of 2,1. Only Cyclin E was associated with worse prognosis when we analyzed disease free survival, with age-adjusted ratio of 1,5. All other molecular markes did not correlated with survival.
Conclusions: Our study shows that cyclin E expression shows the stronger statistical association with overall survival and disease free survival in early stages of NSCLC. Other prognostic markers may play a role in latter stages of tumor progression.

1473 Bronchioloalveolar Adenocarcinomas Versus Other Well Differentiated Primary Pulmonary Adenocarcinomas: Unique Immunohistochemical Phenotypes Identified by High Throughput Microarray

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Background: Bronchioloalveolar adenocarcinomas (BACs) of lung are reported to carry better prognoses than other adenocarcinomas. Strict adherence to WHO criteria for the diagnosis of BAC results in retention of less than 20% of cases previously diagnosed as BAC in the category. We compare immunohistochemical reactivity of 30 lesions originally classified as BACs (10 true WHO BACs and 20 carcinomas reclassified as non-BACs) with antibodies to Bcl-2, Bcl-6, EGFR, Ki-67, P16, and P53 using a high throughput tissue microarray.
Design: Thirty carcinomas originally diagnosed as BAC at ENH between 1995 and 2004 were reviewed by two pathologists (CLS, CDS) and reclassified by WHO criteria as either BAC or non-BAC (N-BAC) neoplasms. After reclassification, tissue microarrays were created using multiple 0.6 mm cores of paraffin-embedded carcinoma and non-neoplastic lung from each resection. Slides from the arrays were then studied by immunohistochemistry with antibodies to Bcl-2, Bcl-6, EGFR, Ki-67, P16, and P53. The slides were reviewed (blinded) for immunopositivity by the same pathologists. Results were statistically analyzed.
Results: Statistical analysis showed a significantly (p=0.007) increased liklihood of a carcinoma from the original group being reclassified as a N-BAC if the carcinoma showed P16 immunoreactivity (odds ratio [OR] 12). Likewise, tumors had an OR of 9.3 for N-BAC vs BAC when immunoreactive for Bcl-2 (p=0.015) and an OR of 7 when diffusely immunopositive for P53 (p=0.045). A nonsignificant trend (p=0.122) for N-BACs to exhibit higher Ki-67 labeling indices (greater than 2%) (OR 4.3) was noted.
Conclusions: BACs and other low-grade pulmonary adenocarcinomas vary in their patterns of immunoreactivity. N-BAC low-grade adenocarcinomas are statistically significantly more likely to exhibit immunoreactivity for P16, BCL-2 and P53 than are BACs when classified by WHO criteria. These differences in immunoreactivity may prove to be useful diagnostic adjuncts in the histologic separation of BACs from other low-grade adenocarcinomas and may provide clues to possible differences in patterns of carcinogenesis.

1474 D2-40 Is Expressed on the Luminal Surface of Pulmonary Airspaces in Normal Developing and Adult Lung but Is Lost in Conditions Associated with Intra-Alveolar Infiltrates

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Background: The D2-40 antigen is a glycosylated sialomucin that is strongly expressed by lymphatic endothelial cells. Recently we have also observed expression of this antibody on the luminal surface of pulmonary airspaces in sections of lung. The aim of the study was to assess the expression of D2-40 antigen in normal lung development and in various pathological conditions.
Design: Formalin-fixed, paraffin-embedded non-autolyzed lung tissue was selected from 34 fetal/neonatal autopsy cases ranging in gestational age from 13 to 41 weeks and from 10 adult cases. In the fetal/neonatal group, 24 cases were histologically normal, whereas 10 were abnormal (2 pneumonia, 2 pulmonary hypoplasia, 2 intra-alveolar hemorrhage, 2 meconium aspiration, 1 patchy atelectasis, 1 pulmonary interstitial emphysema). In the adult group, 5 cases were histologically normal, whereas 5 showed pneumonia. Immunohistochemical staining was performed on all cases using antibody to D2-40.

Results: All cases of normal fetal/neonatal and adult lung showed diffuse strong expression of D2-40 on the luminal surface of the cells lining the bronchioles and alveoli, whereas the lining of the bronchi did not express D2-40. Moreover, D2-40 was also strongly expressed by lymphatic endothelial cells in all fetal/neonatal and adult cases. In all cases where there was an abnormal infiltrate or foreign material within the airspaces, which included cases of pneumonia, intra-alveolar hemorrhage and meconium aspiration, expression of D2-40 was lost. D2-40 positivity was preserved in cases where there was no intra-alveolar infiltrate (pulmonary hypoplasia, patchy atelectasis and pulmonary interstitial emphysema).
Conclusions: Since production of the D2-40 antigen occurs as early as 13 weeks gestation which is prior to the production of surfactant which begins at approximately 20-24 weeks gestation, this indicates that D2-40 is produced by type I pneumocytes or their precursors. Expression of D2-40 in alveoli is lost when there is damage to the alveolar lining cells by an abnormal infiltrate or by foreign material. These results suggest that D2-40 may have a cell membrane protective function.

1475 Comparative Immunohistochemical Analysis of Mucoepidermoid and Adenoid Cystic Carcinomas of Pulmonary and Salivary Gland Origin

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Background: Pulmonary mucoepidermoid carcinoma (PMEC) and pulmonary adenoid cystic carcinoma (PACC) are thought to resemble their salivary gland counterparts. However, due to their rarity, the immunophenotype of PMEC and PACC is not well established. The goal of this study was to define the immunophenotype of PMEC and PACC and potentially identify specific markers useful for the separation of primary pulmonary from metastatic salivary gland tumors.
Design: A tissue microarray (TMA) block was constructed of cores from 14 PMEC, 14 PACC, 12 salivary gland mucoepidermoid carcinomas (SGMEC), and 13 salivary gland adenoid cystic carcinomas (SGACC). 12 cores of normal salivary gland tissue and 9 cores of normal bronchial submucosal glands were included as controls. The immunohistochemical markers CK7, CK20, TTF-1, K903, p63, smooth muscle actin (SMA), CD117a were applied to TMA deparaffinized sections according to the manufacturer instructions. Each core was scored for intensity (0 to 4+) and distribution (0 to 3+) of staining.
Results: The majority of PMEC and PACC, similar to SGMEC and SGACC, were immunoreactive with CK7, while they were non-reactive with CK20 and TTF-1 (see table). The squamoid areas of PMEC and SGMEC were highlighted with K903 and p63. A distinct pattern of staining with K903 decorating the luminal epithelial cells and with p63 and SMA decorating basal/myoepithelial cells was observed in both lung and salivary gland adenoid cystic carcinomas.
Conclusions: PMEC and PACC display similar to their salivary gland counterparts' immunophenotype. They are immunoreactive with CK7, K903, and p63, and negative with TTF-1 and CD20 in the majority of cases. This similarity precludes separation of pulmonary from salivary gland origin of these tumors with current readily available immunohistochemical markers, but may be helpful in their separation from the conventional pulmonary adenocarcinomas.

