Kamata T et al. Frequent BRAF or EGFR Mutations in Ciliated Muconodular Papillary Tumors of the Lung. JTO. 2016. 11: 261-265.

Summarized and presented by Dr. Liping Liu

**Background and Purpose:** Ciliated muconodular papillary tumor (CMPT) is a recently described entity in the peripheral lung parenchyma characterized by an overall papillary or glandular architecture and vaguely organized mixture of ciliated columnar cells, mucous cells, and basal cells, with abundant mucus. The architectural complexity mimics adenocarcinoma of the lung and causes diagnostic challenges. CMPTs consistently follow a benign clinical course, even after limited surgery. The histogenesis of CMPT remains poorly understood.

**Methods:** 10 CMPT were identified in National Cancer Center Hospital in Tokyo, Japan (2006-2014). DNA was extracted from 10 tumor specimens (4 fresh frozen, 6 FFPE samples). Mutations at 2790 hot spots in 50 cancer-related genes were analyzed using iron proton platform. BRAF and EGFR mutations were confirmed by high-resolution melting analysis (HRMA). IHC specific for BRAF V600E mutation was performed.

**Results:** All tumors showed typical morphology and were disease free at a mean f/u of 43 months (range, 2–88). One or more mutations were detected in 8 (of 10, 80%) cases. Mutated genes: BRAF (50%), EGFR (30%), PTPN11 (20%), CTNNB1 (10%), IDH1 (10%), TP53 (10%). The mutations in BRAF were two types of missense mutations (V600E in 4 cases, G606R in 1 case); all 3 EGFR mutations were the same in frame deletion (delE746-T751/S752V). All BRAF and EGFR mutations were mutually exclusive. BRAF IHC: all 3 epithelial components (i.e., ciliated columnar cells, mucous cells, and basal cells) showed similar cytoplasmic staining in all 4 cases with BRAF V600E mutations.

**Discussion:** The high incidence of BRAF mutations in CMPTs is in contrast to the rare incidence in lung adenocarcinomas. For EGFR mutation, although both CMPT and lung adenocarcinoma have exon 19 deletions, the exact loci are different. The presence of driver gene alterations in CMPTs does not dictate their clinical behavior, because gene mutations can be present in both benign and malignant lesions. Given that BRAF V600E is expressed in all 3 cell types of the CMPT suggests a neoplastic nature of all cell types. Although the histologic features of mixed papillomas resemble those of CMPTs, these two tumors can be differentiated by their locations (central versus peripheral) and their associations with the bronchial lumen (endobronchial versus nonendobronchial). BRAF and EGFR mutational assays in mixed papillomas may clarify their nosological relationship with CMPTs.

**Take home message:** The presence of frequent driver mutations of BRAF or EGFR in CMPTs support the notion that these lesions are neoplastic rather than reactive or metaplastic.
Austin MC et al. DNA Yield From Tissue Samples in Surgical Pathology and Minimum Tissue Requirements for Molecular Testing. Arch Pathol Lab Med. 2016;140:130–133.

Summarized and presented by Dr. Liping Liu

**Purpose:** The study used measurements of tissue area and volume to assess whether samples will produce enough DNA to perform even the most complex multigene assays.

**Method:** FFPE samples processed for the 3 most common molecular assays were selected:
- EGFR mutation analysis, performed on needle core biopsies of lung tissue, n=174.
- KRAS mutation analysis, endoscopic mucosal biopsies or resections of colonic tissue, n=115
- BRAF mutation analysis, larger skin or lymph node excisional biopsies, n=77.

366 samples received during a 3-year period. 3 samples yielded insufficient DNA - included in study data set.

Samples: (i) tissue curls (10 curls, each 10µm thick), (ii) variable number of unstained tissue slices on slides (10 µm). H&E slide - used to estimate the area of submitted tissue and the % tumor cells. The estimated tissue volume (in mm$^3$) was calculated as area of tissue (in mm$^2$) x section thickness (in mm) x number of slides or curls. The precision of this method is limited by natural variation in tissue area that occurs with progressive leveling through the paraffin block, but empirical observation of tissue on the unstained slides suggests that this effect is limited.

DNA was extracted and quantitated by absorbance at 260 nm. It is important to note that absorbance measurements typically overestimate the amount of DNA by a factor of 2 relative to fluorescence assays, so the 1- µg figure that was selected is the equivalent of 0.5 µg in a laboratory using fluorescence assay to quantify the amount of extracted DNA. This was confirmed by quantifying DNA in a subset of samples using the Qubit fluorescence assay.

To determine the average DNA yield per unit of tissue volume, linear regression analysis was performed on DNA yield versus tissue volume for each of the 3 sample types in the study. The average DNA yield per mm$^3$ of tissue is equal to the slope of the regression line (y=mx).

**Results:** Most (80%) samples that produced < 1 µg of DNA came from samples with < 4 mm$^3$ tissue. All tissues for EGFR and KRAS with volumes >8 mm$^3$ and all samples for BRAF with volume > 6 mm$^3$ yielded >1 µg of DNA. DNA yield of ≥1 µg was obtained from 90% of the tissue samples with volume ≥4 mm$^3$ and 100% of tissue samples with volume ≥8 mm$^3$.

**Minimal tissue requirement:** If the desired failure rate is ≤10%, tissue volume of 4 mm$^3$ is required; for a failure rate of ≤ 1%, 8 mm$^3$ is required. Assuming a loss of 1 mm$^3$ of tissue during slide preparation, a minimum volume of either 5 or 9 mm$^3$ is needed to achieve these goals.

Guidance for interventional radiology: For an 18-gauge needle commonly used in these procedures, the internal diameter is 0.838 mm, corresponding to an internal volume of 5.52 mm$^3$ per cm of core length. Assuming that viable tissue of sufficient average tumor cellularity (>10%) is obtained and entirely submitted, a single core 9 mm long will produce 1 µg of DNA 90% of the time, and 2 or more cores with a combined length of 18 mm will produce 1 µg or more of DNA 99% of the time.

