UPDATES IN THE SURGICAL PATHOLOGY OF LUNG CANCER

Four “Ps” of Pulmonary Cytopathology: Procedural, Predictive, Personalized and Participatory

American Society of Cytopathology
Companion Society Symposia
United States and Canadian Academy of Pathology (USCAP)

Sunday, March 18, 2012
Vancouver, BC

William Travis, M.D.
Department of Pathology
Memorial Sloan Kettering Cancer Center
New York, NY

travisw@mskcc.org
Worldwide, lung cancer is the most common cause of major cancer mortality in men and the second most common in women.\textsuperscript{1} Recent therapeutic advances have led to a revolution in the lung cancer field in discovering therapeutically tractable oncogene dependency, that have major implications for patient evaluation and approach to diagnosis. The recently published IASLC/ATS/ERS Classification of Lung Adenocarcinoma addresses these issues.\textsuperscript{2} This classification was developed based on an evidence-based approach by an international multidisciplinary panel including pathologists, oncologists/respiratory physicians, radiologists, molecular biologists, and thoracic surgeons.\textsuperscript{2} Multiple new approaches are outlined that will have a major influence on clinical practice for pathologists as well as the entire multidisciplinary team caring for patients with lung cancer.

Since 70\% of patients with lung cancer present with advanced stage disease,\textsuperscript{2} their diagnosis is usually established based on small biopsies, cytology specimens or both, and the primary therapy is chemotherapy; the remaining patients usually have resectable lesions and surgery is the primary treatment. To address all types of patients with lung cancer, there are two major components to the classification based on the type of specimen: 1) small biopsies and cytology (Table 1) or 2) resection (Table 2). Because previous WHO classifications did not provide specific criteria and terminology for pathologic diagnosis of lung cancer in small biopsies and cytology,\textsuperscript{2} this new classification is more clinically relevant as it addresses these small specimens and addresses molecular testing.

In recent years, three therapeutic advances for advanced NSCLC have made accurate histologic diagnosis a critical step for developing an individualized approach to management. The first, relates to tyrosine kinase inhibitors as first line therapy in patients with advanced lung adenocarcinoma with \textit{EGFR} mutations.\textsuperscript{3} For this reason, in the new classification \textit{EGFR} mutation testing is recommended for advanced lung cancer patients with a histologic diagnosis of adenocarcinoma. Second, patients with adenocarcinoma or NSCLC, not otherwise specified (NSCLC-NOS) are more responsive to pemetrexed than those squamous cell carcinoma.\textsuperscript{4} Third, squamous cell carcinoma is associated with life threatening hemorrhage in patients treated with bevacizumab; therefore, this drug is contraindicated in lung cancer patients with this histology.\textsuperscript{5} So a pathologic diagnosis of adenocarcinoma or squamous cell carcinoma will determine patient eligibility for \textit{EGFR} mutation testing and for specific therapies. In all of these clinical trials the pathologic diagnoses were based on light microscopy with or without mucin stains but not on the basis of immunohistochemical stains.\textsuperscript{3-5}

Other emerging genetic advances hold future promise for molecular targeted therapies such as 1) crizotinib which appears to be effective in adenocarcinoma patients with EML4-ALK translocations\textsuperscript{6} and 2) the recent discoveries of FGFR1 amplification and \textit{DDR2} mutations in squamous cell carcinoma,\textsuperscript{7,8} which may render patients exquisitely sensitive to FGFR inhibition and dasatinib respectively.

**CLASSIFICATION BASED ON SMALL BIOPSIES AND CYTOLOGY**

Given the need to individualize lung cancer therapy based on the histologic type of lung cancer and molecular status, the pathologist’s role and approach to lung cancer diagnosis in small biopsies and cytology have been affected substantially.\textsuperscript{9}
The need to classify non-small cell carcinoma (NSCLC) further to distinguish squamous cell carcinoma from adenocarcinoma has not existed until recently so the frequency of NSCLC-not otherwise specified (NOS) has been increasing up to 20-40%. Pathologists now need to make an effort to make a more specific diagnosis using special stains.

Tumors that show morphologic squamous or adenocarcinoma differentiation can be classified as squamous cell carcinoma or adenocarcinoma, respectively. However, for those tumors that lack clear differentiation by morphology and would be classified as NSCLC-NOS in the past, now need to be evaluated by immunohistochemistry. The recommendation is to use a single adenocarcinoma marker such as TTF-1 and a single squamous marker such as the recently described p40 antibody. Intensive investigation of other adenocarcinoma (napsin-A) and squamous (p63, CK5/6) immunohistochemical markers are ongoing. Cytology is a powerful tool for subclassifying lung cancers in the separation of adenocarcinoma versus squamous cell carcinoma with high diagnostic accuracy that can exceed 90%. If a NSCLC-NOS by light microscopy shows a staining pattern that clearly points to adenocarcinoma (TTF-1 positive, p40 negative), the tumor can be classified as NSCLC, favor adenocarcinoma (Table 1). If such a tumor stains with a squamous pattern (p40 positive, TTF-1 negative), then it can be called NSCLC, favor squamous cell carcinoma. If the tumor does not show clear differentiation by immunohistochemistry, it should remain classified as NSCLC-NOS. These terms and criteria will help track the former NOS tumors that are being reclassified with the addition of special stains for future clinical trials as the current trial data are based on light microscopic diagnoses without special stains. Advanced stage tumors classified as adenocarcinoma, NSCLC, favor adenocarcinoma or NSCLC-NOS should be tested for EGFR mutation; mutation positive patients are eligible for tyrosine kinase inhibitor therapy. If there is no EGFR mutation, recent data suggest these patients are candidates for EML4-ALK fusion testing, and if this testing is positive, these patients may benefit from the FDA-approved drug crizotinib, and if negative these patients are eligible for pemetrexed or bevacizumab based chemotherapeutic regimens.

