PULMONARY PATHOLOGY JOURNAL CLUB
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Discussion articles
Munfus-McCray et al. Comparison of EGFR and KRAS mutations in primary and unpaired metastatic lung adenocarcinoma with potential chemotherapy effect.

Purpose: To compare prevalence of EGFR and KRAS mutations in primary and metastatic lung adenocarcinomas, and to compare treatment naïve patients with patients treated with chemotherapy.

Methods: Retrospective comparison of 379 primary (213) and unpaired metastatic (166) lung adenocarcinomas collected over a period of 2 and 2.5 years, respectively, at Johns Hopkins.

- 305 surgical specimens, 75 FNA cell blocks
- EGFR sequencing (exons 18 to 21) performed at Genzyme
- KRAS sequencing (codons 12/13, exon 2) performed at Hopkins

Results:

- Median age 74 (23-90)
- 198 (52%) women
- chemotherapy variable: carboplatin-based (37), cisplatin based (21), other (6), unknown (6)
  - # of cycles 1-18 (mean 6)
  - interval from last does to mutational analysis: mean = 9 months (<1 – 72 months)

<table>
<thead>
<tr>
<th>Mutations</th>
<th>Primary adenocarcinoma</th>
<th>Metastatic adenocarcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prechemotherapy a</td>
<td>Postchemotherapy a</td>
</tr>
<tr>
<td>EGFR</td>
<td>31 (17.6%)</td>
<td>6 (16.2%)</td>
</tr>
<tr>
<td>KRAS</td>
<td>64 (36.4%)</td>
<td>20 (54.1%)</td>
</tr>
<tr>
<td>WT</td>
<td>81 (46.0%)</td>
<td>11 (29.7%)</td>
</tr>
<tr>
<td>Total</td>
<td>176 (100%)</td>
<td>37 (100%)</td>
</tr>
<tr>
<td>P</td>
<td>.1142</td>
<td></td>
</tr>
</tbody>
</table>

a They are unpaired cases.

- Prevalence of EGFR and KRAS mutations were
  - NOT DIFFERENT in treatment naïve primary vs metastatic tumors
  - NOT DIFFERENT in treated versus untreated primary tumors
  - DIFFERENT (P < 0.5) between treated and untreated metastatic tumors

Take-home message: Weak retrospective, unpaired study design with highly variable treatment regimens, but at last corroborates previous observations suggesting that the fidelity of the mutational landscape may be altered with treatment.

**Purpose:** To study the effect of a histologically based 3-tier grading system on rates of regional lymph node and distant metastases using a variation on the terminology proposed in the 2011 IASLC adenocarcinoma consensus statement.


- In-situ and minimally invasive adenocarcinomas excluded for this study.
- Two patients who underwent neoadjuvant therapy with extensive tumor response excluded (not clear if they included patients who underwent neoadjuvant therapy without a significant response)
- Histologic growth patterns were assessed in 5% increments and assigned a “predominant” (undefined) histologic type
  - acinar category was subdivided into tubular and cribriform groups
- Also assessed, mitotic rate (#/10 hpf), nucleolar size (grade 0 – 3: inconspicuous, <, =, > RBC), nuclear variation (< 2x, 2x, 2x), necrosis (grade 0 – 3: < 1mm, 0.5 mm, 0.5-5 mm, >5mm), desmoplasia, lymphovascular (± D2-40), venous/arterial invasion (± elastic stain)

**Results:**

- n = 125; remarkably NO data on stage/tumor size!

