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Patología pulmonar

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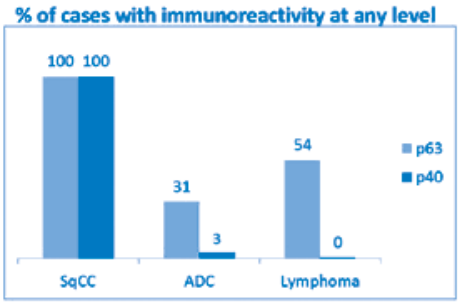
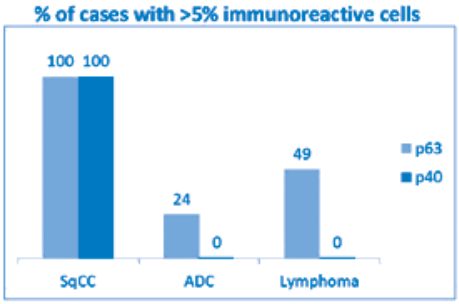
USCAP & AACR HIGHLIGHTS 2012

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

Justin A Bishop, Julie Teruya-Feldstein, William H Westra, Giuseppe Pelosi, William D Travis, Natasha Rekhtman. The Johns Hopkins Medical Institutions, Baltimore, MD; Memorial Sloan-Kettering Cancer Center, New York, NY; Fondazione IRCCS National Cancer Institute and University of Milan School of Medicine, Milan, Italy

- **Background:** Immunohistochemistry has recently emerged as a powerful ancillary tool for differentiating lung adenocarcinoma and squamous cell carcinoma – a distinction with important therapeutic implications. While the most frequently recommended squamous marker p63 is extremely sensitive, it suffers from low specificity due to its reactivity in a substantial proportion of lung adenocarcinomas and other tumor types, particularly lymphomas. p40 is a relatively unknown antibody that recognizes ΔNp63 – a p63 isoform suggested to be highly specific for squamous/basal cells.
- **Design:** The standard p63 antibody (4A4) and p40 were compared in a series of 470 tumors from the archives of Memorial Sloan-Kettering Cancer Center and The Johns Hopkins Hospital, which included lung squamous cell carcinomas (n=81), adenocarcinomas (n=237), and large cell lymphomas (n=152).
- **Results:** p63 was positive in 100% of squamous cell carcinomas, 31% of adenocarcinomas and 54% of large cell lymphomas (sensitivity 100%, specificity 60%). In contrast, while p40 was also positive in 100% of squamous cell carcinomas, only 3% of adenocarcinomas and none of large cell lymphomas had p40 labeling (sensitivity 100%, specificity 98%). The mean percentage of p63 versus p40-immunoreactive cells in squamous cell carcinomas was equivalent (97% vs. 96%, respectively, p=0.73). Rare adenocarcinomas with p40 labeling had reactivity in no more than 5% of tumor cells, whereas the mean (range) of p63-positive cells in adenocarcinomas and lymphomas was 26% (1-90%) and 48% (2-100%), respectively.





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

Category: Pulmonary

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

Kenneth J Bloom, Paul Choppa. Clarient, A GE Healthcare Company, Aliso Viejo, CA

- **Background:** Non-small cell lung cancer (NSCLC) is the leading cause of cancer-related deaths worldwide. Two-thirds of patients present with advanced disease and have an average survival of less than 1 year with standard chemotherapy. Studies have demonstrated that exon 19 deletion or L858R substitution in the EGFR gene are the most powerful predictive biomarkers in patients treated with erlotinib or gefitinib. This has led to the recommendation that EGFR mutational status be evaluated prior to initiating chemotherapy. We sought to determine the frequency and distribution of EGFR mutations in our laboratory over the past several years.
- **Design:** From June 2009 to October 2011 we have evaluated the mutational status of EGFR in 18246 formalin fixed paraffin embedded non-small cell lung cancer samples using an allele specific PCR procedure that is capable of detecting 29 of the most prevalent mutations in exons 18-21 of EGFR, (QIAGEN EGFR PCR kit). The assay uses a real-time PCR platform and is capable of detecting mutations at a sensitivity of 1-5% in a background of non-mutated alleles.
- **Results:** Mutations were identified in 2435 (13.3%) of NSCLC samples tested. Of the 2435 samples with a detectable mutation 1252 (51.41%) harbored exon 19 deletions, 84 (3.45%) were mutations at codon 719, 80 (3.29%) were insertions in exon 20, 827 (33.96%) were L858R, 80 (3.29%) were L861Q, 25 (1.03%) were S768I, 24 (0.986%) were T790M, 2 (0.082%) 19 deletion + L858R, 3 (0.12%) exon 19 deletion + T790M, 19 (0.78%) L858R + T790M, 1 (0.041%) T790M + L861Q, 5 (0.21%) T790M + G719, 8 (0.33%) L858R + S768I, 1 (0.041%) L861Q + S768I, 19 (0.78%) S768I + G719 and 5 (0.21%) L861Q + G719.