In the new classification, a limited use of special stains is recommended for NSCLC-NOS by light microscopy to try further classifying these tumors. Minimizing special stains is helpful to maximize the amount of tissue available for molecular testing. A new responsibility for pathologists, in addition to making a correct diagnosis is to manage these small biopsies and cytology specimens strategically so there is sufficient tissue preserved for molecular studies.

A new emphasis is placed on the need for a multidisciplinary approach to lung cancer diagnosis. One of the central proposals in this classification is that each institution should have a comprehensive strategy that addresses how to obtain these small specimens, how to process them in the pathology laboratory, how to preserve material for molecular testing, sending specimens to the molecular laboratory for expedited testing and the reporting the results in a pathology report. It may be useful to have a multidisciplinary committee to develop this strategy and to monitor issues in an ongoing fashion.
Major changes are also recommended for adenocarcinomas diagnosed in resection specimens (Table 2): 1) the term bronchioloalveolar carcinoma (BAC) should not be used anymore, because tumors previously classified as BAC are represented by five different tumors in this classification; 2) for solitary tumors measuring ≤3cm, new concepts of *adenocarcinoma in situ (AIS)* and *minimally invasive adenocarcinoma (MIA)* have been introduced for lesions that have no invasion or ≤5mm invasion, respectively; these patients should have 100% or near 100% disease free survival (DFS); 3) for invasive adenocarcinomas, comprehensive histologic subtyping is recommended for evaluation with classification according to the predominant subtype; 4) micropapillary adenocarcinoma is proposed as a new subtype with a poor prognosis; 5) the term lepidic replaces BAC for tumors with a predominant component formerly called non-mucinous BAC, and the term *lepidic predominant adenocarcinoma (LPA)* is recommended along with discontinuing the term “mixed subtype”; and 6) invasive mucinous adenocarcinoma (IMA) is the term used to replace those formerly classified as mucinous BAC. IMA are strongly correlated with *KRAS* mutation. *EGFR* mutations are reported to be associated with AIS, LPA, papillary and micropapillary patterns and EML4-ALK translocations with acinar, cribriform and signet ring patterns. 2,16 The classification stratifies resected tumors into prognostically significant histologic subtypes with excellent (AIS and MIA), intermediate (lepidic, acinar and papillary) and poor prognosis (solid, micropapillary, invasive mucinous and colloid). 17-19

In summary this classification outlines many new approaches in lung cancer diagnosis that underscore the importance of histology and genetics in individualizing treatment for patients with lung cancer. Evidence-based recommendations are made that will transform the clinical practice of all physicians involved with lung cancer diagnosis.

**Acknowledgements:**

I would like to acknowledge the contributions of all the IASLC/ATS/ERS panel members who contributed to this work and were co-authors of the article published in primary article in the Journal of Thoracic Oncology, especially Dr. Elisabeth Brambilla who contributed to development of this document.
TABLE 1: IASLC/ATS/ERS CLASSIFICATION FOR SMALL BIOPSIES/CYTOLOGY†

<table>
<thead>
<tr>
<th>2004 WHO Classification</th>
<th>Morphology/Stains</th>
<th>IASLC/ATS/ERS Terminology</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADENOCARCINOMA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed subtype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acinar</td>
<td>Morphologic</td>
<td>Adenocarcinoma, describe</td>
</tr>
<tr>
<td>Papillary</td>
<td>adenocarcinoma</td>
<td>identifiable patterns</td>
</tr>
<tr>
<td>Solid</td>
<td>patterns clearly</td>
<td>present (including</td>
</tr>
<tr>
<td>Bronchioloalveolar</td>
<td>present (including</td>
<td>micropapillary pattern</td>
</tr>
<tr>
<td>carcinoma, nonmucinous</td>
<td>morphologic</td>
<td>not included in 2004 WHO</td>
</tr>
<tr>
<td>Bronchioloalveolar</td>
<td>adenocarcinoma</td>
<td>classification)</td>
</tr>
<tr>
<td>carcinoma, mucinous</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No 2004 WHO counterpart</td>
<td>Morphologic</td>
<td>Adenocarcinoma with</td>
</tr>
<tr>
<td>– most will be solid</td>
<td>adenocarcinoma</td>
<td>lepidic pattern (if pure,</td>
</tr>
<tr>
<td>adenocarcinomas</td>
<td>patterns not</td>
<td>add note: an invasive</td>
</tr>
<tr>
<td></td>
<td>present (supported</td>
<td>component cannot be</td>
</tr>
<tr>
<td></td>
<td>by special stains,</td>
<td>excluded)</td>
</tr>
<tr>
<td></td>
<td>i.e. +TTF-1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Non-small cell carcinoma,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>favor adenocarcinoma</td>
</tr>
<tr>
<td>SQUAMOUS CELL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CARCINOMA</td>
<td>Morphologic</td>
<td>Squamous cell carcinoma</td>
</tr>
<tr>
<td></td>
<td>squamous cell</td>
<td></td>
</tr>
<tr>
<td></td>
<td>patterns clearly</td>
<td></td>
</tr>
<tr>
<td></td>
<td>present</td>
<td></td>
</tr>
<tr>
<td>No 2004 WHO counterpart</td>
<td>Morphologic</td>
<td>Non-small cell carcinoma,</td>
</tr>
<tr>
<td>– not otherwise specified</td>
<td>squamous cell</td>
<td>favor squamous cell</td>
</tr>
<tr>
<td>(NOS)‡</td>
<td>patterns not</td>
<td>carcinoma</td>
</tr>
<tr>
<td></td>
<td>present (supported</td>
<td></td>
</tr>
<tr>
<td></td>
<td>by stains i.e. +p40</td>
<td></td>
</tr>
<tr>
<td>LARGE CELL CARCINOMA</td>
<td>No clear</td>
<td>Non-small cell carcinoma,</td>
</tr>
<tr>
<td></td>
<td>adenocarcinoma,</td>
<td>not otherwise specified</td>
</tr>
<tr>
<td></td>
<td>squamous or</td>
<td>(NOS)‡</td>
</tr>
<tr>
<td></td>
<td>neuroendocrine</td>
<td></td>
</tr>
<tr>
<td></td>
<td>morphology or</td>
<td></td>
</tr>
<tr>
<td></td>
<td>staining pattern</td>
<td></td>
</tr>
</tbody>
</table>