**Take home message:** Two 1-cm cores should be obtained to maximize the likelihood of having enough DNA for most complex multigene molecular assays.

Purpose:
- To characterize histopathologic features useful in distinguishing end-stage pulmonary sarcoidosis (ESPS) from UIP findings in lung explants from patients with a preop diagnosis of sarcoidosis were compared to age- and sex-matched control groups with UIP

Methods:
- Explants from 12 patients-clinical diagnosis of pulmonary sarcoidosis (5-double, 7-single) (1991-2012)
- Diagnosis based on criteria proposed in the joint statement on sarcoidosis by ATS/ERS/World Association of Sarcoidosis and Other Granulomatous diseases
- 10 age- and sex-matched explants with postop diagnosis of UIP
- 3 pulmonary pathologists documented: Extent and pattern of fibrosis, presence/quantity of fibroblast foci and granulomas (per 10 Hpf), distribution/morphology of granulomas, presence of granulomas in hilar/mediastinal LNs, presence/extent of HC
- Extent of fibrosis and HC was scored: 1=1-25%, 2=26-50%, 3=51-75%, 4=76-100%
- GMS, AFB on selected sections

Results:

<table>
<thead>
<tr>
<th>Feature</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Definition</td>
<td>ESPS</td>
<td>Diseases other than ESPS</td>
<td>Overlapping histologic features of sarcoidosis and other disease</td>
<td>UIP (9 IPF, 1 SLE)</td>
</tr>
<tr>
<td>#</td>
<td>8</td>
<td>2</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Sex</td>
<td>3 women</td>
<td>2 women</td>
<td>2 women</td>
<td>matched</td>
</tr>
<tr>
<td>Age (yo)</td>
<td>36-58</td>
<td>56, 59</td>
<td>42, 49</td>
<td>matched</td>
</tr>
<tr>
<td>Time of diagnosis (yrs prior to explant) and clinical findings</td>
<td>4-26 6/8 prior tissue diagnosis 8/8 imaging c/w sarcoidosis PFT obstruction - restriction (3), - combined (1) 6/8 PHT</td>
<td>4-22 Prior tissue diagnosis Imaging: (1) extensive interlobular septal thickening peripheral and basilar predominant, tractionbronchiectasis, HC; (2) upper and mid-zone emphysema PFT: (1) diffusion impairment, (2) obstruction No PHT</td>
<td>4-16 Tissue dx Imaging: (i) UL reticulation, traction bronchiectasis; LL predominant diffuse GGOs; (ii) HC and cystic changes, lymphadenopathy PFTs 2/2 restriction, diffusion impairment PHT 1/2</td>
<td></td>
</tr>
<tr>
<td>Non-necrotizing granuloma</td>
<td>8/8-well formed with peripheral concentric fibrosis none (i) Rare, mainly along interlobular septa</td>
<td>0/10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pathology Features</td>
<td>ESPS</td>
<td>UIP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------------</td>
<td>------</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Distribution of fibrosis</td>
<td>Lymphangitic distribution</td>
<td>Randomly distributed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibroblast foci</td>
<td>Absent or rare</td>
<td>Frequent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Architectural distortion</td>
<td>Absent or mild</td>
<td>Extensive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Granuloma in the lung</td>
<td>Present, lymphangitic distribution</td>
<td>Absent, unless complicated by infections</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Granuloma in lymph nodes</td>
<td>Present</td>
<td>Absent</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Take Home Points:**
- Histologic findings in ESPS are distinctly different from UIP
- Patients with sarcoid might develop another fibrosing lung disease

Background:
- Some treat LCNEC similar to SCLC, others have different treatment regimens
- No randomized trials investigating optimal treatment of LCNEC

Purpose:
- To evaluate clinical presentation, prognosis, currently applied treatment of LCNEC to other lung cancer subtypes

Methods:
- Retrospective population-based study (2003-2012) from Netherland Cancer Registry (NCR)
- Only cases that had histologic confirmation of diagnosis: LCNEC, SCLC, SQCC, AdCa

Results:
- 999/59,283 (1.7%) were LCNEC
- Total incidence of LCNEC as proportion of all lung cancers – 0.9%
- Annual occurrence of LCNEC increased by 255% (sharpest increase in 2008)
- SCLC were more advanced than LCNEC; LCNEC stage distribution was comparable to AdCa except lower % stage I
- LCNEC had less N2/3 disease (52%) than SCLC (79%, p<0.001), but more than SQCC (45%, p<0.001), comparable to AdCa (p=0.1)
- In stage IV patients, LCNEC had less N2/3 disease (69%) than SCLC (80%, p<0.001), comparable to SQCC and AdCa (66% both, p=0.3 and 0.34).
- LCNEC mets in liver (47%), bone (32%), brain (23%), adrenal (19%), lung (14%), pleura (7%), extrathoracic LNs (16%). Fewer liver and more brain mets than SCLC
- Median OS: LCNEC - 8.7 months, SCLC - 7.1, SQCC - 13.1, AdCa - 11.8; all different from LCNEC;
- If treated with surgery – no difference in OS between LCNEC and SCLC
- Treatment: stage I/II LCNEC – 87.3% surgery (same as AdCa, more than SCLC and SQCC). Adjuvant chemo-23.2% LCNEC – more in SCLC (75.4%), less in SQCC (15.3%) and AdCa (13.5%)
- Stage III LCNEC - chemoradiation 30.6%, surgery 21%, chemo 14.5%, no treat 19.4% - different from SCLC (chemoradiation 52.9%, surgery 0.9, chemo 26.9, no treat 14.0) but comparable to AdCa (chemoradiation 29.1%, surgery 24.4%, chemo 17.1%, no treat 15.6%) and SQCC
- Stage IV – 45.7% LCNEC no treat – more than in SCLC and AdCa, comparable to SQCC; chemo in 38.1% LCNEC – less than in SCLC, more than in SQCC, comparable to AdCa