<table>
<thead>
<tr>
<th>Adenocarcinomas by Predominant Histologic Pattern</th>
<th>N (%)</th>
<th>N – “pure”?</th>
<th>Age ± SD</th>
<th>Women:Men</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lepidic</td>
<td>9 (7.2%)</td>
<td>0</td>
<td>69 ± 9</td>
<td>7:2</td>
</tr>
<tr>
<td>Tubular</td>
<td>57 (29.6%)</td>
<td>1 (2.7%)</td>
<td>67 ± 10</td>
<td>20:17</td>
</tr>
<tr>
<td>Cribriform</td>
<td>34 (27.2%)</td>
<td>3 (8.8%)</td>
<td>62 ± 10</td>
<td>17:17</td>
</tr>
<tr>
<td>Papillary</td>
<td>11 (8.8%)</td>
<td>1 (9.1%)</td>
<td>71 ± 8</td>
<td>4:7</td>
</tr>
<tr>
<td>Mucinous</td>
<td>11 (8.8%)</td>
<td>7 (63.6%)</td>
<td>66 ± 12</td>
<td>4:7</td>
</tr>
<tr>
<td>Solid</td>
<td>23 (18.4%)</td>
<td>9 (39.1%)</td>
<td>64 ± 8</td>
<td>12:11</td>
</tr>
<tr>
<td>TOTAL</td>
<td>125</td>
<td>21 (16.8%)</td>
<td>66 ± 10</td>
<td>64:61</td>
</tr>
</tbody>
</table>

*micropapillary component in 13 (10.4%) of cases, 10 of which were cribriform predominant; micropapillary component accounted for 5% to 30% of affected tumors

- Solid and cribriform patterns showed highest correlation with mitotic rate, nucleolar grade, LVI and necrosis (papillary a close third). No correlation between pattern and desmoplasia/anisonucleosis.
- Rates of LN mets highest in solid > cribriform tumors; distant mets highest in solid > mucinous tumors
- In multivariate analysis, mitotic activity and % solid component showed strongest correlation with level II LN or distant mets (followed by nucleoli, vasc invasion, desmoplasia, necrosis, anisonucleosis)

<table>
<thead>
<tr>
<th>Grade (n)</th>
<th>Predominant Histologic Pattern</th>
<th>Adverse “cytologic” Features (≥20% solid or micropap, mit &gt; 1/10 hpf, vasc invasion, nucleolomegaly (≥ gr 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>well differentiated (40)</td>
<td>lepidic, tubular, papillary &amp;</td>
<td>absent</td>
</tr>
<tr>
<td>moderately differentiated (46)</td>
<td>cribriform &amp;/or</td>
<td>present</td>
</tr>
<tr>
<td>poorly differentiated (39)</td>
<td>≥ 20% solid</td>
<td>present/absent</td>
</tr>
</tbody>
</table>

- No LN/distant mets in well diff, highest rates in poorly diff, intermediate in mod diff.

**Take-home message:** In resected lung adenocarcinomas grading matters and is predicated largely on histologic pattern: solid (and micropapillary?) is worst, cribriform ain’t great, and lepidic/tubular/papillary are best.

**Purpose:** Previously published subset analysis of phase III trial showed longer survival with cisplatin + pemetrexed in patients with non-squamous cell histology while patients with squamous cell carcinoma lived longer with cisplatin + gemcitabine. This study was intended to further assess relationship between chemotherapeutic agents, histology, and outcomes in patients treated with different platin doublets for advanced stage NSCLC.

**Methods:** Retrospective analysis of previously published 2002 ECOG phase III trial comparing 4 different platin-based regimens in patients with advanced stage NSCLC.

- n = 1,139
- stage IIIB with malignant pleural/pericardial effusion, stage IV, or recurrent NSCLC
- no prior chemotherapy
- randomly assigned to cisplatin + paclitaxel (CP – reference arm), cisplatin + gemcitabine (CG), cisplatin + docetaxel (CD), or carboplatin + paclitaxel (CbP)
- primary endpoints overall (OS) and progression free (PFS) survival.

**Results:**

- adenocarcinoma (AC) – 647 (56.8%); squamous cell carcinoma (SCC) – 224 (19.7%); large cell carcinoma (LCC) – 74 (6.5%); NOS/other – 194 (17.0%)