Immunohistochemistry results

	CK7	CK20	TTF-1	K903	p63	SMA	CD117a
PMEC	14/14	0/14	0/14	12/14	9/14	0/14	9/14
PACC	13/14	0/14	0/14	11/14	12/14	11/14	11/14
SGMEC	8/9	2/8	0/10	9/9	10/11	0/9	1/9
SGACC	10/10	0/10	0/10	8/9	10/10	9/9	10/10

1476 D2-40 Is a Novel New Marker of Malignant Mesothelioma (MM): Tissue Microarray Study of 45 MM Versus 409 Lung Carcinomas and Primary Non-Mesothelial Neoplasms of the Pleura and Chest Wall

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Background: The differentiation of MM from other histologically similar pleural and pulmonary neoplasms has important clinical ramifications, but can be difficult. D2-40 is a novel monoclonal antibody that reacts with a fixation-resistant epitope on lymphatic endothelium. D2-40 expression has also been observed in mesothelial cells and this sialoglycoprotein has been proposed as a potential marker for MM (Mod Path 2004; 17 (suppl 1): 335A). To further evaluate D2-40 as a diagnostic marker for MM, we evaluated its expression in tissue microarrays of MM, lung carcinomas and non-mesothelial primary neoplasms of the pleura and chest wall.
Design: Tissue microarrays (TMAs) were prepared from 45 MM, 404 lung carcinomas [351 non-small cell lung carcinomas (NSCLC), 53 small cell (SCC)], and 5 non-mesothelial primary neoplasms of the pleura and chest wall [1 benign solitary fibrous tumors (SFT), 3 malignant SFT, 1 desmoid tumor]. Immunohistochemical staining with a monoclonal antibody to D2-40 (1:75, Signet Laboratories) was performed on the TMAs. Staining results were scored by percentage of tumor cells staining (1 = <33%, 2 = 33-66%, 3 = > 66%) and staining intensity (0 = negative, 1 = weak, moderate = 2, strong = 3).
Results: 32/45 (71%) MM were epithelial and 13/45 (29%) were sarcomatous. 100% of epithelial MM showed moderate or strong granular membranous and cytoplasmic staining for D2-40 in 70-100% of cells. None of the sarcomatous MM expressed D2-40. Weak reactivity for D2-40 was observed in 44/340 (13%) NSC, all of which were squamous cell carcinomas. One half of all squamous cell carcinomas were immunoreactive for D2-40 (44/88 = 50%). 1/11 (9%) large cell neuroendocrine carcinomas showed very focal weak staining. No SCC, SFT or desmoid tumor expressed D2-40.
Conclusions: D2-40 expression is useful for differentiating epithelial MM from non-mesothelial primary neoplasms of the pleura and chest wall and from most NSCLC. A weak reaction to D2-40 may also be seen in some squamous cell carcinomas of the lung. D2-40 does not help in the differential diagnosis of sarcomatous MM.

1477 Comparison of DAX-1 and Androgen Receptor Expression in Small Cell Carcinoma and Carcinoid Tumors of the Lung

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Background: The DAX-1 (**d**osage-sensitive sex reversal, **a**drenal hypoplasia critical region, on chromosome **X,** gene **1**) gene is a member of the nuclear hormone receptor superfamily with important roles in gonadal and adrenal differentiation. Mutations in DAX-1 cause X-linked adrenal hypoplasia congenita associated with hypogonadotropic hypogonadism, but limited information is available about its functions in other organs. Recent reports suggest an adverse relationship between DAX-1 expression and prognosis in ovarian cancer, possibly through alterations in steroid production (*Cancer Sci 2003; 94:980-5),* and a possible relationship to failure of endocrine therapies in breast cancer *(Breast Cancer Res 2004; 6: R140-8).* Expression of DAX-1 and AR in neuroendocrine carcinomas has not been previously reported, and was the subject of this investigation.
Design: Tissue microarrays were constructed from 53 small cell carcinomas (SCC) and 54 typical carcinoid tumors (CAR), using three samples of formalin-fixed paraffin-embedded tumor tissue from each case. Immunohistochemistry for DAX-1 (1:100, Santa Cruz Biotechnology) and AR (1:40, BD Pharmingen) was performed on recut sections of the microarrays. The percentage of tumor cells with nuclear staining was scored as 1 = <33%, 2 = 33-66%, 3 = >66% and nuclear staining intensity was scored as 0 = negative, 1 = weak, 2 = moderate and 3 = strong.
Results: Diffuse (66-100% of tumor cells) moderate or strong nuclear staining for DAX-1 and AR was observed in 51/53 (96%) and 49/53 (92%) of the SCC, respectively. Although all 54 of the CAR demonstrated diffuse moderate or strong nuclear immunoreactivity for DAX-1, only 54% of the CAR showed nuclear staining for AR, with weak positivity in approximately 33% of tumor cells.
Conclusions: SCC strongly express both DAX-1 and AR, while CAR express DAX-1 strongly and AR weakly. These findings suggest the possibility of new therapeutic hormonal treatment modalities for these neoplasms

1478 Significance of b-Catenin Expression in Non-Small Cell Lung Cancers

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Background: b-catenin is a member of a group of cytoplasmic protein molecules taking part in cell-cell adhesion. Decreased b-catenin expression in non-small cell lung cancers (NSCLC) has been associated with shortened patient survival. Decreased b-catenin expression has been reported in squamous cell carcinomas and adenocarcinomas of the lung, particularly in poorly differentiated tumors of both histological types. The current study was performed to expand upon this knowledge base by evaluating 340 NSCLC with 5-year clinical follow-up for b-catenin expression.
Design: Tissue microarrays (TMA) comprised of triplicate punch samples from 340 NSCLC were stained by immunohistochemistry for b-catenin (1:300, BD Transduction, San Diego, CA). Staining was evaluated based on percentage of tumor cells stained (1 = <33%, 2 = 33-66%, 3 = >66%) and staining intensity (0 = negative, 1 = weak, 2 = strong). For each tumor, a mean value was calculated from the individual ratings of the three punch samples, and this mean value was used for statistical analysis. Patient survival and staging data was sought for all of the samples. Statistical analysis was performed using Kaplan-Meier curve analysis and Pearson correlation.
Results: 60-100% of the tumor cells revealed positive staining with b-catenin. Staining was membranous and granular, with only a minority of tumor cells also showing cytoplasmic staining (<10%). No nuclear staining was seen. Clinical information for our database of 340 NSCLC revealed an average patient age of 65 yrs., with an average follow-up of 50.5 months. 57% of the NSCLC were adenocarcinomas, 26% squamous cell carcinomas, and 17% large cell carcinomas. Stage I and II tumors represented 65% and 17% of the cohort, respectively. We found no correlation between intensity of staining for b-catenin and survival. Likewise, there was no correlation between b-catenin staining and either tumor stage or cell type.
Conclusions: To date, this is the largest series of NSCLC evaluated for b-catenin expression. Expression of b-catenin does not appear to be prognostically significant in NSCLC.