Take Home Points:
- LCNEC occurrence increased
- LCNEC appears different from SCLC, clinically resembling SQCC and adenoCa in early-stage, less N2-3 involvement, better survival in stage I/II but otherwise comparably poor prognosis and metastatic pattern to SCLC
- No treatment guidelines for LCNEC – but apparently (at least in Netherlands) treatment more closely to SQCC and AdCa than SCLC
Articles for notation - Neoplastic


Background:
- Mammalian achaete-scute homolog 1 (human achaete-scute homolog 1 [hASH]) – member of the basic helix-loop-helix family of transcription factors – plays an obligatory role in the development of specific neuroendocrine cell lineages
- hASH1 – reliable marker of aggressive, high-grade neuroendocrine tumors in the GI tract

Purpose:
- To determine hASH1, synaptophysin, chromogranin, CD56 expression in SQCC, ADC, TC, AC, LCNEC and SCLC by IHC

Methods:
- 86 surgically resected or incisionally biopsied NETL (neuroendocrine tumors of the lung) including 37 TC, 14 AC, 11 LCNEC, 24 SCLC
- TMAs of 183 ADC, 101 SQCC of the lung
- hASH1 (clone 24B7.2D11, 1:500, BC Bioscience, San Jose CA) – nuclear stain
- staining intensity scored 0-3, % tumor cell staining
- + stain if ≥ 5% of tumor cell staining

Results:
- ADC, SQCC negative for hASH1; variable + for other neuroendocrine markers (table 1); >14% + for 1 neuroendocrine marker, 4% + for 2 markers, most commonly CD56 (SQCC) and synaptophysin (ADC)
- All neuroendocrine tumor types were hASH1 positive but expression differed by tumor type: mean and median scores of SCLC were significantly higher than TC, AC and LCNEC
- TC: all strongly + chromogranin and synapto; only focally/weakly + hASH1
- AC: chromo and synapto stained positive for more cases and stronger than hASH1
- LCNEC, SCLC: hASH1 stronger and + in more cases than chromo
- hASH1 expression correlated negatively with chromo in NETL

Take Home Points:
- hASH1 shows increased expression in high grade NETL, specifically SCLC
- Nuclear hASH1 stain appears to be specific for neuroendocrine differentiation and can distinguish SQCC/ADC from NETL
- Not a site-specific marker

Background:
- Multiple TTF-1 clones currently available

Purpose:
- To compare labelling profiles of 2 commercially available TTF-1 MAbs-in primary lung AdCa, lung SQCC, lung sarcomatoid Ca and malignant meso (MM).

Methods:
- Biopsy cases
- DAKO clone 8G7G3/1 (mouse MAb), Ventana clone SP141 (rabbit MAb)

Results:

<table>
<thead>
<tr>
<th>Tissue</th>
<th>8G7G3/1 MAb: n (%)</th>
<th>SP141 MAb: n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal lung—alveolar epithelium</td>
<td>7/7</td>
<td>7/7</td>
</tr>
<tr>
<td>Adenocarcinoma of the lung</td>
<td>35/35</td>
<td>35/35</td>
</tr>
<tr>
<td>Atypical squamous lesions—12 SCCs+1 case of bronchial squamous dysplasia</td>
<td>0/13</td>
<td>5+1/12+1 (~46%)</td>
</tr>
<tr>
<td>Pleomorphic/sarcomatoid carcinoma of the lung (spindle-cell component only)</td>
<td>2/12 (17%)</td>
<td>4/12 (~33%)</td>
</tr>
<tr>
<td>Epithelioid malignant mesothelioma</td>
<td>0/66</td>
<td>0/66</td>
</tr>
<tr>
<td>Sarcomatoid mesothelioma</td>
<td>0/19</td>
<td>8/19 (~42%)</td>
</tr>
</tbody>
</table>

TTF-1 staining for sarcomatoid mesotheliomas was weak to focally moderate

Take Home Points:
- Potential for misclassification of cases especially if using the SP141 clone
- Positive 8G7G3/1 staining of a sarcomatoid pleural lesion strongly favors sarcomatoid carcinoma, this conclusion cannot be drawn from staining with SP141
- Staining in MM is likely real given some data from the gene expression omnibus DataSets that have shown mRNA expression for TTF-1 at low levels in some MM – SP141 expression may represent high sensitivity for very low levels of TTF-1 protein in a variety of tumors other than lung/thyroid AdCa and pulmonary (and extrapulmonary) small cell carcinomas and non-small cell neuroendocrine carcinomas

Purpose:
- To investigate the differential expression of TTF-1, ER, PR, GCDFP-15, p63, and WT-1 to develop an algorithm to distinguish metastatic breast from primary lung cancers.

Methods:
- TMAs of 266 NSCLC, 837 primary breast cancers enriched for triple negative cases
- TTF-1 – clone 8G7G3/1; ER – clone SP1; WT-1 – clone 6F-H2
- Staining intensity (0-3) and percentage (0-3) are added – score 2 = low expression, 3-6 = high expression

Results:
- TTF-1 negative in all breast cancers
- ER+ in 5% lung cancer (most adenoCa, only few had high expression)
- PR+ in 1% lung cancer
- GCDFP-15 + in 25% breast Ca, 1% lung cancer
- WT-1+ in 3% breast cancer, many high expression, 0.4% lung cancer, low expression
- Most specific markers: Lung AdCa: TTF-1 (mean score 4.8 of 6); SQCC: p63 (score 5.5), triple neg breast Ca: cytoplasmic WT-1 (score 2); ER strongly associated with breast Ca if present
- 9% breast Ca show cytoplasmic WT-1 (40% of triple neg) but only 5% lung Ca

Proposed algorithm on biopsy specimen
1. Histologic examination including comparison to previous breast carcinoma
2. Primary panel including TTF-1, ER, PR, HER2
   TTF-1+ = primary lung
   ER and/or HER2+ = primary breast
3. TTF-1- and triple negative tumors – proceed to secondary panel (p63, WT-1, GCDFP-15 +/- additional markers such as GATA3
   p63+ only (especially if strong) = lung >>> breast
   GCDFP-15+ only = breast >>> lung
   WT-1 cytoplasmic + only = breast >>> lung
   p63 (especially if scanned) and WT-1 = breast >> lung
   p63, WT-1, GCDFP-15- = breast = lung
   Try additional markers such as GATA3 for breast