<table>
<thead>
<tr>
<th></th>
<th>AC (647)</th>
<th>SCC (224)</th>
<th>LCC (74)</th>
<th>NOS/other (194)</th>
<th>ALL (1,139)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age – median</td>
<td>61</td>
<td>63</td>
<td>63</td>
<td>61</td>
<td>61</td>
</tr>
<tr>
<td>(range)</td>
<td>(25-86)</td>
<td>(39-79)</td>
<td>(44-78)</td>
<td>(32-83)</td>
<td>(25-86)</td>
</tr>
<tr>
<td>Women (%)*</td>
<td>41</td>
<td>27.2</td>
<td>31.1</td>
<td>38.1</td>
<td>37.1</td>
</tr>
<tr>
<td>Disease stage</td>
<td>IIB</td>
<td>IV/recurrent</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(%)</td>
<td>15.6</td>
<td>12.1</td>
<td>10.8</td>
<td>7.7</td>
<td>13.3</td>
</tr>
<tr>
<td>median PFS (mos)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>overall</td>
<td>3.8</td>
<td>3.6</td>
<td>4.2</td>
<td>2.9</td>
<td></td>
</tr>
<tr>
<td>CP</td>
<td>3.7</td>
<td>2.6</td>
<td>3.5</td>
<td>2.8</td>
<td>3.4</td>
</tr>
<tr>
<td>CG</td>
<td>4.4</td>
<td>4.3</td>
<td>4.5</td>
<td>3.4</td>
<td>NS</td>
</tr>
<tr>
<td>CD</td>
<td>3.7</td>
<td>3.1</td>
<td>4.2</td>
<td>3.6</td>
<td></td>
</tr>
<tr>
<td>CbP</td>
<td>3.5</td>
<td>3.7</td>
<td>3.9</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>median OS (mos)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>overall</td>
<td>8.3</td>
<td>8.1</td>
<td>7.4</td>
<td>7.2</td>
<td>NS</td>
</tr>
<tr>
<td>CP</td>
<td>9.1</td>
<td>6.9</td>
<td>6.1</td>
<td>6.0</td>
<td></td>
</tr>
<tr>
<td>CG</td>
<td>8.1</td>
<td>9.4</td>
<td>9.7</td>
<td>7.9</td>
<td></td>
</tr>
<tr>
<td>CD</td>
<td>7.7</td>
<td>8.1</td>
<td>6.8</td>
<td>8.2</td>
<td></td>
</tr>
<tr>
<td>CbP</td>
<td>7.6</td>
<td>9.3</td>
<td>8.3</td>
<td>6.9</td>
<td></td>
</tr>
</tbody>
</table>

*women had higher incidence of AC (62.6% vs 53.4%) and lower incidence of SCC (14.4% vs 22.8%) than men

- no difference in OS or PFS across histologic groups
- no difference in PFS or OS within each chemotherapy arm across histologic groups
- no difference in PFS or OS within each histologic group across chemotherapy arms

**Take-home message:** When it comes to advanced stage NSCLC it’s all bad news and histology doesn’t matter much beyond predictors for treatment sensitizing mutations (the latter an editorial comment – does not follow from data in this paper!).

Purpose: To evaluate histologic findings in lung biopsies from patients suspected of having pulmonary graft-versus-host disease (GVHD).

Methods: Uncontrolled, retrospective case based study of primarily transbronchial lung biopsies (TBBx) from patients thought to have pulmonary GVHD.

- inclusion criteria (see results)
  1. history of hematopoietic stem cell transplant
  2. abnormal chest radiology
  3. clinically “suspected” GVHD in GI tract and/or skin
  4. infectious etiologies “excluded” by culture (bacteria, viral) and special stains (AFB, GMS, CMV, HSV)
  5. “diagnostic findings present”

- 2 excluded: insufficient material (1), lack of “diagnostic” findings (1)

- histologic findings assessed in bronchioles/small airways and alveolar compartment
  airways: intraepithelial lymphocytes (CD3 stains - #/100 epithelial cells) and eosinophils, epithelial atypia, apoptosis, fibrosis (Masson trichrome)
  alveolar compartment: fibrin, organizing pneumonia (OP), interstitial inflammation (1+ [bronchiolocentric] to 3+ [diffuse thickening without bronchiolocentricity]), atypical reactive pneumocytes, apoptosis, vasculitis, eosinophils, plasma cells

- classified by “dominant” histologic pattern
  acute lung injury – intra-alveolar fibrin, reactive pneumocytes, septal edema, no OP
  OP – with/without fibrin
  CIP – absence of OP or fibrin

Results: 17 biopsies from 14 patients (2 biopsies in 1 patient, 3 biopsies in another).