1479 Expression of Markers of Cell Growth and Survival in Large Cell Neuroendocrine Lung Carcinoma (LCNC) of the Lung

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Background: Large cell neuroendocrine carcinoma (LCNC) of the lung is a rare and aggressive type of neuroendocrine lung cancer. Because of its infrequent occurrence, little has been reported regarding the expression of markers of cell growth and survival in these tumors. To address this issue, we investigated the expression of beta-catenin, FKHR, FRAT2, DAX-1, and p-AKT in a tissue microarray of 11 cases of resected LCNCs.
Design: Eleven surgically resected LCNCs were identified from a database of 5418 lung cancers (0.2%). A tissue microarray was created with triplicate punches from formalin-fixed, paraffin-embedded tissues from the resection specimens. Recut sections were immunostained for beta-catenin (1:300 BD Transduction), FKHR (1:40 Cell Signaling), FRAT2 (1:50 Santa Cruz Biotechnology), DAX-1 (1:100 Santa Cruz Biotechnology), and p-AKT (1:50 Cell Signaling) on a Dako Autostainer using the Envision + Labelled Polymer Universal Kit (DakoCytomation, Carpenteria, CA). Intensity of immunopositive tumor cells was scored as weak, moderate, or strong.

Results: Beta-catenin, FKHR, FRAT-2, DAX-1, and p-AKT were expressed in a majority of cells in all 11 cases. Beta-catenin was strongly expressed in 2 cases (18%), moderately expressed in 3 (27%), and weakly expressed in 6 (55%). FKHR was strongly expressed in 4 cases (36%), moderately expressed in 4 (36%), and weakly expressed in 3 (27%). DAX-1 was strongly expressed in 5 cases (45%) and moderately expressed in 6 cases (55%). P-AKT was strongly expressed in 1 case (9%), moderately expressed in 2 (18%), and weakly expressed in 8 (73%). 8 of the 11 cases (73%) were positive for FRAT2 and 3 were negative.
Conclusions: Our findings indicate that these proteins involved in cell growth, proliferation, and survival (beta-catenin, FKHR, FRAT2, DAX-1, and p-AKT) are expressed by most LCNCs and may play a role in their development. These proteins may serve as potential targets for therapy in these aggressive cancers.

1480 Expression of Anti-Oxidant Enzymes in Surgically Resected Small Cell Carcinoma (SCC) of the Lung

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Background: Reactive oxygen species from tobacco smoke and environmental pollutants, and host-protective anti-oxidant enzymes, are believed to be important in the pathogenesis of non-small cell lung cancer. Pharmacologic manipulation of anti-oxidant enzymes has been proposed as a potential prevention or therapy for some types of cancers, including non-small cell lung carcinomas. We investigated the frequency of anti-oxidant enzyme expression in SCC to determine if these enzymes may also play a role in the pathogenesis of SCC.
Design: Formalin-fixed, paraffin-embedded sections from 19 surgically resected SCC were immunostained for the following anti-oxidant enzymes: cyclooxygenase-2 (COX-2, Cayman Chemical, 1:150), glutathione-s-transferase (GST pi, DakoCytomation, 1:100), and inducible nitrous oxide synthase (iNOS, Neomarkers; 1:40) using Envision + Labeled Polymer Detection kit for mouse or rabbit (DakoCytomation). Immunoreactivity for these proteins was assessed as negative or positive and compared with patient sex, age and smoking history.
Results: Patients consisted of 8 females, 11 males with average of 69.6 pack-year smoking history. Immunoreactivity for anti-oxidant enzymes was identified in SCC as follows: COX-2 = 16/19 (84%), GST pi = 14/19 (74%) and iNOS = 18/19 (95%). 3/3 SCC negative for COX-2 were in males with an average age of 50.3 years as compared to an average age of 67.4 years for males with SCC positive for COX-2. 3/5 of SCC negative for GST pi were in females and 1/1 SCC negative for iNOS was in a male.
Conclusions: Anti-oxidant enzymes COX-2, GST pi and iNOS are expressed in the majority of resected SCC in our series, indicating a possible role for these enzymes in the development of SCC. Although the numbers are small, there is a suggestion that males with SCC that lack COX-2 expression may develop their cancers at a younger age. Further investigation is needed to determine if these findings represent a greater susceptibility to damage from reactive oxygen species in this subset of SCC patients.

1481 Chest Radiograph Stage, Racial and Gender Differences in Granuloma Burden and Distribution in Sarcoidosis

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Background: Pulmonary sarcoidosis is a granulomatous disease of uncertain etiology. Diagnosis is supported by bronchoscopic biopsy and assessment of disease burden is based on clinical and radiographic data. The purpose of our study is to determine if bronchoscopic biopsy results, in patients with sarcoidosis, correlate with (1) gender, (2) race, (3) age, or (4) chest radiograph stage at presentation.
Design: We retrospectively studied 260 previously untreated patients with clinical sarcoidosis. All bronchoscopic biopsies were reviewed for clinical biopsy type (transbronchial vs. endobronchial), number of pieces, type of biopsy tissue present (peribronchial vs. alveolar), number and distribution of granulomas, and presence of necrosis. Biopsy results were analyzed in relation to patient age, gender, race, and chest radiograph stage, using general linear statistical models.
Results: Granuloma burden (the number of granulomas per biopsy piece) correlated with race (p=0.022), gender (p=0.010), and chest radiograph stage (p=0.0033). Granuloma burden also correlated with chest radiograph alveolar pattern (p=0.0033) and combined chest radiograph interstitial and alveolar pattern (p=0.0009). The distribution of granulomas within the biopsy (peribronchial vs. alveolar) correlated with chest radiograph stage (p=0.022 and p=0.012, respectively). Granuloma burden did not correlate with biopsy type (endobronchial vs. transbronchial), location of granulomas within the biopsy, or age. Granuloma necrosis was uncommon and did not correlate with race, gender, age, chest radiograph stage, type of biopsy, granuloma burden or granuloma distribution.
Conclusions: Granuloma distribution and granuloma burden reflect chest radiograph stage in patients with sarcoidosis. There are racial and gender differences in granuloma burden in patients with sarcoidosis. These differences may reflect the underlying pathology of sarcoidosis and support differences in therapeutic management.