Take Home Points:
- If ER, TTF-1 negative, adding p63, GCDFP-15 and WT-1 may be helpful with high level p63 expression favoring lung, while GCDFP-15+ or cytoplasmic WT-1 favors breast
- Did not test mammoglobin (literature - reportedly 80% breast Ca+ and only 0-1.2% lung Ca) – should be part of the algorithm!!!
- Did not test GATA3 (literature – reportedly 67-95% breast Ca, 0.3-8% lung AdCa, 12% lung SQCC)

Background:
- Lung carcinoma nodules in contralateral lobes without distant mets and tumors with pleural nodules and/or malignant effusion staged as pM1a.
- Lung carcinomas with distant extrapulmonary mets are pM1b
- pM1a and pM1b are stage IV or pStage IV disease
- multiple tumors should be considered synchronous primaries if they are of different histopathologic cell types
- AJCC does not describe explicit pathologic criteria to distinguish synchronous primaries pT(m) from intrapulmonary metastases present in the same lobe (pT3) or contralateral lobes (pM1a) when the tumors are of the same cell type
- Comprehensive histopathologic evaluation (CHE) described by Travis and Girard to help distinguish synchronous primary lesions from intrapulmonary mets
- Staging of these tumors is inconsistent – some staged as pT1(m) – patients were treated solely with surgery; others with similar pathology were staged pM1a – received postop chemotherapy for stage IV disease

Purpose:
- To compare survival data of patients with synchronous bilateral NSCLC who underwent resection in the absence of malignant effusion, pleural tumor, or extrapulmonary mets with patients with pStages I through IV disease

Methods:
- Consecutive patients who underwent resection of synchronous bilateral NSCLC and mediastinal LN resection and w/o history of extrapulmonary mets, pleural tumor or malignant pleural or pericardial effusion at diagnosis
- Synchronous bilateral NSCLC = neoplasms that were resected within 6 months of each other
- Tumors were also evaluated using the CHE method (Travis)
- Within each adenoCa the % of histologic patterns (acinar, papillary, micor papillary, solid, lepidic) were evaluated by 2 pathologists in 5% increments
- Evaluated keratinization, clear cell or signet ring cell cytology, desmoplasia, inflammation
- Group I: synchronous neoplasms with different histologic features/proportions of histologic patterns; group II: identical histologic features/proportions of growth patterns
- AIS, MIA = group I lesions since they do not metastasize

Results:
- 18 cases, 10 women, age 57-83 yo (mean, 69.5)
- Total of 44 NSCLC (32 invasive adenoCa, 3 adenosquam Ca, 1 SQCC, 1 AIS
- Sublobar resection (n=33), lobectomies (n=11); all surgical margins negative
- Number of tumors resected within this 6-months period – 2-5/patient
- Tumor sizes: 0.2-5 cm (median, 1.3 cm)
- LN status: 33 (75%) pN0; 1 (2.3%) pN1; 2 (4.5%) pN2, 8 (18.2%) pNX
- 0.6% of patients who had surgery for lung cancer at that institution had synchronous bilateral NSCLC
- 13 (72.2%) no additional treatment; 5 (27.8%) had chemo and/or radiation
• Based on comparison of % of histologic patterns and comparison of additional histologic features – 40 tumors from 16 patients = group I; 4 tumors = group II; therefore, 16 (88.9%) patients had pT(m) lesions, 2 (11.1%) had pM1a lesions
• 10-yr OS group I 54.9%; group II 66.7% - no significant difference
• No difference in 10-yr OS between patients with or without adjuvant therapy
• pStage I: survival of patients with resected synchronous bilateral NSCLC is similar to controls in cancer registry
• pStages II-IV: 10-yr OS is better in patients with resected synchronous bilateral NSCLC than control group (p=0.047, p=0.0004, p<0.0001)

**Take Home Points:**
• Patients with resected synchronous bilateral NSCLC should be staged as pT(m)
• Literature generally supports aggressive surgical treatment for synchronous lung cancer in patients w/o malignant pleural effusion, pleural nodules or distant metastases

**Purpose:**
- Well differentiated thymic SQCC need to be separated from other more aggressive thymic carcinomas and from thymomas and other benign or malignant neoplasms that can show cystic changes

**Methods:**
- All resection specimens
- Mucicarmine, PAS, PAS-D
- CK5/6, p40, Pax8 (polyclonal)
- Staged according to TNM for thymic carcinoma proposed by Weissferdt and Moran

**Results:**
- 6 patients, 48-75 yrs (mean 61.5); dyspnea, SOB, chest pain
- No hx of malignancy or paraneoplastic syndrome
- Complete surgical resection, negative margins
- Ill-defined tumors, 4-9 cm (mean, 6.5)
- All predominantly cystic, only limited solid areas
- Cyst walls were lined by squamous epithelium with varying degrees of atypia
- Mucicarmine, PAS, PAS-D negative
- CK5/6, p40, Pax8 strongly positive
- All T1N0M0
- Follow up 1-2 yrs, all 4 patients with f/u alive, no evidence of recurrence or met

**Take Home Points:**
- Diff diag:
  - Multiloculated thymic cyst with or without pseudoepitheliomatous hyperplasia – look for frank areas of SQCC or evidence of keratinization;
  - Cystic thymoma (dual cell population of lymphocytes and epithelial cells, prominent perivascular spaces, lobulated architecture)
  - Mucoc Ca (mucous secreting cells, no keratinization, possibly t(11;19) translocation resulting in MECT1-MAML2 fusion);
  - Metastatic SQCC of lung, exclude by clinical and radiologic means