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

  Category: Pulmonary

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

Chris MJ Conklin, Kenneth J Craddock, Cherry Have, Ming Tsao, Christian Couture, Diana N Ionescu. University of British Columbia, Vancouver, BC, Canada; University Health Network, Toronto General Hospital, Toronto, ON, Canada; IUCPQ (Hôpital Laval), Quebec City, QC, Canada; BC Cancer Agency, Vancouver, BC, Canada

- **Background:** The discovery of EML4-ALK fusion gene in patients with NSCLC was a breakthrough in targeted therapy for lung cancer with significant clinical implications. ALKR is however only seen in a small percentage of NSCLC making identification of these patients challenging and costly.
- **Design:** Using immunohistochemistry (IHC) with mouse monoclonal 5A4 antibody (Ab) from Nikirei Biosciences and fluorescence in situ hybridization (FISH) we screened a tissue microarray built from 593 resected surgical specimens from stage I NSCLC (243 lung adenocarcinoma (ACA), 272 squamous cell carcinoma (SQC), 35 large cell carcinoma, 32 non-small cell carcinoma NOS, and 6 other). IHC was scored as 0 (no staining), 1+ (faint cytoplasmic staining), 2+ (moderate, smooth cytoplasmic staining) and 3+ (intense, granular cytoplasmic staining) in >10% of tumor cells. IHC positive cases were 3+ only. Suspicious and positive cases were confirmed by IHC and FISH on whole section (WS).
- **Results:** Results by FISH were available on 273 cases and by IHC on 385 cases. A total of 11 cases, either positive (N=2) or suspicious (N=9) by at least one methodology, were identified. Cases suspicious by FISH (N=5) were however not suspicious by IHC and cases suspicious by IHC (N=4) were not suspicious by FISH. One case was positive on TMA as well as on WS by IHC and FISH. The only other unequivocally positive FISH case, with an atypical pattern (loss of 5' ALK signal), was not positive or suspicious by IHC. The average age of our 5 males and 5 females was 63 years. There were 4 ACA, 5 SQC and 1 NSCLC NOS, and 3 of all were positive for TTF1.
- **Conclusions:** IHC screening for ALKR in NSCLC with 5A4 antibody may not necessarily identify all cases with gene rearrangement by FISH. As small biopsy/cytology samples are inherently limited for molecular testing the question of finding the best strategy to identify ALKR as well as other clinically relevant molecular anomalies is critical, both in terms of time and cost. These results are currently in the process of being compared to other primary antibody clones (ALK-1 by Dako, 5A4 by Novocastra and D5F3 by Cell Signaling Technology) and revelation systems (FLEX by Dako, CSA II by Dako and ADVANCE by Dako).

  Category: Pulmonary

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

Javier Hernandez-Losa, Pablo Martinez, Josep Castellvi, Tallada Natalia, Teresa Moline, Maria Angeles Montero, Susana Cedres, Victor Rodriguez-Freixinos, Enriqueta Felip, Santiago Ramon y Cajal. Hospital Universitari Vall d'Hebron, Barcelona, Spain; VHIR. Universitat Autónoma de Barcelona, Barcelona, Spain