†Modified from reference ²
‡ NSCLC-NOS pattern can be seen not only in large cell carcinomas but also when the solid poorly differentiated component of adenocarcinomas or squamous cell carcinomas are sampled but do not express immunohistochemical markers or mucin
**TABLE 2: IASLC/ATS/ERS CLASSIFICATION OF LUNG ADENOCARCINOMA IN RESECTION SPECIMENS†**

**PREINVASIVE LESIONS**

Atypical adenomatous hyperplasia

Adenocarcinoma *in situ* (≤3 cm formerly BAC)
- nonmucinous
- mucinous
- mixed mucinous/non-mucinous

**MINIMALLY INVASIVE ADENOCARCINOMA** (≤3 cm lepidic predominant tumor with ≤5 mm invasion)
- nonmucinous
- mucinous
- mixed mucinous/non-mucinous

**INVASIVE ADENOCARCINOMA**

Lepidic predominant (formerly non-mucinous BAC pattern, with >5 mm invasion)

Acinar predominant

Papillary predominant

Micropapillary predominant

Solid predominant with mucin production

**VARIANTS OF INVASIVE ADENOCARCINOMA**

Invasive mucinous adenocarcinoma (formerly mucinous BAC)

Colloid

Fetal (low and high grade)

Enteric

†From reference 2
Reference List


(10) Ou SH, Zell JA. Carcinoma NOS is a Common Histologic Diagnosis and is Increasing in Proportion Among Non-small Cell Lung Cancer Histologies. *J Thorac Oncol* 2009;4:1202-1211.


UPDATES IN THE SURGICAL PATHOLOGY OF LUNG CANCER

Four “Ps” of Pulmonary Cytopathology: Procedural, Predictive, Personalized and Participatory

American Society of Cytopathology, USCAP Vancouver, BC; March 18, 2012

William D. Travis, M.D.
Attending Thoracic Pathologist
Memorial Sloan Kettering Cancer Center
New York, NY
RATIONALE FOR NEW ADENOCARCINOMA CLASSIFICATION

- Lung cancer – most frequent cause major cancer incidence/mortality worldwide
- Adenocarcinoma – the most common histologic subtype
- Widely divergent clinical, radiologic, molecular & pathologic spectrum
- Bronchioloalveolar carcinoma (BAC) – confusing used many different ways despite 99/04 WHO; mucinous/nonmucinous
- Rapid evolving molecular advances (EGFR)
IASLC/ATS/ERS ADENOCARCINOMA CLASSIFICATION: MULTIDISCIPLINARY APPROACH

- Prior WHO classifications: by pathologists
- Due to remarkable advances in past 10 yrs: oncology, molecular, radiology, surgery: need for integrated multidisciplinary approach
- International Association for the Study of Lung Cancer (IASLC); American Thoracic Society (ATS), European Respiratory Society (ERS)
- Panel: Pathologists, Oncologists, Radiologists, Molecular Biologists, Surgeons
“The Lung Adenocarcinoma Oncogenome”

Pie chart of mutually exclusive mutations (2010)

- MEK (2008)
- ERBB2 (2004)
- BRAF (2002)
- EML4-ALK (2007)
- NF1 (2008)
- KRAS (1987)
- EGFR (2004)

+ Overlapping mutations: p53 (30%), LKB1 (15%), PIK3CA (2%)

Courtesy of Marc Ladanyi, MSKCC
International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society International Multidisciplinary Classification of Lung Adenocarcinoma