Background:
- LMO2=LIM Domain Only 2 – evolutionarily conserved protein involved in scaffolding of transcription factors necessary for hematopoiesis and angiogenesis
- LMO2 expression - suggested as potentially useful in discriminating thymoma from T-ALL

Purpose:
- To test the usefulness of LMO2 expression to distinguish thymoma from T-ALL

Methods:
- 15 thymoma (5 AB, 5 B1, 5 B2), 30 T-ALL
- whole tissue sections from 15 thymomas
- biopsy specimens from 15 bone marrows (B5 fixative and decal) and clot sections in 5 of the 15 bone marrow specimens in which LMO2 was weak
- TMA with 15 T-ALL (7 thymus, 6 LNs, 1 skin, 1 testicle)

Results:
- Thymoma: thymocytes in all 15 cases negative for LMO2
  Thymic epithelial cells – LMO2 weakly +
  B cell aggregates when present – LMO2 expression in mantle zone and follicular B cells
  11 cases with adjacent benign thymic tissue – thymocytes negative for LMO2;
  weak LMO2 expression in thymic epithelial cells, strongly + in occasional B cells
- T-ALL: abnormal T lymphoblasts uniformly LMO2 + in all bone marrow clots
  abnormal T lymphoblasts LMO2 + in all 15 bone marrow core bxs – but in 5 cases staining for LMO2 and TdT was weak (decalcification artifact)
  10/15 cases of TMA – strong nuclear staining for LMO2; 4/5 cases without apparent expression of LMO2 had only few lymphoblasts and prominent crush artifact
  Targeted LMO2 FISH probes applied to 16 T-ALL cases – no rearrangement; 2 cases (both LMO2+ by IHC) had trisomy 11 by conventional cytogenetics – and an extra LMO2 signal at 11p13 in one of the cases

Take Home Points:
- LMO2 IHC could be a reliable marker to distinguish T-ALL from non-neoplastic thymocytes and T-cell precursors.
- Use T-cell lineage markers and/or markers of immaturity (TdT) to determine LMO2 expression in target cell population (because thymic epithelial cells, B cells express LMO2)

Purpose:
- To investigate the histomorphologic and molecular features in a series of thoracic MTs.
- Correlate clinical and pathologic features based on this series and cases in literature

Methods:
- 8 cases of primary thoracic MTs
- Diagnosis: keratin+ and/or EMA+ together with S100+ or myogenic markers (calponin, SMA)
- IHC score: + if >50% tumor cells; focal <50%, negative no stain
- Tumors were assessed for borders, architectural pattern, stromal characteristics, cytologic features, nuclear pleomorphism, mitotic activity, LVI, necrosis
- “well circumscribed”=tumor-parenchymal interface sharply defined or tumor was entirely endobronchial/tracheal with no infiltration of the bronchial/tracheal wall
- “infiltrative borders” = lobulated and irregular tumor-parenchymal interface with extension into adjacent parenchyma.
- FISH: all cases screened in a stepwise manner in the following order using probes flanking EWSR1, FUS, PLAG1, HMGA2
- Cases that showed rearrangement in EWSR1 or FUS were screened for partners with probes for PBX1, PBX3, KLF17, ZNF444, POU5F1, CREB1, ATF1, NR4A3

Results:
- Age 27-77 (mean, 54), no sex predominance, 5/8 smoking hx
- 4/5 symptomatic (hemoptysis or non-bloody cough)
- 4/8 tumors in large airways (endobronchial or endotracheal) – tended to be smaller than intraparenchymal or subpleural tumors; 2.0 vs 6.6 cm).
- CT (n=4): MTs in large airways – well circumscribed, homogeneous, polypoid, sessile; intraparenchymal: well-defined nodules to irregular masses with calcifications
- All cases surgically managed: lobectomy (n=5); 1 case with adjuvant chemoradiation
- Stage: T1a (n=3), T1b (n=2), T2a (n=1), T3 (n=2)
- F/U 15-110 months (mean 58) – metastases (n=2), no death of disease
- Gross: tumors within large airways: sessile polyps, submucosal masses, compression of adjacent airways. No capsule, homogeneous cut surface – few cystic and/or hemorrhagic
- Micro: not encapsulated, majority were well circumscribed (n=5); most had a mixture of histologic patterns, with solid sheets or nested growth pattern, and associated hyalinized or myxoid stroma. Reticular pattern and chondroid matrix rare (n=1); osteoclast-like giant cells and psammomatous calcifications focal (n=1)
- Cytologic characteristics varied: clear cells most common (n=5) > epithelioid (n=4) > plasmacytoid (n=2); nuclear pleomorphism typically mild; no frank cytologic atypia
- Necrosis and/or LVI in n=5
- Mitotic activity 0-6/mm(2)
- IHC: all keratin+ and/or EMA+ and S100+ or myogenic markers+; TTF-1- uniformly
- FISH (n=4): EWSR1 rearrangements (n=3) (partnered with PBX1 or ZNF444, n=1 each) and FUS (n=1); compared with fusion-negative cases, tumors with EWSR1 or FUS
rearrangements occurred in patients who were on average 8 yrs younger and more commonly female with more cases having necrosis and a slightly higher mean mitotic activity; all fusion-positive tumors had spindle and clear cell components, an epithelioid component was present in all fusion-negative tumors

- Combined dataset – series + literature: univariate analysis: patients with metastasis or necrosis had worse 5-yr survival. Number of mitoses correlated with outcome.

**Take Home Points:**

- Thoracic MTs display a range of morphology and clinical behavior with a subset harboring EWSR1 or FUS rearrangements – might be useful for distinction from other tumors
- Tumors of adulthood
- Typically occur in the tracheobronchial tree
- No case had glandular differentiation – distinction from lung adenocarcinoma

Purpose:
- To examine the spatial distribution of different markers and cell populations in thymic carcinoma, by IHC.