- **Background:** ALK rearrangement represents a novel molecular target in a subset of non small cell lung cancers (NSCLC). Our aim is to explore fluorescence in situ hibridation (FISH) and immunohistochemistry (IHC) as diagnostic methods, prevalence and clinical outcomes of ALK rearrangement patients in a selected population of NSCLC.
- **Design:** Patients with NSCLC previously screened for EGFR mutation at our institution between June 2006 and January 2010 were selected. ALK rearrangement was identified by using FISH and the value of IHC (D5F3 monoclonal antibody-mAb) was explored. For IHC ALK protein expression positivity was defined as tumor-specific staining of any intensity in ≥10% of the tumor cells.
- **Results:** 99 patients were identified with median age was 61.5 years (range 35-83), 80% were adenocarcinomas, 7% squamous and 13% NOS carcinomas. 51% patients were female. All were caucasian. 32% of the patients were never smokers and 30% former smokers. 7 (7%) patients were ALK rearranged positive by FISH, 13 (13%) were EGFR mutant, and 65 (65.6%) were wild type (WT/WT) for both ALK and EGFR. ALK rearrangements and EGFR mutations were mutually exclusive. ALK rearranged patients tend to be younger than EGFR mutated or WT/WT patients (median age of 56.7, 63 and 62.3 years, respectively). Patients with ALK positive tumors were predominantly never smokers (71.4%) and adenocarcinomas (71.4%). ALK positive and EGFR mutant patients have a better survival than WT/WT. All patients with ALK FISH negative tumors were negative for ALK IHC. Out of 7 patients positive for ALK FISH, 5 were also positive for ALK IHC, 1 negative and in the other there was not enough tissue to perform the analysis. The ALK FISH positive cases were analyzed by IHC with 5A4 mAb obtaining the same results.

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

  Category: Pulmonary

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

Kyuichi Kadota, Yi-Chen Yeh, Kei Suzuki, Camelia S Sima, Valerie W Rusch, Andre L Moreira, Prasad S Adusumilli, William D Travis. Memorial Sloan-Kettering Cancer Center, New York

- **Background:** A newly proposed IASLC/ATS/ERS lung adenocarcinoma classification emphasizes the prognostic significance of histologic subtypes. In this classification, however, one limitation is that the majority of patients (approximately 40%) are classified into the acinar predominant subtype. We investigated whether cribriform pattern can further stratify the prognosis of histologic subtypes in stage I lung adenocarcinoma.
- **Design:** H&E stained slides of 540 stage I lung adenocarcinoma patients (2002-2009) were reviewed. Tumors were classified into histologic subtypes according to the IASLC/ATS/ERS classification: adenocarcinoma in situ (AIS), minimally invasive adenocarcinoma (MIA), lepidic, papillary, acinar, micropapillary, solid predominant, invasive mucinous and colloid adenocarcinoma. The percentage of cribriform pattern was recorded in 5% increments, and we added cribriform predominant subtype. Log-rank test was used to analyze the association between histologic variables and recurrence-free probability (RFP).
- **Results:** Patients with AIS/MIA (n=26) experienced no recurrence (3-year RFP: 100%). Patients with lepidic predominant tumors (n=76) had the low-risk of recurrence (3-year RFP: 97%). Patients with acinar (n=193), and papillary predominant tumors (n=102) had the intermediate-risk of recurrence (3-year RFP: 90% and 91%, respectively). Patients with micropapillary (n=46), solid predominant (n=71), and invasive mucinous/colloid adenocarcinoma combined group (n=26) had the high-risk for recurrence (3-year RFP: 59%, 72%, and 62%, respectively). The 3-year RFP of cribriform predominant subtype (n=16, 70%) was comparable to the high-risk group for recurrence. When looking at RFP according to cribriform pattern percentage in all cases, patients with ≥30% cribriform pattern (n=31) had marginally lower RFP (3-year RFP: 77%) than <30% cribriform pattern (n=509, 86%, p=0.047). Within patients with acinar predominant subtype, patients with ≥30% cribriform pattern (n=25) had significantly lower RFP (3-year RFP: 75%) than <30% cribriform pattern (n=168, 92%, p=0.001).
- **Conclusions:** Cribriform pattern further stratified the acinar predominant tumors into two prognostically distinct subsets. In addition, cribriform predominant tumors may be considered as a poorly differentiated or high grade category with a high-risk for recurrence.