1482 The Utility of C4d as a Diagnostic Adjunct in Collagen Vascular Disease Associated Pulmonary Fibrosis

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Background: Usual interstitial pneumonia (UIP) and non-specific interstitial pneumonia (NSIP) represent the two most common patterns of idiopathic interstitial pneumonia. These patterns are also seen in patients with collagen vascular disease, although morphologic assessment alone does not distinguish these cases from idiopathic disease. The use of immunoflourescence has demonstrated immunoglobulin deposition in some collagen vascular disease associated cases, suggesting immune-mediated pulmonary injury. We hypothesized that C4d, a stable component of complement activation, could distinguish collagen vascular disease associated pulmonary fibrosis from idiopathic cases.
Design: A total of 50 patients with established diagnoses of pulmonary fibrosis (21 NSIP pattern, 29 UIP pattern) with comprehensive autoimmune serological workup were retrieved from the Department of Surgical Pathology archives at Columbia University. Of the 50 patients, there were 24 patients with evidence of autoimmune disease. Immunohistochemistry for C4d was performed according to standardized procedures. The sections were interpreted by two pathologists and the consensus result reported. Statistical analysis was performed using the two-tailed Fisher exact test.
Results: C4d deposition was seen within alveolar interstitium in 58% (14 of 24) of collagen vascular associated cases and 31% (8 of 26) of idiopathic cases; this difference was not statistically significant (p= 0.09). Among the cases with UIP pattern, C4d deposition was present in 7 of 10 (70%) patients with collagen vascular disease and 6 of 19 (32%) with idiopathic disease; this difference was not significant (p=.06). Similarly among NSIP cases, 8 of 14 (57%) patients with collagen vascular disease and 1 of 7 (14 %) with idiopathic disease had C4d deposition (p=.06).
Conclusions: Between both UIP and NSIP pattern cases, a greater proportion of C4d positive cases were seen in collagen vascular disease associated lung fibrosis; however, this difference did not achieve statistical significance. Therefore, the presence of C4d deposits was not significantly more common in collagen vascular disease associated IPF in our series.

1483 Epidermal Growth Factor Receptor (EGFR) Enhanced Expression in Chemoresistant Non-Small-Cell Lung Carcinoma (NSCLC) after Platinum-Based Therapy

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Background: EGFR is a 170kDa transmembrane glycoprotein with tyrosine kinase activity. At diagnosis, 40-80% of NSCLCs express EGFR. Novel therapeutic strategies for NSCLC patients include targeting EGFR. Biological parameters are mostly evaluated using tissue obtained at diagnosis. In lung cancer, data comparing EGFR expression for the same patient before and after platinum-based chemotherapy progression are lacking. The aim of the present study is to investigate intrapatient changes in EGFR expression.
Design: We analyze EGFR immunohistochemistry expression in specimens from NSCLC patients for whom tumor samples are available both at diagnosis and after progression to platinum-based therapy. These patients are included in an ongoing phase II pharmacodynamic study using Erlotinib in second-third line treatment, in which tumor biopsies are performed at study entry. Immunostainig is performed by EGFR monoclonal antibody (DakoCytomation). Tumors are considered EGFR-positive if any membrane staining is observed in at least 10% or more tumor cells.
Results: To date, eleven patients have been analyzed: eight men, three women; six large-cell carcinomas, three adencarcinomas and two squamous cell carcinomas. In the diagnostic samples, ten specimens (90.9%) were found to express EGFR. The EGFR-negative patient was male and had an adenocarcinoma. After platinum-based chemotherapy progression samples from all eleven patients expressed EGFR. Overall, the number of positive EGFR stained cells increased in the progression samples. Furthremore, there was a marked increase in activated serine/threonine protein kinase Akt expression in tumor progression samples.
Conclusions: NSCLC samples show increased expression of EGFR after progression following platinum-based chemotherapy. These data support considering EGFR a molecular target for new therapies.

1484 p63 Is a Useful Marker in Distinguishing Pleural Malignant Mesothelioma (PMM) from Pulmonary Squamous Cell Carcinoma (SCC) Involving Pleura

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Background: Distinguishing poorly differentiated SCC involving pleura from PMM can occasionally present a diagnostic challenge. Unlike pulmonary adenocarcinoma for which specific markers have been identified, there are no specific markers to distinguish SCC from poorly differentiated PMM that not uncommonly are negative for calretinin. Expression of CK5/6 by PMM is not helpful because the majority of SCC are also CK5/6 positive. Recently, expression of p63, a member of the p53 family, is reported in non-small carcinoma of the lung including 97% of SCC. We undertook the present study to evaluate the possible utility of p63 in differentiating SCC from PMM.
Design: Paraffin sections from 48 previously confirmed cases of PMM were stained with p63 antibody (NeoMarkers). All these cases were obtained from the authors' consultation files. Sections of multi-tissue control blocks were used as quality controls.
Results: None of the mesothelioma cases studied revealed immunoreactivity for p63. Nuclear staining of basal layer of squamous epithelium included in multi-tissue control sections was observed in all sections.
Conclusions: The expression of p63 by the vast majority of SCC and its absence in PMM may prove useful in distinguishing SCC from calretinin negative PMM.

1485 CD138 Is Not a Helpful Marker in Distinguishing Spindle Cell Adenocarcinoma (SCA) from Malignant Mesothelioma (MM): A Paraffin Immunohistochemical Study

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Background: Morphologic distinction between SCA involving pleura and MM is extremely difficult, especially in those cases in which the pattern of pleural involvement by SCA simulates MM (Pseudomesotheliomatous adenocarcinoma). Unlike epithelial MM, mixed and particularly sarcomatoid variant of MM can be negative for mesothelioma-associated antigens such as calretinin. In a recent study, all the SCA were found to be immunoreactive for CD138. Another recent study indicates that CD138 is consistently absent in MM. We undertook this study to evaluate the expression of CD138 in MM and its possible application in distinguishing SCA from MM.

Design: Forty-seven cases of morphologically, immunohistochemically, and clinically well characterized cases of MM were obtained from the authors' consultation files. Paraffin sections from all the cases were stained with anti-CD138 antibody (Clone B-B4, Serotec). Sections of multi-tissue control blocks were used as positive and negative controls.

Results: Expression of CD138 was identified in 13 of the 47 (28%) cases of MM including seven epithelial, one sarcomatoid, and five mixed variants. The expression ranged from strong membrane and cytoplasmic to paranuclear (Golgi) and brush border-like patterns. Cases with less than 5% staining of tumor cells regarded negative.

Conclusions: In contrast to a previously published study, our results indicate that CD138 is expressed in approximately 1/3 of MM and, therefore, it has a limited value in distinguishing MM from SCA.

1486 Epidermal Growth Factor Receptor (EGFR) Gene Amplification in Pleural Malignant Mesothelioma (MM): A Chromogenic In Situ Hybridization (CISH) Assay

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Background: Epidermal growth factor receptor (EGFR) is reportedly overexpressed in several solid tumors including MM. With the availability of anti-EGFR antibodies the accurate assessment of EGFR status has become an important test in many laboratories. Currently, in most centers, paraffin immunohistochemistry is the preferred method for assessing EGFR expression. Expression of EGFR protein has been previously reported in MM. However, to our knowledge, EGFR gene amplification status in MM has not been previously studied using CISH assay.

Design: Forty-five cases of well-characterized MM including 30 epithelial, 12 mixed and three sarcomatoid, were obtained from the authors' consultation file. EGFR gene amplification was analyzed by a CISH method using paraffin embedded tissue sections and EGFR probes as recommended by the manufacturer (Zymed Laboratories Inc.) Sections of a known positive case of high-grade Glioma were used as positive control.