Methods:
- 5 thymic carcinomas, stage I or II

Results:
- CD5+ in tumor cells of all cases; positive cells in areas of contact with the lymphoid stroma, in which a number of lymphocytes expressing the same CD5 antigen were also present; these cells did not express CD20
- CD5+ tumor cells were in the peripheral area alone in 60% of cases; in 40% the cells extended to the central areas; no case had CD5+ tumor cells only in the central area
- Range of % CD5+ area in tumors: 40-90% (mean, 71).
- The level of CD5 expression in CD5-negative areas of thymic carcinoma was comparable to normal thymic epithelial cells (TEC);
- mitotic activity and Ki-67 LI were higher in both, CD5+ and CD5- areas of thymic carcinoma compared to benign TEC.
- Mitotic activity and Ki-67 LI were higher in CD5+ areas than in CD5- areas of thymic carcinoma.
- P53 and Bcl-2 expression levels were higher in thymic carcinoma than benign TEC – but no differences between CD5+ and CD5- areas in thymic carcinomas.
- No differences in E-cadherin expression

Take Home Points:
- CD5-expressing cells might induce their own proliferation, as it is a self-reactive molecule with hemophilic mechanism
- Findings suggest strongly that the tumor cells are related to further proliferation with induction of CD5
- The ability of CD5 induction to be possibly related to growth and tumor occurrence, and the thymic microenvironment may play an important role
- Inhibition of CD5-mediated pathway may be a useful target to delay tumor growth

Purpose:
- To evaluate the proportion of micropapillary components in consecutive resected adenoCa and to compare the clinicopathological, IHC and genetic differences of mucinous and non-mucinous MPC to elucidate clinicopathologic differences of mucin production in lung MPC

Methods:
- Surgically resected tumor; histology according to current WHO; IMA excluded
-Extent of micropapillary component: none (<5% of the tumor), focal (5-10%), moderate (10-50%), extensive (≥50%)
- Micropapillary component classified as mucinous or non-mucinous subtype
- EGFR, KRAS, BRAF, HER2 mutational analysis; ALK by IHC, if + - RT-PCR and/or FISH

Results:
-694 patients with consecutively resected primary lung adenoCa: 7.8% AIS, 3.7% MIA; 577 cases of invasive adenoCa: 37.9% papillary predominant, 13.7% solid with mucin production; 13.1% lepidic, 10.5% acinar, 7.9% mucin
-Micropapillary component in 48.7% (320 of 657 evaluable cases): 55 cases of mucin predominant, 172 papillary, 38 acinar, 30 solid, 25 lepidic.
-29.7% focal, 56.6% moderate, 13.8% extensive micropapillary component
-Cases with micropapillary component: 20.9% mucinous, 79.1% non-mucinous subtypes
-Tumors with micropapillary component exhibited more aggressive pathologic features and high proportion of EGFR mutations and ALK rearrangements
-Tumors with mucinous micropapillary component exhibited more aggressive pathological features, a higher proportion of HER2 mutations and ALK rearrangements and a lower proportion of EGFR mutations
-Among 67 tumors with mucinous micropapillary component, including micropapillary non-predominant cases, TTF-1+ in 89.6% cases and HNFalpha + in 16.4%
-Median f/u 111.7 months (2.3-150)
-No recurrence in any of the 80 patients with AIS or MIA
-Patients with micropapillary component had worse prognosis;
-Univariate analysis: presence of micropapillary component - associated with increased risk of death; no difference in survival depending on presence/absence of mucinous micropapillary component
-Multivariate analysis: no difference in survival depending on presence/absence of micropapillary component.

Take Home Points:
- Micropapillary component in lung adenoCa is unfavorable for prognosis but not in multivariate analysis
-Mucin production in lung MPC correlates with HER2 mutation or ALK rearrangement
-HNF4alpha is a reliable marker for IMAs and is also expressed in other adenoCa subtypes
-Mucinous micropapillary component seems to be of type II pneumocyte/Clara cell lineage (because TTF-1+, HNFalpha-)

Background:
- Previous case report of a malignant mesothelioma with MET amplification associated with MET receptor expression suggesting that inhibition of MET might be used as a targeted therapy in selected MM patient.

Purpose:
- To validate that finding in a larger cohort of MM

Results:
- 60 MM (male, 66.7%, median age, 60 yrs)
- Epithelioid (n=36), sarcomatoid (n=12), biphasic (n=8), desmoplastic (n=2), papillary (n=2)
- 30 cases from TMA, 30 from FFPE
- 5 (8.3%) cases with abnormal FISH results: 1/5 cases (epithelioid MM) had MET amplification (about 8 MET signals on >70% cells, MET/CEP7 ratio=4.0); 4/5 cases (epithelioid MM) had high polysomy of MET (range, 4-10 spots of MET in about 60-80% of MM cells)
- 55 (91.7%) FISH-negative cases – disomic for MET
- MET amplification case - moderate expression of MET protein cytoplasmic and membranous in MM cells
- High gene polysomy – low staining of MET protein

Take Home Points:
- MET amplification is rare (1.7% of total cases)
- MET polysomy is more frequent (6.7% of total cases)
- Highly polysomic status in MM might be predictive for targeted therapy (previous report by Catenacci et al – case of durable complete response in metastatic gastric cancer treated with anti-MET-TKI receptor monoclonal antibody in which the primary tumor had high MET polysomy)
- In vitro assays showed inhibition of MET receptor with PHA-665752 or Perifosine directly inhibiting the EGFR/MET-AKT axis
- MET gene amplification and polysomy associated with MET receptor overexpression might reinforce new possibilities of treatments in vivo
Articles for notation – Non-neoplastic


Purpose:
- Hypothesis: Individuals with objectively identifiable known markers of asbestos exposure have a higher incidence of histological UIP than those w/o asbestos exposure

Methods:
- Retrospective; 1718 cases who underwent at least lobectomy for pleuropulmonary tumors
- Clinical data: suspicion of asbestos exposure based on occupational hx; presence of pleural plaques based on sequential surgical, pathological, radiological HRCT reports
- Pathologic criteria for asbestosis: (i) diffuse lung fibrosis (mostly UIP pattern); (ii) > 2 asbestos bodies/cm² in specimen
- Division into asbestos-exposed group (criteria: presence of mal. pleural mesothelioma, pleural plaques, asbestos bodies in specimen) and non-asbestos exposed group
- Iron staining on some specimens – if > 1 asbestos body/specimen = positive