  Category: Pulmonary

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

Kyuichi Kadota, Jun-Ichi Nitadori, Kei Suzuki, Camelia S Sima, Akihiko Yoshizawa, Valerie W Rusch, William D Travis, Prasad S Adusumilli. Memorial Sloan-Kettering Cancer Center, New York

- **Background:** The majority of lung adenocarcinoma express thyroid transcription factor-1 (TTF-1), but lack of TTF-1 expression correlates with worse prognosis. We investigated whether TTF-1 expression can correlate with a newly proposed IASLC/ATS/ERS lung adenocarcinoma classification and further stratify prognosis of histologic subtypes in stage I lung adenocarcinoma.
- **Design:** H&E stained slides of 506 stage I lung adenocarcinoma patients (1995-2005) were reviewed. Tumors were classified into histologic subtypes according to the IASLC/ATS/ERS classification. We constructed a tissue microarray and performed immunohistochemistry for TTF-1. In all, 459 cases with adequate cores were available for TTF-1 expression analysis. Intensity of staining was scored as 0 (no expression), 1 (mild), 2 (intermediate), and 3 (strong). According to the intensity score, TTF-1 expression was divided into two groups: low (0-1) or high (2-3). Log-rank test was used to analyze the association between histologic variables and recurrence-free probability (RFP).
- **Results:** High TTF-1 expression was identified in 100% (8/8) of adenocarcinoma in situ and minimally invasive adenocarcinoma, 96% (25/26) of lepidic predominant, 88% (113/129) of papillary, 87% (188/215) of acinar, 75% (9/12) of micropapillary, 77% (44/57) of solid, 44% (4/9) of invasive mucinous, and 33% (1/3) of colloid adenocarcinoma. High TTF-1 expression was more frequent in tumors with low architectural grade (p<0.001). The 5-year RFP of tumors with low TTF-1 expression (n=67, 69%) was lower than high TTF-1 expression (n=392, 85%, p=0.001). Among tumors with the acinar subtype, low TTF-1 expression (n=27) was associated with lower RFP (5-year RFP: 70%) compared to high TTF-1 expression (n=188, 86%, p=0.013). Among patients with other each histologic subtype, there was no significant difference of RFP between low and high TTF-1 expression.
- **Conclusions:** TTF-1 expression was significantly correlated with architectural grade based on histologic subtype. In addition, TTF-1 expression correlated with recurrence, and further stratified the acinar predominant tumors into two prognostically distinct subsets.

  Category: Pulmonary

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

Hartmut Koeppen, Tom Januario, Ellen Filvaroff, Penny Towne, Racheal James, Patrick Roche, Xiaoling Xia, Jiping Zha, Bob Yauch. Genentech, Inc., South San Francisco, CA; Ventana Medical Systems, Inc., Tucson, AZ

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

Category: Pulmonary

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

Ainura Kyshtoobayeva, Kenneth J Bloom. Clarient, A GE Healthcare Company, Aliso Viejo, CA

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

Category: Pulmonary

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

Hiroshi Minato, Mana Fukushima, Nozomu Kurose, Takayuki Nojima. Kanazawa Medical University, Uchinada, Ishikawa, Japan

- **Background:** The differentiation of MM from RMC can occasionally present a diagnostic challenge, especially on a small biopsy specimen. Recently several new biomarkers, such as GLUT-1, CD146, and IMP3, have emerged as good candidates for distinguishing MM from RMC. Each study using each marker has reported its high sensitivity and specificity for discrimination between MM from RMC; however, the markers rarely studied by other groups and have not comparatively analyzed. Moreover, CD146 was studied only in cytologic specimens. We, therefore, performed comparative immunohistochemical analysis in MM and RMC cases with antibodies for GLUT-1, CD146, IMP3, EMA and Desmin.
- **Design:** Paraffin-embedded sections from 31 cases of previously confirmed MM and 40 cases of benign lung tissues with RMC were used. 15/31 (48%) MM were epithelioid, 11/31 (36%) were biphasic, and 5/31 (16%) were sarcomatoid. Antibodies used were GLUT-1 (polyclonal, Abcam), CD146 (N1238, Abcam), IMP3 (L523S, Dako), EMA (E29, Dako) and Desmin (D33, Dako). Staining results were scored by percentage of mesothelial or tumor cells staining. When more than 5% of mesothelial/tumor cells were stained with each antibody, the stain was defined as positive.
- **Results:** As positive markers for MM, GLUT-1, CD146, IMP3, and EMA showed sensitivity of 84%, 61%, 94%, and 74%, respectively, and specificity of 100%, 100%, 88%, and 88%. As a negative marker for MM, Desmin showed sensitivity of 48%, and specificity of 97%. In MMs, proportion of positive tumor cells for GLUT-1 was from 10 to 95% (mean 47%); CD146, 6 to 95% (32%); IMP3, 6 to 100% (61%); EMA, 6 to 100 (60%). In RMCs, proportion of positive mesothelial cells for IMP3 was from 10 to 25% (mean 17%); EMA, 10 to 35% (19%); Desmin, 6 to 30% (12%).