William D. Travis, MD, Elisabeth Brambilla, MD, Masayuki Noguchi, MD, Andrew G. Nicholson, MD, Kim R. Geisinger, MD, Yasushi Yatabe, MD, David G. Beer, PhD, Charles A. Powell, MD, Gregory J. Riely, MD, Paul E. Van Schil, MD, Kavita Garg, MD, John H. M. Austin, MD, Hisao Asamura, MD, Valerie W. Rusch, MD, Fred R. Hirsch, MD, Giorgio Scagliotti, MD, Tetsuya Mitsudomi, MD, Rudolf M. Huber, MD, Yuichi Ishikawa, MD, James Jett, MD, Montserrat Sanchez-Cespedes, PhD, Jean-Paul Sculier, MD, Takashi Takahashi, MD, Masahiro Tsuboi, MD, Johan Vansteenkiste, MD, Ignacio Wistuba, MD, Pan-Chyr Yang, MD, Denise Aberle, MD, Christian Brambilla, MD, Douglas Flieder, MD, Wilbur Franklin, MD, Adi Gazdar, MD, Michael Gould, MD, MS, Philip Hasleton, MD, Douglas Henderson, MD, Bruce Johnson, MD, David Johnson, MD, Keith Kerr, MD, Keiko Kuriyama, MD, Jin Soo Lee, MD, Vincent A. Miller, MD, Iver Petersen, MD, PhD, Victor Roggli, MD, Rafael Rosell, MD, Nagahiro Saijo, MD, Erik Thunnissen, MD, Ming Tsao, MD, and David Yankelewitz, MD

Journal of Thoracic Oncology 6(2):244-485, 2011
Personalized Therapy For Lung Cancer Patients is Driven by Histology and Genetics

- Predictive of response
  - EGFR mutation (adenocarcinoma) – EGFR TKI’s
  - Adenocarcinoma or NSCLC-NOS – pemetrexed
  - EML4-ALK translocation (adenocarcinoma) – crizotinib

- Predictive of toxicity
  - Bevacizumab – contraindicated in life-threatening hemorrhage in squamous carcinoma
Results in \textit{EGFR} mutation positive and negative patients (All Asian, 94\% Never Smokers)

- \textbf{EGFR mutation positive}
  - Gefitinib (\(n=132\))
  - Carboplatin / paclitaxel (\(n=129\))
  - HR (95\% CI) = 0.48 (0.36, 0.64)
  - \(p<0.0001\)
  - No. events gefitinib: 97
  - No. events Chemo: 111

- \textbf{EGFR mutation negative}
  - Gefitinib (\(n=91\))
  - Carboplatin / paclitaxel (\(n=85\))
  - HR (95\% CI) = 2.85 (2.05, 3.98)
  - \(p<0.0001\)
  - No. events gefitinib: 88
  - No. events Chemo: 70

# First Line TKI Therapy in *EGFR* Mutated NSCLC

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Treatmen t Arm</th>
<th>Control Arm</th>
<th>Stage</th>
<th>Median PFS</th>
<th>Median OS</th>
<th>Indication</th>
<th>Study Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mok T N Engl J Med 361:947, 2009 IPASS</td>
<td>1217</td>
<td>Gefitinib</td>
<td>Carboplatin/Pactilaxel</td>
<td>IIIB/IV</td>
<td>5.7 vs 5.8 mo (HR for EGFR mutated pts 0.48; HR for nmutated pts 2.84)</td>
<td>18.6 vs 17.3 months ($P = NS$)</td>
<td>First-line</td>
<td>Randomized Phase III</td>
</tr>
<tr>
<td>Mitsudomi T Lancet Oncol 11:121, 2010 WJTOG3405</td>
<td>177 (M+)</td>
<td>Gefitinib</td>
<td>Cisplatin, Docetaxel</td>
<td>IIIB/IV</td>
<td>9.2 vs 6.3 mo ($P &lt; 0.001$)</td>
<td>First-line</td>
<td>Randomized Phase III</td>
<td></td>
</tr>
<tr>
<td>Maemondo M N Engl J Med 362:2380, 2010</td>
<td>230 (M+)</td>
<td>Gefitinib</td>
<td>Carboplatin, Paclitaxel</td>
<td>IIIB/IV</td>
<td>10.8 vs 5.4 mo (HR 0.3, $P &lt; 0.0001$)</td>
<td>30.5 vs 23.6 months ($P = NS$)</td>
<td>First-line</td>
<td>Randomized Phase III</td>
</tr>
<tr>
<td>Zhou C Lancet Oncol 12:735, 2011 Optimal</td>
<td>165 (M+)</td>
<td>Erlotinib</td>
<td>Carboplatin/Gemciatbine</td>
<td>IIIB/IV</td>
<td>13.6 vs 4.6 mo (HR 0.16, $P &lt; 0.0001$)</td>
<td>First-line</td>
<td>Randomized Phase III</td>
<td></td>
</tr>
<tr>
<td>Rosell R J Clin Oncol 29: Suppl 7503 Eurtac</td>
<td>153 (M+)</td>
<td>Erlotinib</td>
<td>Platinum-based chemothera py</td>
<td>IIIB/IV</td>
<td>9.4 vs 5.2 mo (HR, 0.42, $P &lt; 0.0001$)</td>
<td>22.9 vs 18.8 months ($P = 0.42$)</td>
<td>First-line</td>
<td>Randomized Phase III</td>
</tr>
</tbody>
</table>
CLINICAL RECOMMENDATION

- In patients with advanced lung adenocarcinoma we recommend testing for EGFR mutation (strong recommendation, moderate quality evidence).

- Remarks: This is a strong recommendation because potential benefits clearly outweigh harms. This recommendation assumes that correct classification by EGFR mutation status is associated with important benefit based upon randomized phase 3 clinical trials of EGFR TKI therapy which demonstrate a predictive benefit for response rate and progression-free survival, but not overall survival, as well as subset analyses of multiple additional studies.