Results: In 17 of the 45 cases (38%) multiple copies of the EGFR gene (average 6-7 signals) were identified, indicating gene amplification. The cases with amplified EGFR gene included 13 epithelial, three mixed, and one sarcomatoid subtypes. In the remaining 28 cases only 2 intranuclear dot-like peroxidase positive signals were present consistent with non-amplified gene.

Conclusions: Our study reveals EGFR gene amplification in a significant proportion of MM. The rate of EGFR gene amplification appears lower than EGFR protein overexpression. This finding suggests that in addition to gene amplification, other mechanisms may be involved in upregulation of EGFR protein. Therefore, EGFR immunohistochemistry may be a preferred method in selection of patients for anti-EGFR antibody treatment.

1487 Prolonged Survival in Malignant Mesothelioma: A Study of Sixteen Cases

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Background: Prolonged survival in diffuse MM is rare. We have studied 16 cases with survival in excess of 3 years, in order to evaluate clinico-pathological features which enhance survival.

Design: Sixteen MM cases with a survival range from 42.69 months to 74 months with a mean survival time of 48.46 months were evaluated for clinical and histopathological features. MM were classified according to the WHO classification [2004]. The following immunohistochemical and molecular markers were studied; EMA, p53, desmin, CD10. SV40 DNA sequences were studied by PCR analysis from paraffin embedded specimens and were sequenced. *P16/CDKN2A* and *RASSF-1A* genes was assessed by the method of methylation specific PCR [MSP] Occupational histories were evaluated by a group of asbestos epidemiological experts.

Results: The patients were 10 men and 6 women with an average age at diagnosis of 66.7 years. Asbestos exposure was identified in 9/11cases. Clinical and anatomical location and immunohistochemical profiles (EMA, desmin, CD10) were similar to mesothelioma with poor prognosis. All were characterized by small nodules (with a size less than 2 cm). Histological types comprised epithelioid 15 (including 1 pleomorphic) and one biphasic MM with osteocartilaginous differentiation. Except 2, all showed a prominent chronic inflammatory infiltrate.

Molecular positive results were as follows; SV40 (2), *P16/CDKN2A* (1), and *RASSF-1A*(2) methylation. There was no correlation between SV40, p53 expression, *P16/CDKN2A* and *RASSF-1A* methylation .

Conclusions: Favorable prognostic factors were small size(less than 2cm), epithelioid histological type, and prominent chronic inflammatory infiltrate. Immunohistochemical and molecular markers did not differ significantly from mesothelioma with poor prognosis.

1488 Immunophenotypic Profile of SV40-Induced Diffuse Malignant Mesothelioma (DMM) in Hamsters: Similarities with Human DMM

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Background: DMM is a deadly cancer of serosal membranes incurable with current therapies. Animal models of DMM are needed for investigation of specific pathways in DMM pathogenesis and to provide a preclinical system to test therapeutic interventions. SV40 is an oncogenic DNA virus known to cause tumors in laboratory animals, including DMM. However, phenotypic features of SV40-induced DMM are not known.

Design: Seventy Syrian golden hamsters 21 days of age were inoculated by the intraperitoneal route with SV40 (10^7 plaque-forming units/animal) and followed for 8 months or 12 months. Control animals of the same age were inoculated intraperitoneally with cell culture media. Tumors were excised, formalin-fixed and paraffin-embedded and sections stained with H and E. Eight tumors with histologic features of epithelial and biphasic DMM were immunostained for markers used in the differential diagnosis of human DMM. Staining for DMM markers Calretinin (1:100, DakoCytomation), HBME-1 (1:400, DakoCytomation), and CK 5/6 (1:100, Zymed), adenocarcinoma marker CEA (1:200, DakoCytomation) and epithelial markers positive in DMM and other tumors EMA (1:2000, DakoCytomation), AE1/AE3 (1:200, DakoCytomation) and CAM 5.2 (1:1000, Zymed) was performed using an Envision+ Labelled Polymer Universal Kit (DakoCytomation). Staining for DMM marker Mesothelin (1:400, Vision Biosystems) was performed using Mach3 Mouse Probe-Polymer Kit (Biocare Medical).

Results: Malignancies (DMM and sarcomas) developed only in animals infected with SV40 and not in controls, both in experiments held for 8 months (15/42, 36% vs. 0/32, 0%; p=0.001) and for 12 months (10/28, 36% vs. 0/46, 0%; p=0.001). Presence of SV40 tumor antigen in tumors was confirmed by immunohistochemical and indirect immunofluoresece staining. Eleven tumors (44%) had histopathologic features of epithelial or biphasic DMM. Eight SV40-induced DMM showed the following immunophenotypic features: AE1/AE3= 7/8 (88%); CAM 5.2= 4/8 (50%); mesothelin= 6/8 (75%); HBME-1= 8/8 (100%); calretinin= 2/8 (25%); EMA= 1/8 (13%); CK 5/6= 0/8 (0%); CEA= 0/8 (0%).

Conclusions: SV40-induced DMM in hamsters has histopathologic features and immunohistochemical phenotype similar to human DMM and may serve as a model for therapeutic investigations.

1489 Comparison of Fungal Culture Versus Surgical Pathology Examination in the Detection of Histoplasma in Surgically Excised Solitary Pulmonary Granulomas

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Background: Pulmonary infection with Histoplasma capsulatum is endemic in the geographical region served by our institution. Granulomatous pulmonary disease secondary to Histoplasma infection can manifest as a solitary pulmonary nodule. In some cases, these nodules require diagnostic surgical excision, with triage by intra-operative frozen section and submission of material for culture. Although microbiologic culture is often considered the "gold standard" in the diagnosis of infectious disease, we noted that fungal cultures and direct smears of the tissue are often negative in spite of the presence of Histoplasma yeast forms in the surgical pathology material. We sought to determine if routine fungal culture is of diagnostic value in this particular setting.

Design: Retrospective review of surgical pathology and clinical microbiology reports of thirty consecutive lung wedge excisional biopsies that demonstrated granulomatous inflammation at the time of frozen section.

Results: Sixteen cases demonstrated fungal organisms consistent with Histoplasma species via Gomori's methenamine silver stain (GMS) or periodic acid Schiff with diastase (PAS-D) on surgical pathology. Of these 16 cases, 13 were tested in the microbiology lab using direct smear examination (Calcofluor white stain) and fungal culture; Histoplasma was detected in one case (1/13). Ten cases revealed no fungal organisms via GMS and/or PAS-D on surgical pathology. Of these, 7 were tested in the microbiology lab, and Histoplasma was not detected in any of them (0/7). One case had no GMS/PAS-D staining performed or microbiologic examination. One case demonstrated Aspergillus species on both surgical pathology and microbiology. One case demonstrated Coccidioides species on surgical pathology, but no material was sent to microbiology. Finally, one case demonstrated fungal forms consistent with either Blastomyces or Cryptococcus species on surgical pathology, with Blastomyces species confirmed by microbiologic examination.