- 9 men (mean age 55 ± 3 yrs, range 28 – 66 yrs): 5 women (mean age 54 ± 5 yrs, range 40 – 64 yrs)
- time since txplt 4-82 months (mean 19 months)
- 10 pathologic + 4 clinical diagnoses of extrapulmonary GVHD
- At least 3 (21%) patients failed to satisfy inclusion criteria: 1 “not performed” for CT findings; 1 “None” and another “eye” for “other organ involvement”
- SOB (8), “acute respiratory failure” (2), asymptomatic (2), and “Obstructive symptoms” (2)
- CT (13) showed various combinations of ground glass, consolidation, nodules and “nonspecific opacities” that were localized in 2 (RUL and LLL)
- 16 TBBx and 1 VATS, including 1 TBBx “with only bronchiolar mucosa that was assigned to CIP because a prior biopsy from this patient lacked fibrin or OP pattern.”

- acute lung injury – 3; OP – 4; CIP – 7
  o pathology findings
    small airways (14): lymphocytosis (50/100 epith cells), epith atypia apoptotic bodies, eos
    alveolar compartment (16): interstitial T cells, pneumocyte atypia, apoptosis, “perivasculitis”, eos, plasma cells
  o outcomes
    acute lung injury – 3/3 DOD (ARDS); OP – no BOS (5.5 ± 2.8 mos); CIP – 3 w BOS (11 – 43 mos)

Take-home message: Beyond obliterative/constrictive bronchiolitis, we still haven’t a clue how to diagnose GVHD in the lung and this paper doesn’t get us any closer but instead muddies the waters.
Articles for notation

**Neoplastic diseases - histology**

Sumiyoshi et al. Pulmonary adenocarcinomas with micropapillary component significantly correlate with recurrence, but can be well controlled with EGFR tyrosine kinase inhibitors in the early stages. Lung Cancer 2013; 81: 53-9.

**Take-home message:** Retrospective analysis of 440 resected adenocarcinomas showed that those with ≥ 5% micropapillary carcinoma (MPC) had significantly decreased *disease-free survival* compared to patients without a micropapillary component, but that did not extend to a difference in *overall survival* in stage IA patients. There was no correlation between the percentage of MPC and tumor recurrence beyond the minimum ≥ 5% threshold. No difference in rates of KRAS and EGFR mutations when comparing MPC+ to MPC- tumors.


**Take-home message:** Retrospective review of reports (no re-review of slides) for 190 patients who underwent resection for NSCLC showed that presence of lymphatic invasion negatively impacted survival in patients with stage IA and IB disease, but not those with higher stage disease. In both univariate and multivariate analysis lymphatic invasion was a statistically significant negative prognostic factor.

**Neoplastic diseases - immunohistochemistry**


**Take-home message:** Prospective analysis of TMAs constructed from 209 resected lung tumors (207 patients) using LOTS of immunostains showed nothing new, affirming that CK5/p63 is good combination for squamous cell carcinomas (SCC), TTF1/napsin A good for adenocarcinoma (AC), ER shows up in as many as 15% of ACs and rarely in SCC, and NE markers shows up in some adenocarcinomas (12%) and SCCs (8%).


**Take-home message:** Analysis of 75 NSLCs (47 squamous cell carcinomas and 28 adenocarcinomas) comparing an antibody directed against plakophilin-1 (PKP1) to antibodies for keratin-15 (K15) and desmoglein-3 (DSG3) on FFPE sections showed the highest sensitivity (100%) for K15 membrane staining and the highest specificity for PKP1 (100%) and DSG3 (100%) membrane staining. Not compelling enough to replace much simpler tools like CK5(/6) and p63/p40.

Agaimy et al. ISL1 expression is not restricted to pancreatic well-differentiated neuroendocrine neoplasms, but is also commonly found in well and poorly differentiated neuroendocrine neoplasms of extrapancreatic origin. Mod Pathol 2013; 26: 995-1003.