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

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

Category: Pulmonary

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

Jose Ramirez, Marc Campayo, Maria Luisa Cabanas, Nuria Vinolas, Ramon Marrades, Laureano Molins, Mariano Monzo. Hospital Clinic. IDIBAPS, CIBERES. Universitat de Barcelona, Barcelona, Spain; Universitat de Barcelona, Barcelona, Spain

- **Background:** The transcription factor SOX2 is overexpressed in many solid tumours, including NSCLC. miR-145 and the miR-302-367 cluster are involved in stemness through SOX2 regulation. miR-145 plays an important role in SOX2 translation, and SOX2 regulates the expression of the miR-302-367 cluster. We have analyzed the expression of miR-145 and the miR-302-367 cluster in tumour and paired normal tissue samples from resected NSCLC patients and correlated our findings with time to recurrence (TTR).
- **Design:** We analyzed the expression of miR-145 and miR-302-367 cluster in 70 tumour and 70 paired normal tissue samples from NSCLC patients who had undergone complete surgical resection from 2007 to 2009. We focused in 36 Adenocarcinoma and 28 Squamous Cell Carcinoma. RNA was obtained from fresh frozen tumour and normal tissue using the Trizol method and microRNA expression was detected using TaqMan MicroRNA Assays.
- **Results:** Patients(p) characteristics: stage I, 12 (17.1%) stage II, 14 (20%) stage III; 36 (51.4%). 36 (51.4 %) Adenocarcinoma (ADC), 28 (40%) squamous cell carcinoma (SCC) 6 (8.6 %) NSCLC NOS. With a mean follow-up of 17 months (m), 23 p (32.9%) had relapsed. miR-145 expression was downregulated (P<0.001), and miR-367 expression was upregulated (P<0.001) in tumour compared to normal tissue samples. Mean TTR for p with low miR-145 levels was 18.4 m vs 28.2 m for p with high miR-145 (P=0.015). Mean TTR for p with low miR-367 levels was 29.1 m vs 23.4 m for p with high miR-367 (P=0.048). Concerning the prognostic differences between the main histological types, there was no correlation between the overexpression of both miRNAs in ADC cases. Conversely, there was a significant prognostic value of miR-367 for TTR (p=0.012) in SCC cases, but it was just a trend with miR-145 (p=0.071) for TTR.

- **Conclusions:** In our study we have demonstrated a relationship between expression of miR-145 and miR-367 and TTR in SCC patients. On the other hand these microRNAs have no prognostic significance in ADC patients. Both conclusions support the importance of distinguishing SCC and ADC cases in all the studies with miRNAs.

Supported by a grant from F.I.S - 080135

Category: Pulmonary

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

Natasha Rekhtman, Paul K Paik, Maria E Arcila, Laura J Tafe, Geoffrey R Oxnard, Andre L Moreira, Travis D William, Maureen F Zakowski, Kris G Mark, Marc Ladanyi. Memorial Sloan-Kettering Cancer Center (MSKCC), New York, NY; Dartmouth Hitchcock Medical Center, Lebanon, NH; Dana-Farber Cancer Institute, Boston, MA