Conclusions: Surgical pathology examination of solitary granulomatous pulmonary disease detected Histoplasma organisms with greater sensitivity than culture and direct smear. There were no false-negative surgical pathology diagnoses when compared to microbiological results. These findings suggest that routinely sending granulomatous material for fungal culture is not necessary in this particular setting.

1490 Lymphomatoid Granulomatosis: Appraisal of the Historical Differential Diagnosis

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Background: In the 60's and early 70's Dr. Averill Liebow collected cases in which he considered the novel disease which was to be called lymphomatoid granulomatosis (LYG). We have examined these historic cases in detail histologically in conjunction with immunohistochemistry and in-situ hybridization, techniques not available in his

era. Our goal was to examine and refine the criteria for the diagnosis of LYG with the parameters and newer techniques in the context of the differential diagnoses considered by Dr. Liebow.
Design: We performed immunohistochemical staining for LMP-1 and in-situ hybridization for EBER on 11 complete cases included in Dr. Liebow's original series for LYG with available paraffin blocks in the Averill Liebow Pulmonary Pathology Collection at UCSD. We also performed additional immunohistochemical stains with CD3, CD20, and CD68. Histological parameters were examined and correlated with LMP-1 and EBER status in these 11 cases. These parameters included necrosis, vascular involvement, enumeration of the constellation of inflammatory cells, the cytologic atypia of lymphoid cells, and the presence of organizing pneumonia.
Results: Among these 11 historic cases, five cases were positive for LMP-1 and/or EBER; Three cases were postitive for both LMP-1 and EBER and two cases were positive for EBER only. Six cases were negative for both LMP-1 and EBER. The histologic parameters that correlated best with LMP-1 and EBER positivity were confluent necrosis with massive vascular involvement, mixed inflammatory infiltrates with an increase in small lymphocytes and variable plasma cells but no confluent PMNs, and the absence of lymphoid follicles or BALT. Secondary changes such as organizing pneumonia were also common in EBER and LMP-1 positive cases. The histologic differential for LMP-1 and EBER negative cases included malignant lymphomas of both low and high grade and infectious pneumonia in concert and in isolation. Wegener's granulomatosis and so called pseudolymphomas were also considerations.
Conclusions: In the correct histologic context EBER and LMP-1 positivity are the most sensitive and specific parameters within this historical differential diagnosis emphasizing the current viewpoint that LYG is an EBV-driven process with a reactive inflammatory infiltrate. This is analagous to lymphoproliferative disease in the setting of immunosuppression.

1491 Specific Involvement of EGFR Muataion in Adenocarcinoma with Features of Terminal Respiratory Unit

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Background: Recently, two groups published novel mutations of the epidermal growth factor receptor (EGFR), that are closely associated with clinical response to gefitinib. Because bronchioloalveolar feature is reported as a predictor of gefitinib response, we thus examined morphological characteristics of EGFR-mutated lung cancers.
Design: A consecutive series of 241 NSCLCs were examined using RT-PCR coupled direct sequencing.
Results: EGFR mutation was detected in 97, all except one of which were adenocarcinomas. In addition to prevalence in females and non-smokers, majority of EGFR-mutated adenocarcinomas expressed TTF-1 and surfactant protein (94% and 77%, respectively). Both correlations showed highly statistical significance ($P < 0.001$). Furthermore, EGFR mutation was detected in some cases of atypical adenomatous hyperplasia.
Conclusions: We previously noted that terminal-respiratory-unit (TRU) type adenocarcinoma is different from the other types in terms of molecular pathway for carcinogenesis and phenotypic profiles (AJSP 26, 767-73, 2002). TRU features are characterized by its cellular morphology and expression of TTF-1 and surfactant proteins. This specific involvement of EGFR mutation further suggests that TRU type adenocarcinoma is a distinct subset of pulmonary adenocarcinoma.

1492 The Lack of Expression of Human Herpesvirus 8 in Primary Pulmonary Hypertension

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Background: Primary pulmonary hypertension (PPH) is a fatal disease characterized by impaired regulation of pulmonary hemodynamics and abnormal vascular growth. This disease has been the focus of considerable research, but no definitive etiology has been found. A recent study reports that the vasculotropic virus, human herpesvirus 8 (HHV-8) may play a role in the pathogenesis of plexiform lesions and in PPH.
Design: We analyzed 32 formalin-fixed, paraffin-embedded lung tissue samples from patients with pulmonary hypertension (PH) with plexiform lesions. These included: 26 patients with PPH with plexiform lesions, 2 patients with AIDS-associated plexiform PPH (PPH-AIDS) and 4 patients with secondary PH with plexiform lesions due to congenital shunts (PH-C). Control tissues included: 1 patient with pulmonary veno-occlusive disease (PVOD), 8 patients with PH secondary to emphysema (PH-EMPH) and 11 normal donor lungs not used in transplantation (NL). The presence of HHV-8 protein was studied by immunohistochemistry (Latent nuclear antigen LNA-1 encoded by ORF73; Advanced Biotechnologies). Twenty (20) of these specimens (8-PPH, 2- PH-C, 2-PPH-AIDS, 4-PH-EMPH, 4-NL) and one primary lung culture from a PPH lung explant specimen were analyzed by Taqman polymerase chain reaction (PCR) for HHV-8 DNA. Ultrastructural analysis for HHV-8 virus was performed on endothelial cells from the one primary lung culture from a PPH lung explant specimen. Five (5) cases of Kaposi's sarcoma served as positive controls for both immunohistochemical and PCR analyses.
Results: All 32 plexiform PH samples and 20 control samples were negative for HHV-8 protein by immunohistochemistry. All 20 formalin-fixed samples (12 plexiform PH and 8 controls) and the one primary PPH lung culture sample were negative for HHV-8 DNA by PCR. No viral particles were found in the endothelial cells from the PPH primary lung culture by ultrastructural analysis. All five cases of Kaposi's sarcoma were positive for HHV-8 by both immunohistochemistry and PCR.
Conclusions: Our data suggest the vasculotropic virus HHV-8 is not present in lungs with plexiform PH, including PPH and secondary plexiform PH, or in one primary culture of a PPH lung, by immunohistochemical, PCR and ultrastructural analyses. Its role in the pathogenesis of PPH needs further investigation.