Results:
- 50 controls w/o occupational hx or histologic UIP – no tissue asbestos bodies
- 133 cases with occupational hx – 50 in asbestos-exposed, 83 in non-asbestos exposed group
- 183 asbestos-exposed cases: 165 - pleural plaques, 11 - MPM, 34 - tissue asbestos bodies
- 57 histologic UIP cases – 4 had > 2 tissue asbestos bodies/cm² (2 cement factory worker, 1 asbestos spray worker, 1 bus driver) – those fulfilled pathological criteria for asbestosis, 21 had tissue asbestos bodies but did not fulfill the pathologic criteria for asbestosis
- Asbestos-exposed group (n=183) – male predominance (91%), older age (69.7 yrs), higher smoking rate and pack years (90%, 46.6), higher rate of positive occupational hx (27%), higher rate of histologic UIP (31%) than non-asbestos-exposed group (n=1535)
- Asbestos-exposed group - higher rate of histological UIP (31%) than occupational hx subgroup of non-asbestos-exposed group (14%)
- Incidence of histological UIP – 14% - of these – 35% had clinical disease (most IPF, 4 asbestosis, 2RA-related UIP); majority had subclinical ILD
- Histological UIP group – higher incidence rate of being male (89%), elderly, smoker, occupational hx (14.9%), having had lower lobe lobectomy, asbestos exposure (23.8%), pleural plaques alone, asbestos bodies alone than the non-histological UIP group
- Univariate analysis: being male, elderly, smoking hx, occupational hx, asbestos exposure, asbestos bodies, pleural plaques – risk factors for histological UIP
- Multivariate analysis: being elderly, smoking hx, asbestos exposure, asbestos bodies were independent risk factors for histological UIP

Take Home Points:
- UIP more common in asbestos-exposed than non-asbestos exposed patients
- Asbestos exposure, especially if tissue asbestos bodies are present, is an important risk factor for histological UIP
- Asbestos exposure causes not only asbestosis but also histological UIP, complicating the distinction between IPF and asbestosis
Huo Z et al. Organizing pneumonia components in non-specific interstitial pneumonia (NSIP): a clinicopathological study of 33 NSIP cases

Purpose:
- To correlate proportion of OP components in NSIP with prognosis and histologic patterns
- To analyze the clinical, radiological, pathological implications of OP components in NSIP

Methods:
- 33 cases of NSIP with and w/o OP
- Multidisciplinary review of each case (pathologist, radiologist, pulmonologist)
- 4 pathologists reviewed slides – consensus
- HRCT reviewed by 2 radiologists - consensus

Results:
- 33 NSIP = 13 cellular, 15 mixed, 5 fibrosing
- Architecture preserved in all; lesions temporally uniform
- OP components in 78.8% cases; proportions: <10% (n=13), 10-20% (n=8), >20% (n=5); OP proportion correlated with histologic subtype (more common in cellular NSIP); no correlation with NSIP etiology
- No hyaline membranes, eosinophilic infiltrates, granuloma, viral inclusions, fungus
- Patients age: 20-65yo; 12 men; all had respiratory symptoms (32/33 dyspnea; 31/33 cough; 8/33 sputum; 2/33 chest pain; some had fever (9/33), fatigue (4/33, other symptoms: joint pain, joint swelling, rash, Raynaud’s phenomenon)
- 8 secondary NSIP (1 amiodarone, 7 CTD)
- 25 idiopathic; 76% ANA
- PFTs available in 30 patients; 4 normal, 25 restrictive, 1 mixed – 19 of these had mixed/fibrosing NSIP; diffusing capacity (available in 27 patients) – all diffusely abnormal; PFT dysfunction more common in mixed/fibroting than cellular NSIP
- BAL fluid: increased lymphocytes in 21, normal in 4; CD4+/CD8+ ratio decreased in 24/25; 1 normal
- Radiologic features: lower lobe predominance (90.9%), equal severity in 9.1%; 69.7% predominantly peripheral distribution; 30.3% diffuse or predominantly central: GG attenuation (n=63.6%; 10 had OP ≥ 10%; 11 had OP <10%), irregular linear or reticular pattern (72.7%), traction bronchiectasis or bronchiolectasis (75.8%), irregular focal consolidations (36.4%; 10 had OP ≥10%, 2 <10%); patchy shadows (36.4%), no HC
- 16 received corticosteroids, 13 received corticosteroids + an immunosuppressive agent
- 29 available for f/u (18 months): 17 improved, 5 stabilized, 7 disease progression-2 of these 7 died due to disease progression after 1 or 21 months – both mixed/fibroting pattern; prognosis correlated with histologic patterns but not with OP components or NSIP etiology.

Take Home Points:
- OP component is common in NSIP – more often and more extensive in cellular pattern; no effect on prognosis, not associated with etiology
- The irregular focal consolidation on HRCT may be indicative of presence of OP components

Purpose:
- To increase sensitivity for detection of malignancy, to reduce the need for more invasive surgical sampling
- To increase the sensitivity for detection of active TB and improve discrimination between TB and sarcoidosis in granulomatous lymphadenitis