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

Category: Pulmonary

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

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- **Background:** Epidermal growth factor receptor (EGFR) mutations, and to a lesser extent EGFR copy number variations, have been correlated with response to EGFR tyrosine kinase inhibitors (TKIs). In contrast, KRAS mutations have been recently associated with resistance to TKIs. Most of these studies have been carried out on adecarcinomas (ACs) due to the association of this histologic type with the presence of alterations in the EGFR gene pathway. The aim of this study was the molecular characterization of a series of lung squamous cell carcinomas (SCCs) and the possible implications on TKIs therapy.
- **Design:** A series of 47 surgically resected paraffin embedded SCCs were reviewed and their histological classification further confirmed by IHC (CK7, CK5/6, CK903, CK20, p63 and TTF1). The presence of EGFR and KRAS mutations was analyzed by direct sequencing and the incidence of EGFR copy number variations determined by fluorescent in situ hybridization (FISH). These results were then compared to those found in a series of 48 ACs and their statistical significance analyzed using Fisher's exact test.
- **Results:** Despite there were no EGFR or KRAS mutations found in this series of SCCs, there was a high percentage of cases (55%) presenting EGFR copy number variations, which is not statistically different from that found in the series of ACs (p=0.14). These FISH positive SCCs included 6 cases with EGFR amplification and 20 cases with high polysomy.
- **Conclusions:** Our results confirm the absence of EGFR and KRAS mutations in lung SCCs observed in other series. Nonetheless, the significant number of EGFR copy number variations observed, and the possible correlation with TKIs sensitivity cannot be overlooked and should be further analyzed. This study suggests that FISH may be an appropriate methodology to assess the EGFR status of SCCs.

Table 1: Univariate analysis to correlate molecular alterations with histologic type.			
	SCCs (n=47)	**ACs (n=48)**	**P value**
EGFR FISH			
(+)	26	34	0.14
(-)	21	14	
EGFR mutations			
wild-type	47	42	0.03
mutated	0	6	
KRAS mutations			
wild-type	43	31	0.0008
mutated	0	9	
not assessed	4	8	

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

Category: Pulmonary

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

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- **Background:** The recent introduction of next generation (NGS) DNA sequencing to clinical samples has enabled the discovery of novel and unanticipated genomic-derived targets of therapy response and resistance for patients with NSCLC.
- **Design:** DNA was extracted from 4 x 10 µm FFPE sections from 45 primary NSCLC (26 female; 19 male; mean age 68 years; 24% Stage I; 13% Stage II; 5% Stage III; 16% Stage IV; 46% Stage unknown). The exons of 145 cancer-related genes were fully sequenced using the Illumina HiSeq 2000 (Illumina, San Diego, CA) to at an average sequencing depth of 253X and evaluated for point mutations, insertions/deletions (indels), specific genomic rearrangements and copy number alterations (CNA). Samples included 5% fluid cellblocks; 5% regional lymph nodes; 3% pericardial biopsy and 87% lung biopsies or resections. There were 42 adenocarcinomas (27acinar, 12 lepidic, 2 mucinous, 1 papillary), 1 large cell carcinoma, and 2 squamous cell carcinomas. In 23 adenocarcinomas, the NGS results were compared with commercial laboratory allele-specific PCR genotyping on the same tissue blocks.
- **Results:** In the comparison study of EGFR status, the NGS result was concordant with commercial laboratory genotyping in 23/23 (100%) cases. In 22 additional NSCLC samples, NGS revealed 53 total genomic alterations, including 14 (64%) base substitutions, 2 (9%) INDELs, 6 (27%) CNA, and 0 (0%) rearrangements. Genomic alterations associated with sensitivity or resistance to targeted therapies for NSCLC were found in 16/22 (73%) of cases including 10 *KRAS*, 4 *STK*11, 3 *JAK*2, 2 *PIK3CA*, 2 *BRAF*, 2 *EGFR*, 1 *NF1*, 1 *TSC1*, 1 *TSC2*, 1 *CCNE*1, 1 *PTCH*, 1 *CDK4*, 1 *CCND*1, 1 *BRCA2*, 1 *CDKN2*A, and 1 *ATM* mutation. In comparison with the COSMIC database, NGS results were similar for most genes except for a lower rate of *EGFR* mutations (9% vs. 21%), a higher rate of *KRAS* mutations (41% vs. 16%) and an unprecedented rate of *JAK2* mutations (14% vs. 0%). 7/22 (32%) of the NSCLC had 2 or more potentially actionable alterations after NGS.
- **Conclusions:** Deep sequencing of clinical NSCLC samples is completely concordant with traditional hot-spot genotyping and also uncovers an unexpected number of genomic alterations that could influence therapy selection for this disease. Broad-based, deep sequencing of cancer-related genes results in sensitive detection of all classes of genomic alterations in NSCLC and can reveal actionable genomic abnormalities that inform treatment decisions.