1493 Morphologic Features of Lung Adenocarcinomas in Relation to Responsiveness to Epidermal Growth Factor Receptor (EGFR) Inhibitors and EGFR Mutational Status

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Background: Bronchioloalveolar carcinoma (BAC) is an adenocarcinoma of the lung where cells grow along the walls of pre-existing alveoli. The World Health Organization (WHO) criteria restrict the term BAC to cases that show exclusively this pattern. Pure WHO-type BAC is uncommon (about 3%) but a BAC component may be seen in about 20% of adenocarcinomas. It has been shown that the clinical pattern and pathologic stage, but not the histologic features, predict outcome for patients with BAC. Patients classified as pure BAC, BAC with invasion, and adenocarcinoma with BAC features show no significant differences in survival on conventional therapy. However, recently adenocarcinoma with BAC component and never smoker status have been linked to regression of disease with the EGFR inhibitors, gefitinib (Iressa) or erlotinib (Tarceva), and with the presence of EGFR kinase domain mutations.
Design: We studied 29 tumors including 14 Iressa responders (IR) and 13 Tarceva responders (TR). These were compared to tumors from nonresponders to the same drugs. Exons 18 to 24 of EGFR were subjected to direct sequencing to identify EGFR mutations.
Results: All IR and TR tumors were peripherally located well to moderately differentiated adenocarcinomas with few BAC. Nuclear clearing was seen in 15 cases. Three cases showed focal necrosis, and metastases were present in 10 cases. Among the comparison group of nonresponder tumors, most were high grade and only one had good BAC morphology. Necrosis was seen in all nonresponder tumors, and optically clear nuclei were uncommon (squamous cell carcinoma was over represented in this group).

Histology	IR	TR
Pure BAC	1	3 (1 pure mucinous)
BAC with focal invasion	1	3
Adenocarcinoma with BAC features	7	6
Adenocarcinoma	6	1

EGFR kinase domain mutations were as follows: IR: mutated (9), no mutation (4), and unknown (3). TR: mutated (4), no mutation (3), and unknown (3).
Conclusions: Histologic features can be useful in selecting patients for treatment with EGFR inhibitors. Among the responders, we find no histologic differences between IR and TR tumors. The nonresponder tumors appear to be of higher grade, but overlaps exist between the two populations.

1494 High-Density Tissue Microarray-Based Appraisal of the Prognostic Value of Thyroid Transcription Factor-1 and Surfactant Precursor Protein B in Non-Small Cell Lung Cancers

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Background: A member of the Nkx2 family of homeodomain-containing proteins, thyroid transcription factor-1 (TTF-1) has important functions in lung development and is frequently expressed in non-small cell lung cancers (NSCLCs). This high-throughput microarray study was performed to assess the relationships between expression of TTF-1 and surfactant precursor protein B (SPPB), which is regulated by TTF-1, and patient survival.
Design: Dual color immunohistochemistry for TTF-1 (1:60, DakoCytomation) and SPPB (1:15, Novocastra) was performed on high-density tissue microarray slides containing samples from 351 NSCLCs with more than five years of clinical follow-up. Nuclear staining was evaluated for TTF-1 and cytoplasmic staining for SPPB. Staining intensity was graded as 0, 1+ (weak), 2+ (moderate) or 3+ (strong), and the percentage of tumor cells staining was scored semi-quantitatively as follows: 0=<10%, 1=10-50%, 2=>50%. Information about patient survival and tumor stage was collected. Statistical analysis was performed using Kaplan-Meier analysis and Pearson correlation.
Results: As expected, more adenocarcinomas expressed TTF-1 (83.1%) and SPPB (37.6%) than squamous cell carcinomas (TTF-1 23.0%, SPPB 4.6%) and large cell carcinomas (TTF-1 45.2%, SPPB 4.8%). For the entire cohort, nuclear TTF-1 staining correlated strongly with improved survival (p=0.004), and the best survival occurred with TTF-1 (+)/SPPB (-) tumors. These relationships also held true for early stage NSCLCs as a group, and a striking relationship was found between TTF-1 expression and improved survival (p=0.0001) in stage I and II adenocarcinomas. Cytoplasmic SPPB staining was associated with a trend towards lower survival for the group as a whole (p=0.11) and for patients with early stage NSCLC (p=0.17).
Conclusions: These findings suggest that nuclear TTF-1 expression is predictive of improved patient survival in NSCLCs, particularly in stage I and II adenocarcinomas. SPPB expression has a smaller negative association with survival.

1495 Stromal Cell-Derived Factor-1 Expression in 351 Non-Small Cell Lung Cancers: A Tissue Microarray and Immunohistochemical Assessment of Its Relationship to Patient Survival

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Background: The alpha-chemokine stromal cell-derived factor-1 (SDF-1) plays an important role in stem cell trafficking, and several recent reports suggest a function for SDF-1 in regulating the metastasis of non-small cell lung cancer (NSCLC). Its relationship to prognosis, however, is not clear.

Design: High-density tissue microarrays containing 351 NSCLCs with more than five years of clinical follow-up were immunostained for SDF-1 (1:25, R&D Systems) using standard avidin-biotin techniques. Staining intensity was graded as 0, 1+ (weak), 2+ (moderate) or 3+ (strong), and the percentage of tumor cells staining was graded semi-quantitatively as follows: 0=no tumor cells staining, 1= 1-32% staining, 2=33-66% staining, 3=>66% staining. For each tumor, an overall percentage of cells stained was calculated as the mean of the individual percentages recorded for the three samples of each tumor in the microarray. Information about patient survival and tumor stage was obtained. Statistical analysis was performed using Pearson correlation and Kaplan-Meier analysis.

Results: Staining intensity was graded as weak, moderate, and strong in 11.9%, 51.5%, and 27.8% of the NSCLCs, respectively. 20.0% showed 1-32% of cells staining, 18.7% fell between 33 and 66%, and 52.1% of tumors showed >66% expression. Only 9.6% of the tumors were negative. For the entire cohort, strong cytoplasmic SDF-1 staining intensity correlated significantly with improved survival (p=0.03). For stage I and II adenocarcinomas, enhanced survival also correlated positively with the overall percentage of tumor cells staining (p=0.03).

Conclusions: Increased SDF-1 expression may predict better patient survival in NSCLCs. Its wide expression in NSCLCs may indicate a role for SDF-1 in the pathogenesis of these neoplasms.

1496 SV40 and Malignant Mesothelioma: A Molecular and Immunohistochemical Study of 83 Cases from USA and Two Different Regions of Turkey

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Background: Simian virus (SV40) is an oncogenic DNA virus and may act as co-carcinogens with asbestos in the etiology of malignant mesothelioma of the pleura and peritoneum. Several studies showed DNA sequences specific for SV40 in large series mostly from United States, however, a literature search did not reveal any finger prints of the virus on the environmental malignant mesothelioma cases from Turkey.

Design: In this study, we chose 40 pleural malignant mesothelioma cases from Southeastern region, 17 cases from Middle Anatolia of Turkey, as well as 26 cases from United States to detect DNA sequences by using nested polymerase chain reaction (PCR) using the primer pairs, the SV primer set directed at the LTAg gene sequence unique to SV40 and the PYV primer set directed at a sequence shared by SV40 and papovavirus strains BK and JC, respectively. The presence of DNA was established by amplification of a 250 bp product from the betaglobin gene. We also used an avidin-biotin immunoperoxidase technique to detect SV40 T antigen (TAg) in our series.