**Take-home message:** Insulin gene enhancer binding protein islet-1 (ISL1) can be seen in neuroendocrine neoplasms other than those arising in the pancreas, including several neuroendocrine lung tumors in this study: 13/13 (100%) primary small cell carcinomas, 8/10 (80%) of metastatic small cell carcinomas, 2/10 (20%) carcinoids, and 1/5 (20%) atypical carcinoids. Not sure whether or when this might prove useful information but felt like it might!


**Take-home message:** Usual encyclopedic review with 277 references. Covers pretty much everything including all of the keratin antibodies, epithelial cell adhesions molecules etc. If it ain’t listed here, it ain’t worth knowing about! Great reference when you want to know specificity for these things.
Neoplastic diseases – molecular diagnostics/precision medicine


Take-home message: Analyzed abnormal checkpoint gene with forkhead-associated domain and ring finger (CHFR) methylation using fresh frozen tissue from 165 resected adenocarcinomas also analyzed for mutations in EGFR and KRAS and EML4-ALK fusion. All were mutually exclusive, with EGFR mutations (29%) being the most common followed by CHFR hypermethylation (10%), KRAS mutations (8%), and EML4-ALK translocations (6.7%). Only CHFR hypermethylation was significantly correlated with lymphatic invasion and a poor prognosis.


Take-home message: Tested a large (373) cohort of adenocarcinomas using IHC, FISH and RT-PCR. Nearly perfect concordance between IHC and FISH, except for two IHC-positive/FISH-negative cases that proved to have variant EML4-ALK fusions by RT-PCR. Another in a growing list of references, including the work reported from Mayo Minnesota, demonstrating that IHC can effectively detect ALK rearrangements including unique variants that might be missed by FISH.


Take-home message: Comprehensive and carefully controlled meta-analysis comes to the predictable conclusion that KRAS mutations are associated with a worse overall survival in patients with NSCLC, especially those with adenocarcinomas and early stage disease.

Neoplastic diseases - biomarkers


Take-home message: Thymidilate synthase (TS) is primary target of pemetrexed, an antifolate drug, and there is evidence that high levels of mRNA and protein expression might account for poor response in squamous cell carcinomas. Excision repair cross-complementation group 1 (ERCC1) is a DNA repair polypeptide that has been linked with resistance to platinum-based chemotherapy. Turns out in this retrospective study of 41 consecutive patients with advanced lung adenocarcinoma treated with pemetrexed-cisplatin as first line therapy that low expression of both TS and ERCC1 was associated with improved PFS, and in the case of ERCC1 OS as well. When combined, low TS/low ERCC1 predicted for significantly longer PFS and OS when compared to high TS/high ERCC1.


Take-home message: CD151 is a transmembrane protein linked to poor prognosis in a variety of cancers. In this retrospective analysis of 380 NSCLC (245 SCC, 135 ADC) patients using a TMA high CD151 expression was detected in 28.7% of patients and was more common in men, smokers and ADCs (especially the solid subtype). High CD151 expression predicted for shorter OS and DFS and was an independent negative prognostic factor for OS in NSCLCs in general and for ADC in particular.


Take-home message: In this retrospective analysis of 295 surgically resected NSCLCs using whole slides, vimentin was expressed in ≥ 10% of neoplastic cells in 66 (22.4%) of tumors in which vimentin-expressing tumor cells were “almost always detected in poorly differentiated cells at the periphery of tumour clusters at the interface between tumour clusters and stroma. In these cells, vimentin was present in the cytoplasm of
tumour cells, with a particularly recurrent subcellular distribution directed toward the invasion front.” Patients whose tumors showed vimentin expression in > 50% of cells had lower rates of metastases-free survival.

Non-neoplastic diseases

Take-home message: Not all pneumoconiosis are “occupationally” related, this report describing a form of mixed dust fibrosis in a Bhutanese refuse living in Nepal. For at least 5 hours daily since the age of 15 she cooked daily in a small windowless room in a bamboo hut using an open mud stove fueled by firewood or charcoal. She also used a stone grinder to grind grains. So if any of those conditions apply to you, consider respiratory protection . . . or possibly relocation!