Travis WD et al; JTO 6:244-285, 2011
PEMETREXED IS MORE EFFECTIVE IN ADCA AND LARGE CELL CA THAN SQUAMOUS CA

Scaglioni G et al JCO 26:3543-3551, 2008

- Patients Randomized to Pemetrexed

---

- Non-squamous:
  - Median = 9.2

- Squamous:
  - Median = 6.2

---

- Overall Survival p=0.001
- Prog Free Survival (not shown) p=0.004
CRIZOTINIB ALK INHIBITON IN NSCLC

– Kwak EL et al; NEJM 2010; 363:1693
Initial Therapy of Advanced Adenoca or NSCLC-NOS

- Adenocarcinoma
  - Large cell ca
  - NSCLC-NOS

- EGFR Mutation
  - Exon 19 del
    - Exon 21 L858R, L861X
    - Exon 18 G719A/S
- Neg EGFR mut
  - Pos EML4-ALK
- Neg EGFR mut
  - Neg EML4-ALK
- Unknown EGFR Mutation & ALK Status

- Erlotinib/Gefitinib
  - ±
    - Pem/Bev/Cis
- Crizotinib
- Pemetrexed
  - Bevacizumab
  - Cisplatin

Modified from Mark Kris, Chief, Thoracic Oncology, MSKCC
Molecular Characteristics Used to Select Patients for Protocols at MSK

<table>
<thead>
<tr>
<th>Trait</th>
<th>Protocol/Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EGFR</strong></td>
<td>PF-00299804 Erlotinib (Adjuvant)</td>
</tr>
<tr>
<td><strong>EGFR + T790M</strong></td>
<td>BIBW 2992 + Cetuximab</td>
</tr>
<tr>
<td><strong>KRAS</strong></td>
<td>Ridaforolimus</td>
</tr>
<tr>
<td></td>
<td>GI-4000 (G12C,V,D) (Adjuvant)</td>
</tr>
<tr>
<td><strong>MET Amplification</strong></td>
<td>Crizotinib</td>
</tr>
<tr>
<td><strong>EML4-ALK</strong></td>
<td>Crizotinib</td>
</tr>
<tr>
<td><strong>BRAF, MEK</strong></td>
<td>Zelboraf (vemurafenib)</td>
</tr>
<tr>
<td></td>
<td>Selumetinib (AZ6244)</td>
</tr>
<tr>
<td></td>
<td>GSK2118434</td>
</tr>
<tr>
<td><strong>HER2</strong></td>
<td>PF-00299804</td>
</tr>
<tr>
<td><strong>PIK3CA</strong></td>
<td>GSK2141795</td>
</tr>
</tbody>
</table>

Courtesy of Mark Kris, Chief, Thoracic Oncology, MSKCC
## NCI – TCGA – The Cancer Genome Atlas
### Preliminary Analysis 150 Squamous Ca

<table>
<thead>
<tr>
<th>Gene</th>
<th>Event type</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>FGFR1</td>
<td>Amplification</td>
<td>20-25%</td>
</tr>
<tr>
<td>FGFR2</td>
<td>Mutation</td>
<td>5%</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>Mutation</td>
<td>9%</td>
</tr>
<tr>
<td>PTEN</td>
<td>Mutation/Deletion</td>
<td>18%</td>
</tr>
<tr>
<td>CCND1</td>
<td>Amplification</td>
<td>8%</td>
</tr>
<tr>
<td>CDKN2A</td>
<td>Deletion/mutation</td>
<td>45%</td>
</tr>
<tr>
<td>PDGFRA</td>
<td>Amplification/mutation</td>
<td>9%</td>
</tr>
<tr>
<td>EGFR</td>
<td>Amplification</td>
<td>10%</td>
</tr>
<tr>
<td>MCL1</td>
<td>Amplification</td>
<td>10%</td>
</tr>
<tr>
<td>DDR2</td>
<td>Mutation</td>
<td>4%</td>
</tr>
<tr>
<td>ERBB2</td>
<td>Amplification</td>
<td>2%</td>
</tr>
</tbody>
</table>

Possible therapeutic target identified in 63% of Squamous cell ca

Targets need to be validated in pre-clinical models


FGFR1/2, PIK3CA and DDR2 inhibitor trials are planned or ongoing

Hammerman P, JTO 2011; 6: S2, S39
LUNG ADENOCARCINOMA

CLASSIFICATION IN SMALL BIOPSY AND CYTOLOGY SPECIMENS

Because this was never addressed by WHO, by necessity other histologies needed to be addressed
NON-SMALL CELL LUNG CANCER: 70% PRESENT IN ADVANCED STAGE
SMALL BIOPSY/CYTOLOGY LUNG CANCER DIAGNOSIS: IN USA OVER 130,000 CASES IN 2011

- 2011: ACS estimates for USA:
  - 221,130 Lung Cancers
- 85% NSCLC = 187,961 (15% SCLC)
- 70% Advanced Stage = 131,572
  - Unresectable: Diagnosed by small biopsies/cytology
PHASE III STUDY COMPARING CISPLATIN PLUS GEMCITABINE WITH CISPLATIN & PEMETREXED IN ADVANCED NSCLC