Results:
- 88 patients
- Focus on “definite” diagnoses of sarcoidosis, TB, cancer, reactive lymphadenopathy (LA)–sarcoidosis, TB and reactive LA clustered together – most but not all cancer samples clustered separately
- Direct comparison of gene expression data - differentially expressed genes between granulomatous and non-granulomatous LN (488 genes), between TB and sarcoidosis (58 genes), between malignant and reactive (1,223 genes); granulomatous LNs were enriched for genes associated with immunologic processes integral to cell-mediated immunity and granuloma formation and regulated by canonical transcription factors involved in proinflammatory and cytokine responses; genomewide transcriptional profiles of TB and sarcoidosis LN samples were very similar – only 16 genes were higher expressed in sarcoidosis, 42 genes were higher expressed in TB LN samples
- In non-granulomatous cases, malignant LN samples were enriched for genes involved in cell cycle control and extracellular matrix interactions
- Step 1: classification of each sample as granulomatous or nongranulomatous; step 2: granulomatous cases were subclassified as TB or sarcoidosis; nongranulomatous cases were subclassified as cancer or reactive. Excellent specificity across all 4 diagnostic groups; high sensitivity for detection of malignancy (93%) and sarcoidosis (85%). Less sensitivity for reactive (80%) and TB (67%)
- Data from at least 5 genes were required to discriminate granulomatous from nongranulomatous, 19 genes to discriminate TB and sarcoidosis, 150 genes to discriminate cancer and reactive
- Test on specimens that were not “definite” diagnosis – SVM analysis identified “granulomatous disease” in almost all specimens with histologic evidence of granulomas, 2 samples w/o granulomas on final histology
- The majority of “possible cancer” samples were classified as “reactive”; 2 cases as cancer by SVM – in the absence of histologic evidence but later confirmed met cancer in LN
- SVM also predicted presence of “cancer” 4 months before tumor involvement was discernable on histology in another case. One case was also predicted as “cancer” by SVM but no evidence of malignancy on f/u and presumptive diagnosis of sarcoid

Take Home Points:
- It might be possible to discriminate granulomatous from nongranulomatous disease and TB from sarcoidosis using multiplex quantitative PCR analysis of 20-30 genes
- Application of SVM in a 2-step decision tree model was more sensitive than mycobacterial culture for id of TB
- Maybe identifies patients who might benefit from surveillance
Review Articles


- Discussion of new observations some of which have been implemented into the WHO, others are still under investigation
- Distinguish diffuse malignant mesothelioma (DMM) from other MM with better prognosis (localized MM-LMM, well-differentiated papillary MM – WDPM)
- Histologic subtyping of epithelioid mesothelioma
  - Morphologic subtypes: tubulopapillary, papillary, micropapillary, trabecular, solid, pleomorphic
  - Epithelioid DMM with pleomorphic features
    - Aggressive behavior, worse prognosis than other epithelioid MM, survival similar to biphasic and sarcomatoid meso
    - Anaplastic or prominent giant cells, often multinucleated
    - Not reclassified as sarcomatoid meso – they are just regarded as a poor prognostic subset of epithelioid DMM
    - After exclusion of pleomorphic epithelioid MM, combined tubulopapillary & trabecular tumors had more favorable prognosis than solid subtype and the combined solid/micropapillary group
    - Substantial interobserver reproducibility among 2 observers for subtyping of epithelioid DMM
- IHC – see table 2 for sensitivities and specificities and organ-specific stains
  - Recommend a panel of 2 meso markers, 2 adenoCa markers and TTF-1 (no discussion on clone)
  - GATA-3 not helpful because expressed in >50% mesos
- Separation of benign from malignant mesothelial proliferations – major and minor criteria nicely summarized in table 3
  - Invasion for malignant meso is still the key
  - Zonation
  - Problem: surface proliferations might be simply extension of an invasive tumor onto pleural surface – p16 might be helpful
  - Desmoplastic meso: invasion, short storiform pattern (“patternless pattern”)
  - Be aware of “fake fat”
  - IHC not helpful
  - p16 FISH, BAP1 IHC promising
- Sarcomatoid meso with and without heterologous elements
  - If heterologous elements are present – most often osteosarcomatous > mixture of chondrosarcoma and osteosarcoma, rhabdomyosarcoma, and chondrosarcom only
  - If diffuse pleural thickening with heterologous elements, even if the keratin is negative, by convention the diagnosis of MM is preferred over osteosarca, chondrosarca, rhabdomyosarca
  - Diagnosis of MM can be made in the absence of keratin and calretinin expression if the morphologic, clinical and radiologic features are otherwise consistent with meso – there should be diffuse pleural thickening in the absence of an intrapulmonary mass or a history of soft tissue sarcoma
• Well-differentiated papillary mesothelioma (WDPM) = superficial spreading of papillary formations with broad fibrovascular cores, often with myxoid stroma – lined by bland, flattened, or epithelioid mesothelial cells, w/o or with limited invasion of the submesothelial layer
  o Invasion can consist of invasion of stalks of papillae by bland-appearing cells or cytologically higher grade solid foci
  o Correlations with radiology (small translucent nodules) and operative findings
  o WDPM – typically indolent. Most behave clinically benign after complete resection
  o Uncertain whether WDPM can progress to DMM
  o WDPM with invasive foci confined to stalk are prone to recurrences but rarely fatal
• Promising advances
  o Histologic grading – not included in WHO
  o p16 deletion by FISH
    ▪ Never seen in benign mesothelial processes – but not 100% sensitive.
    ▪ Only useful if deletion is present.
    ▪ Can be useful for surface proliferation of underlying DMM
    ▪ Useful to favor DMM in cytologic specimens or pleural effusions – but that needs conformational studies
    ▪ Loss of p16 by FISH – correlated with poorer prognosis
    ▪ p16 IHC and FISH do not correlate
  o BAP1 – inactivating mutations – in sporadic and hereditary DMM
    ▪ 40-60% epithelioid MM; <20% sarcomatoid MM
    ▪ Most are somatic, <5% germline mutations
    ▪ IHC for BAP1 appears reliable to predict mutation
  o Combination of p16 loss by FISH and BAP1 loss by IHC – might be useful to separate benign from malignant mesothelial proliferations
  o STAT6 IHC for SFT – might be useful specifically in high grade SFT and on small biopsies
    ▪ Not specific – positive in a few desmoid tumors and unclassified sarcomas
  o WWTR1-CAMTA1 fusion as a marker for epithelioid hemangioendothelioma
    ▪ Small percentage of these tumors might have the YAP1-TFE3 fusion
  o CTNNB1 mutation and beta catenin IHC in desmoid-type fibromatosis