Category: Pulmonary

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

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- **Background:** Primary lung NE tumors include a spectrum of neoplasms from low grade typical carcinoid (TC), intermediate grade atypical carcinoid (AC) to high grade small cell carcinoma (SCLC) & large cell NE carcinoma (LCNEC). This study uses gene expression profiling to provide molecular signatures of the tumor subtypes and their correlation to clinical features.
- **Design:** Gene expression profiling was performed with the Affymetrix U133 Plus 2.0 array in 120 primary lung NE tumors including 64 TC, 13 AC, 20 SCLC & 23 LCNEC. Hierarchical clustering analysis was performed based on the gene expression of the most variable genes among 1286 genes that were analyzed. Carcinoids were divided into central & peripheral locations. The association of the clusters with clinical and histological characteristics were tested using Fisher's exact test or the Wilcoxon rank-sum test. Differential gene expression (limma package in R) and gene set enrichment analyses were performed. Survival was evaluated using competing risk analysis.
- **Results:** Cluster analysis identified three clusters (1, 2 & 3). Cluster 1 included all of the high grade NE neoplasms and 4 TC (6.2%). Cluster 2 included both TC (37.5%) & AC (38.5%). However, the majority of TC (56.3%) & AC (61.5%) grouped into cluster 3. For carcinoids, cluster 2 was associated with a central location (82.8%) while cluster 3 was associated with peripheral (70.5%) location ($P<0.01$). Supervised clustering analysis revealed many genes in cluster 1 that discriminate SCLC (i.e. ZIC2, ID4) from LCNEC ($P<0.001$). Cluster 1 was highly associated with smoking history and pack/years compared with clusters 2 and 3. Patients with cluster 2 tumors were much younger than patients in cluster 1 & cluster 3 (mean age 55 vs 67 vs 63 yrs, $P<0.01$).The 30-month cumulative incidence of death due to disease was much higher for cluster 1 (27%) than cluster 2 (0%) & cluster 3 (4%) ($P=0.012$).
- **Conclusions:** Gene expression profiling reveals three clusters of genes in lung NE tumors. The high grade NE neoplasms, SCLC and LCNEC share the same cluster while TC and AC are divided between two clusters that correlate with central vs peripheral location. Although SCLC and LCNEC share the same cluster, these tumors are distinguished by expression profiles of many genes. Further study on these genes such as ZIC2 and ID4 may provide important information about these tumors.

Category: Pulmonary

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

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

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

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

There were substantial agreement on predominant histologic subtype between different pathologists (Kappa=0.729), and moderate to substantial agreement on presence or absence of five major histologic patterns (Kappa=0.648 for lepidic,

0.434 for acinar, 0.672 for papillary, 0.643 for micropapillary, and 0.610 for solid pattern).

- **Conclusions:** There is moderate agreement on predominant ADC histologic subtype between FS and final diagnosis. The interobserver agreement is satisfactory. Because FS have a high specificity in identifying micropapillary and solid patterns, recognition of one of these poor prognostic patterns may help a surgeon to consider anatomic rather than limited resection. However, the value is limited by low sensitivity, especially for micropapillary pattern.

Category: Pulmonary

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

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- **Background:** Napsin A is a useful marker in identifying adenocarcinoma of the lung in a tumor of unknown origin. Our preliminary data and literature using a polyclonal antibody to napsin A demonstrated that it was a highly sensitive marker for pulmonary adenocarcinomas. However, expression of napsin A was also observed in a significant percentage of other tumors, including renal cell carcinomas, thyroid papillary carcinomas and esophageal adenocarcinomas. With the availability of a monoclonal antibody to napsin A, we compared expression of the polyclonal and the monoclonal antibodies in tumors from various organs using a single immunostaining system (Dako).
- **Design:** Immunohistochemical evaluation of napsin A (1. Cat No. 760-4446, rabbit polyclonal, prediluted, Ventana; 2. Cat No. CM 338CK, mouse monoclonal, BioCare Medical) expression was performed on 1058 cases of tumors on tissue microarray sections. The staining intensity and distribution were recorded.
- **Results:** The immunostaining results are summarized in Table 1. The sensitivity and specificity for the polyclonal and monoclonal antibody were 83.3% and 95.6%, and 72.6% and 97.9%, respectively

Table 1. Summary of Immunostaining Results

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

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

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

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