PHASE III STUDY COMPARING CISPLATIN PLUS GEMCITABINE WITH CISPLATIN & PEMETREXED IN ADVANCED NSCLC

IN THIS STUDY APPROXIMATELY 20% OF CASES REPRESENT NSCLC-NOS

SPECIAL STAINS ARE INTRODUCED TO CLASSIFY NSCLC-NOS FURTHER

Travis WD et al; JTO 6:244-285, 2011
### TABLE 2: PROPOSED IASLC/ATS/ERS CLASSIFICATION FOR SMALL BIOPSIES/CYTOLOGY

<table>
<thead>
<tr>
<th>2004 WHO Classification</th>
<th>SMALL BIOPSY/CYTOMA/IASLC/ATS/ERS</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADENOCARCINOMA</td>
<td></td>
</tr>
<tr>
<td>Mixed subtype</td>
<td></td>
</tr>
<tr>
<td>Acinar</td>
<td></td>
</tr>
<tr>
<td>Papillary</td>
<td></td>
</tr>
<tr>
<td>Solid</td>
<td></td>
</tr>
<tr>
<td>Bronchioloalveolar cancer (nonmucin)</td>
<td></td>
</tr>
<tr>
<td>Bronchioloalveolar carcinomas (mucinous)</td>
<td></td>
</tr>
<tr>
<td>Terad</td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma with fetal pattern</td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma with ciliated pattern</td>
<td></td>
</tr>
<tr>
<td>Signet ring</td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma with (describe patterns present) and signet ring features</td>
<td></td>
</tr>
<tr>
<td>Clear cell</td>
<td></td>
</tr>
<tr>
<td>Non 2004 WHO counterpart — most will be solid adenocarcinoma</td>
<td></td>
</tr>
<tr>
<td>Morphologic adenocarcinoma patterns not present (supported by special stains)</td>
<td></td>
</tr>
<tr>
<td>Non-small cell carcinoma, favor adenocarcinoma</td>
<td></td>
</tr>
</tbody>
</table>

| SQAMOUS CELL CARCINOMA   |                                  |
| Papillary                |                                  |
| Clear cell               |                                  |
| Small cell               |                                  |
| Basaloid                 |                                  |
| Non 2004 WHO counterpart — most will be squamous cell carcinoma |                     |
| Morphologic squamous cell patterns clearly present | |
| Squamous cell carcinoma  |                                  |

| SMALL CELL CARCINOMA     |                                  |
| Small cell carcinoma     |                                  |

| LARGE CELL CARCINOMA     |                                  |
| Large cell neuroendocrine carcinoma (LCNEC) |                       |
| Non-small cell carcinoma with neuroendocrine (NE) morphology (positive NE markers), possible LCNEC |        |
| Large cell carcinoma with NE morphology (LCNEC) |                       |
| Non-small cell carcinoma with NE morphology (negative NE markers), see comment |        |
| Comment: This is a non-small cell carcinoma where LCNEC is suspected, but data failed to demonstrate NE differentiation | |

| ADENOSQUAMOUS CARCINOMA  |                                  |
| Morphologic squamous cell and adenocarcinoma patterns present; Non-small cell carcinoma, with squamous cell and adenocarcinoma pattern |                     |
| Comment: this could represent adenocarcinoma carcinomas | |

| No counterpart in 2004 WHO classification |                                  |
| Morphologic squamous cell or adenocarcinoma patterns not present; Large adenocarcinomas favor separate glandular and adenocarcinoma components | |

| Sarcomatoid carcinomas | Poorly differentiated NSCLC with spindle and/or giant cell carcinomas (mention of adenocarcinoma or squamous carcinoma are present) |

---

Journal of Thoracic Oncology 6(2):244-485, 2011
<table>
<thead>
<tr>
<th>2004 WHO CLASSIFICATION</th>
<th>2011 IASLC/ATS/ERS CLASSIFICATION</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ADENOCARCINOMA</strong></td>
<td><strong>Morphologic adenocarcinoma patterns clearly present:</strong> Adenocarcinoma, describe identifiable patterns present (including micropapillary pattern not included in 2004 WHO classification)</td>
</tr>
<tr>
<td>Mixed subtype</td>
<td></td>
</tr>
<tr>
<td>Acinar</td>
<td></td>
</tr>
<tr>
<td>Papillary</td>
<td></td>
</tr>
<tr>
<td>Solid</td>
<td></td>
</tr>
<tr>
<td>Bronchioloalveolar</td>
<td></td>
</tr>
<tr>
<td>No 2004 WHO counterpart – most will be solid adenocarcinomas</td>
<td><strong>Morphologic adenocarcinoma patterns not present (supported by special stains):</strong> Non-small cell carcinoma, favor adenocarcinoma</td>
</tr>
<tr>
<td><strong>SQUAMOUS CELL CARCINOMA</strong></td>
<td><strong>Morphologic squamous cell patterns clearly present:</strong> Squamous cell carcinoma</td>
</tr>
<tr>
<td>Papillary</td>
<td></td>
</tr>
<tr>
<td>Clear cell</td>
<td></td>
</tr>
<tr>
<td>Small cell</td>
<td></td>
</tr>
<tr>
<td>Basaloid</td>
<td></td>
</tr>
<tr>
<td>No 2004 WHO counterpart</td>
<td><strong>Morphologic squamous cell patterns not present (supported by stains):</strong> Non-small cell carcinoma, favor squamous cell carcinoma</td>
</tr>
<tr>
<td><strong>LARGE CELL CARCINOMA</strong></td>
<td>Non-small cell carcinoma, not otherwise specified (NOS)</td>
</tr>
</tbody>
</table>

IMMUNOHISTOCHEMICAL MARKERS

- ADENOCARCINOMA (ONE MARKER)
  - TTF-1 (best), Napsin, PE-10

- SQUAMOUS CARCINOMA (ONE MARKER)
  - p40 (best), p63, CK5/6, 34βE12
  - Desmocolin-3 (need more testing)