Results: All US malignant mesothelioma cases except two showed positive immunoreaction for TAg (92%), whereas 14 cases were positive from Middle Anatolia region (82%) and only 6 cases from Southern Anatolia region of Turkey (15%). The intensity of the immunostaining was stronger and more diffuse in US cases. By PCR technique, we noted SV40 DNA sequences in 13 US cases (50%), in 3 Middle Anatolia cases (18%), and in 3 Southeastern Anatolia cases (7.5%) respectively.

Conclusions: This study showed SV40 DNA sequences in environmental malignant mesothelioma cases from two different parts of Turkey, and also confirmed the high incidence of SV40-positive cases in USA. The geographical conditions, type of the inhaled asbestos fibers, and genetic characteristics may play role in the development of malignant mesothelioma in these cases as well as SV40 infection. SV40 contaminated polio vaccination may explain the source of the virus in US cases, however fails to explain the existence of the virus in Turkey and also the higher incidence in Middle Anatolia when compared with Southeastern Anatolia.

1497 Lung Adenocarcinoma with Mixed Subtypes, a Distinct Biologic Entity?

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Background: In the 1999 WHO classification of pulmonary neoplasms, adenocarcinomas with bronchioloalveolar (BAC) and invasive components are classified as "adenocarcinoma with mixed subtypes". However, the clinicopathologic features of this subtype have not been fully defined. Since the majority of small adenocarcinomas are classified in this category and represent a heterogenous group of tumors from minimally to fully invasive cancers, further refinement of the diagnostic criteria is needed. A more accurate subclassification may help define biologically distinct subgroups, thus helping to guide patient care.

Design: 164 cases of primary lung adenocarcinomas resected between 1992-2004 were reviewed and divided into 4 groups. Group I: BAC only (49); Group II: Mixed type with =5 mm invasive component (16); Group III: Mixed type with >5 mm invasive component (63); Group IV: Invasive adenocarcinoma only (36). Tumor size and lymph nodes metastasis were compared. P53 and Ki-67 staining were analyzed in 46 cases (14 from Group I; 9 from Group II; 11 from Group III; 12 from Group IV). The Ki-67 labeling index (LI) was determined by counting 500-1000 tumor cells in three high-power fields (400X) of the most highly labeled areas. P53 overexpression was defined as the presence of nuclear staining in >20% of tumor cells.

Results: Table 1 shows the clinicopathologic characteristics of each group. None of the Group I and II tumors had lymph node involvement. In contrast, 8 of 63 Group III (12.7%) lesions and 21 of 36 Group IV (75%) lesions showed lymph node involvement. The mean Ki-67 LI and frequency of p53 overexpression showed an increasing tendency from group I to IV, with values being higher in groups III and IV than in groups I and II.

Conclusions: Data shows that adenocarcinoma with mixed subtypes (groups II, III) have characteristics between BAC (group I) and invasive carcinoma (group IV), consistent with the idea that this entity is a tumor in progression from BAC to invasive cancer. A subgroup of this entity (group II) has characteristics and biologic behavior more similar to BAC, suggesting that this subgroup could be defined as a form of early invasive cancer, and may be clinically managed as such.

TABLE 1. Clinicopathologic characteristics of each histological group

Histologic subtype	p53 (% of positive lesions)	Ki-67 LI (%) (mean ± SD)	Age (mean ± SD)	Tumor size (mean ± SD) (cm)	Lymph node involvement (%)
I	8	6.2 ± 2.7	67.7 ± 8.4	1.9 ± 1.5	0
II	11	7.5 ± 3.7	65.6 ± 9.5	1.6 ± 0.8	0
III	30	21.3 ± 14.8	66.9 ± 10.2	2.3 ± 1.2	12.7
IV	44	31 ± 19.2	68.5 ± 10.5	3.1 ± 2.6	75

Quality Assurance

1498 Frozen Section (FS) Diagnosis (Dx) in Pediatric Surgical Pathology: A Decade's Experience at a Children's Hospital (CH)

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Background: FS are critical for patient care and are a key quality component in anatomic pathology. Little data exists about the use, discrepancy, and deferral rates of FS Dx in pediatric and adolescent care. The purpose of this study was to analyze indications, discrepancies, and deferrals for all FS performed at a CH during a decade.

Design: All FS Dx for 1995-2004 were reviewed for indications, discrepancies, and deferred Dx. Discrepancies were categorized into major and minor subtypes according to impact on patient care.

Results: 33,524 surgical pathology cases were accessioned, with 2,735 individual FS (8.2%). Most frequent indications included Hirschsprung disease (HD), and questions related to neoplasms (tumor detection, specimen adequacy, triage, classification, margins). 101 discrepancies (3.6%) were identified, of which 7 (0.2%) were major, with potentially significant clinical impact, and 94 (3.4%) were minor. Major discrepancies included tumor, ganglion cell, or fungal detection. Minor discrepancies involved sampling errors, reclassification of benign or malignant neoplasms without clinical consequences, tumor grading, and recognition of ganglion cells or transition zone in suspected HD. Deferrals included tumor classification from generic to specific, identification of organisms, and evaluation of lymph node biopsies for lymphoma.

Conclusions: The FS rate of 8.2% is similar in CH and general hospitals. The major discrepancy (discordance) rate is lower, which may reflect the different indications for FS at CH. HD is a major FS Dx pitfall. The deferral rate is higher in CH and may reflect how a deferred Dx is defined. Traditional definitions of deferred and discordant FS Dx should be refined to reflect increasing use of adjunct techniques, especially in tumor classification. The findings emphasize that in CH, the majority of FS are performed for HD and for tumor classification, triage, detection, and specimen adequacy, but are used infrequently to identify normal or unknown tissue, a lesion in a radiographically directed specimen, or to detect lymph node metastases. The differences in CH FS Dx underscore the importance of education in pediatric surgical pathology.

1499 Clinician Satisfaction with Complete Subspecialization in Surgical Pathology: One Year Follow-Up at the Cleveland Clinic

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Background: Prior to July 2003, the surgical pathologists of the Department of Anatomic Pathology (DAP) at the Cleveland Clinic Foundation (CCF) practiced general surgical pathology with partial subspecialization. Encouraged by subspecialized clinician groups who demanded greater efficiency and expertise, the department re-organized to a complete subspecialization model consisting of 16 services covered by groups of 2-8 pathologists. With this new model, the DAP hoped to improve clinician satisfaction with the DAP services.

Design: Cleveland Clinic physicians were surveyed before and after implementation of subspecialization in the Department of Anatomic Pathology (DAP). Physicians were asked to rate the surgical pathology reports for: 1) Accuracy, 2) Completeness, 3) Timeliness, and the surgical pathologists for 1) Capable of answering questions and 2) Meet my expectations.(Strongly agree, Agree, Disagree, Strongly Disagree). The DAP was rated for overall satisfaction (Very satisfied, Somewhat satisfied, Somewhat dissatisfied, Very dissatisfied). Surveys were mailed to 465 physicians from the Divisions of Surgery and Medicine in April 2003, two months before and one year after implementation of complete subspecialization. Completed surveys were received from 41% of physicians from Surgery and 46% of physicians from Medicine.