- Cocktails – nuclear/cytoplasmic antibodies
  - Adenoca – TTF-1/Napsin
  - Squamous – p63/CK5/6
p40 (ΔNp63) is superior to p63 for the diagnosis of pulmonary squamous cell carcinoma

Justin A Bishop¹, Julie Teruya-Feldstein², William H Westra¹, Giuseppe Pelosi³, William D Travis² and Natasha Rekhtman²

– p40 antibody (5-17, Calbiochem, 1:2000)
NSCLC-NOS
BY LIGHT MICROSCOPY
NSCLC – FAVOR
ADENOCARCINOMA

TTF-1

P63
NSCLC – FAVOR ADENOCARCINOMA TOUCH PREP CYTOLOGY
NSCLC-NOS, FAVOR ADENOCARCINOMA CONFIRMED BY MOLECULAR

- **EGFR mutation** - negative
  - Exon 19 deletion
  - Exon 21 L858R mutation
- **KRAS mutation** - positive
  - G12V
- Results favor adenocarcinoma
CYTOLOGY IS A POWERFUL TOOL FOR CLASSIFYING NSCLC

Suitability of Thoracic Cytology for New Therapeutic Paradigms in Non-small Cell Lung Carcinoma

High Accuracy of Tumor Subtyping and Feasibility of EGFR and KRAS Molecular Testing

Natasha Rekhtman, MD, PhD,* Suzanne M. Brandt, MD,* Carlie S. Sigel, MD,* Maria A. Friedlander, MPA, CT (ASCP),* Gregory J. Riely, MD, PhD,† William D. Travis, MD,* Maureen F. Zakowski, MD,* and Andre L. Moreira, MD, PhD*

J Thoracic Oncol 6:451-8, 2011
NSCLC diagnosed by light microscopy in small biopsies/cytology

- Squamous Cell Carcinoma: 20-30%
- NSCLC-NOS: 20-40%
- Adeno-Carcinoma: 40-50%

Historically, NSCLC-NOS has been encouraged because there was no reason to classify these tumors further. As a result, 20-40% of NSCLC in small biopsies/cytology are currently being diagnosed as NSCLC-NOS.
NSCLC-NOS: 20-40% of NSCLC

NSCLC, Favor Squamous Cell Carcinoma

NSCLC-NOS: <5%

NSCLC, Favor Adeno-Carcinoma

Metastasis or Other Tumor
LIGHT MICROSCOPY

FORMER NSCLC-NOS: 20-40% OF NSCLC

NSCLC-NOS:
- 20-30%
- 20-40%

ADENO-CARCINOMA:
- 40-50%

SQUAMOUS CELL CARCINOMA:
- 20-30%

NEW CLASSIFICATION

NSCLC-NOS
Goal <5%
NEED TO DISCRIMINATE BETWEEN DIAGNOSES BASED ON LIGHT MICROSCOPY VS LM & IHC

- The only validation of histology for EGFR mutation/TKI’s, Pemetrexed and Bevacizumab is by light microscopy alone

- The use of IHC for diagnosis is not validated in clinical trials
EGFR MUTATION SPECIFIC ANTIBODIES (Cell Signaling)

- **Exon 19 deletion**
  - All 20 cases with 15-bp deletion were MS Ab positive (sensitivity 100%, specificity 99%)
  - 35 other than the common 15bp deletion – 49% stained positively (sensitivity 74%)

- **EGFR L858R mutation**
  - 17/18 cases were positive with MS Ab (sensitivity 95%, specificity 99%); better if use 2+/3+ for positive

EGFR EXON 21 L858R MUTATION SPECIFIC AB

EGFR EXON 19 DELETION MUTATION SPECIFIC AB

Each group of thoracic physicians (clinicians, radiologists, surgeons, pathologists, molecular biologists) must develop a strategy to manage tissues:

- Obtaining biopsies or cytology samples
- Optimal processing by laboratories/pathologists for diagnosis AND molecular studies
- Pathologists should be the leader of this
KEY PRINCIPLES

- Minimize diagnostic stains to maximize tissue for molecular studies
- Molecular testing is reliable on FFPE tissues – even very small samples
- Unstained slides (n=10-15) provide adequate DNA if sufficient tumor
- Cytology fluids (i.e. pleural) – cytospin and make cell block (for IHC/molecular)
PERSONALIZED MEDICINE IN ADVANCED LUNG CANCER PATIENTS IS DRIVEN BY HISTOLOGY AND GENETICS
LUNG ADENOCARCINOMA

CLASSIFICATION IN RESECTION SPECIMENS
OLD BAC CONCEPT
FIVE PLACES IN NEW CLASSIFICATION

1. Adenocarcinoma in situ (AIS) which can be non-mucinous and rarely mucinous
2. Minimally invasive adenocarcinoma
3. Invasive adenocarcinoma with predominant nonmucinous lepidic pattern
4. Invasive adenocarcinoma with less than predominant nonmucinous lepidic pattern (probably most formerly clinically advanced adenocarcinomas with BAC pattern)
5. Mucinous adenocarcinoma with lepidic pattern
IASLC/ATS/ERS ADENOCARCINOMA CLASSIFICATION

- PREINVASIVE LESIONS
  - ATYPICAL ADENOMATOUS HYPERPLASIA
  - ADENOCARCINOMA IN SITU (≤3 cm, formerly BAC pattern) †
    - non-mucinous
    - mucinous
- MINIMALLY INVASIVE ADENOCARCINOMA (≤3 cm, a lepidic predominant tumor with ≤5mm invasion)

- INVASIVE ADENOCARCINOMA
  † Size should be specified. AIS and MIA should be completely sampled histologically
ATYPICAL ADENOMATOUS HYPERPLASIA
ADENOCARCINOMA IN SITU NONMUCINOUS
ADENOCARCINOMA IN SITU NONMUCINOUS
IASLC/ATS/ERS ADENOCARCINOMA CLASSIFICATION

- PREINVASIVE LESIONS
  - ATYPICAL ADENOMATOUS HYPERPLASIA
  - ADENOCARCINOMA IN SITU (≤3 cm, formerly BAC pattern) †
    - non-mucinous
    - mucinous

- MINIMALLY INVASIVE ADENOCARCINOMA (≤3 cm, a lepidic predominant tumor with ≤5mm invasion)

- INVASIVE ADENOCARCINOMA
  † Size should be specified. AIS and MIA should be completely sampled histologically
MINIMALLY INVASIVE ADENOCARCINOMA
NONMUCINOUS
MINIMALLY INVASIVE ADENOCA NONMUCINOUS
IASLC/ATS/ERS ADENOCARCINOMA CLASSIFICATION

INVASIVE ADENOCARCINOMA

- **Lepidic pattern predominant** (formerly non-mucinous BAC pattern)
- Acinar pattern predominant
- Papillary pattern predominant
- Micropapillary pattern, predominant
- Solid pattern predominant

*(Comprehensive histologic subtyping: semiquantitative assessment of patterns in 5-10% increments)*
LEPIDIC PREDOMINANT
MICRO-PAPILLARY

SOLID WITH MUCIN

DPAS STAIN
INVASIVE MUCINOUS ADENOCARCINOMA
INVASIVE MUCINOUS ADENOCARCINOMA
Frequent *KRAS* mutations
STAGE I ADENOCARCINOMA (N=514) 
RECURRENCE-FREE SURVIVAL (RFS) BY IASLC HISTOLOGIC TYPE

<table>
<thead>
<tr>
<th>Histologic Type</th>
<th>(N)</th>
<th>5 Year RFS %</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIS (1)</td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>MIA (8)</td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Lepidic NM (29)</td>
<td></td>
<td>90</td>
</tr>
<tr>
<td>Papillary (143)</td>
<td></td>
<td>83</td>
</tr>
<tr>
<td>Acinar (232)</td>
<td></td>
<td>85</td>
</tr>
<tr>
<td>Inv Mucinous Ad (13)</td>
<td></td>
<td>76</td>
</tr>
<tr>
<td>Solid (67)</td>
<td></td>
<td>71</td>
</tr>
<tr>
<td>Micropapillary (12)</td>
<td></td>
<td>64</td>
</tr>
<tr>
<td>Colloid (9)</td>
<td></td>
<td>71</td>
</tr>
</tbody>
</table>

Yoshizawa, A et al; Modern Pathology 24: 653-664, 2011
MICROPAPILLARY ADCA IS AN INDEPENDENT PREDICTOR OF RECURRENCE IN LIMITED RESECTIONS (<=2CM)

COMBINED ARCHITECTURAL AND MITOTIC GRADING

-Kadota K et al (in preparation)
Tumor Size – Difficult to Appreciate Grossly If Lepidic Growth
CASE 1: 68F, Multiple, bilateral GGN
Dx: Adenocarcinoma with lepidic pattern
CASE 1: 68F, Multiple, bilateral GGN

PATHOLOGY

- **Dx:** Adenocarcinoma with lepidic pattern

PATHOLOGY DIFFERENTIAL DIAGNOSIS

- Adenocarcinoma *in situ*
- Minimally invasive adenocarcinoma
- Adenocarcinoma with lepidic pattern
  - Lepidic predominant
  - Invasive predominant
CASE 1: 68F, Multiple, bilateral GGN
CASE 1: 68F, Multiple, bilateral GGN
RADIOLOGIC PATHOLOGIC CORRELATION

- **Most likely diagnosis:**
  - Adenocarcinoma *in situ*
  - Minimally invasive adenocarcinoma

- **Less likely diagnosis**
  - Adenocarcinoma with lepidic pattern
    - Lepidic predominant
    - Invasive predominant
CASE 2: 73F, Left Lung Mass
Adenocarcinoma with lepidic pattern
CASE 2: 73F, Left Lung Mass
Adenocarcinoma with lepidic pattern – possible focus of invasion
CASE 2: 73F, Left Lung Mass

PATHOLOGY

- Dx: Adenocarcinoma with lepidic pattern – focus suspicious for invasion

PATHOLOGY DIFFERENTIAL DIAGNOSIS

- Adenocarcinoma \textit{in situ}
- Minimally invasive adenocarcinoma
- Adenocarcinoma with lepidic pattern
  - Lepidic predominant
  - Invasive predominant
CASE 2: 73F, Left Lung Mass
Part Solid Nodule
CASE 2: 73F, Left Lung Mass
RADIOLOGIC PATHOLOGIC CORRELATION

- Most likely diagnosis:
  - Adenocarcinoma with lepidic pattern
    - Invasive predominant
    - Lepidic predominant

- Less likely diagnosis
  - Adenocarcinoma \textit{in situ}
  - Minimally invasive adenocarcinoma