Pulmonary Pathology Journal Club

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Articles for Discussion

Impact of patient characteristics, prior therapy, and sample type on tumor cell programmed cell death ligand 1 expression in patients with advanced NSCLC screened for the ATLANTIC Study.
Boothman et al, JTO

Background: Checkpoint inhibitors targeting the programmed cell death ligand 1 (PD-L1) pathway have resulted in a significant survival benefit in patients with non-small cell lung carcinoma. Response to these drugs is enhanced in tumors that express high levels of PD-L1. PD-L1 status can be impacted by smoking, EGFR mutation and histology. PD-L1 status can also be variable following treatment or in sampling of metastatic versus primary tumors. Chemotherapy and radiation therapy have been proposed to upregulate PD-L1 expression. The objective of the study was to determine the impact of patient characteristics, sample types and prior therapies on PD-L1 expression as well as concordance of PD-L1 expression in a subset of paired recent and archival samples.

Methods: 1590 patients in the ATLANTIC study were included. The patients had stage IIIB-IV NSCLC and received at least 2 prior systemic treatments, one of which included a platinum based regimen. A subset of patients had PDL1 testing performed on both a recent and archival biopsy specimen. PDL1 was performed using the SP263 assay on formalin-fixed paraffin-embedded tissues. Tumors were classified using a cutoff of greater than or equal to 25% of tumor cells.

Results:

- There was higher rates of PD-L1 positivity in the 1121 patients that smoked (35.1%) compared with the 469 non-smokers (26.2%); p=0.0005.
- Lower rates of PD-L1 positivity were seen in patients with EGFR mutations (26.1%) compared with tumors without EGFR mutations (36.9%); p=0.0002.
- 33.2% of biopsy cases and 28.3% of resected samples were positive for PD-L1; p=0.192
- The prevalence of PDL1 positivity was significantly higher in recent samples from ≤3 months (34.3%) compared with archival samples from; > 3 months – ≤1 year (30.3%), >1 year - ≤3 years (29.4%) and ≥ 3 years (20.2%); p=0.039.
- There were increased PD-L1 positive cases in metastatic samples (36%) compared with samples from primary tumors (29.5%); p=0.005.
- There was no difference in PDL1 status in patients that received tyrosine kinase inhibitors (39.9 %) before sample acquisition and those that did not (39.7%); p=0.969.
- PDL1 positivity was significantly higher in patients who received chemotherapy (41.6%) prior to sample acquisition versus those that did not (29.0%); p = 0.004. This was also seen in those that received radiation therapy (50.4%) prior to sample acquisition compared with those that did not (38.4%); p = 0.013.
- In 123 cases with paired analysis of recent samples (≤ 3 months) compared with archival samples (> 3 month) PD-L1 status was unchanged in 74% of cases and 19.5% of cases

had an increase in PD-L1, while 6.5% of cases had a decrease. There was no difference in this small subgroup based upon prior treatment.

Conclusion: PDL1 status can be impacted by treatment with chemotherapy or radiation therapy prior to sample acquisition and may impact patient selection for additional therapy. Lower rates of PD-L1 positivity were present in archival cases.

Take home message: PD-L1 status is variable and it can change due to patient or treatment characteristics. Ideally PD-L1 testing should be performed on recent specimens <3 months and not archival specimens.

Prognostic impact of the number of metastatic lymph nodes on the eighth edition of the TNM classification of NSCLC.

Katsumata et al, JTO

Background: The current TNM classification for lung cancer classifies the nodal stage based upon anatomical location according to the defined nodal map. Prognostic heterogeneity in patients within the same nodal stage has been identified. Nodal staging in other solid tumors includes the number of metastatic lymph nodes. The objective of the study was to identify the prognostic significance of the number of metastatic lymph nodes within the current TNM classification system.

Methods: 1989 patients treated with lobectomy (n=1889) or pneumonectomy (n=100) with dissection of the hilar and mediastinal lymph nodes for non-small cell lung cancer treated between January 2003 and December 2012 were included in the study. Patients who underwent preoperative treatment and patients with low-grade malignancies (carcinoid, mucoepidermoid carcinoma or adenoid cystic carcinoma) were excluded. Hilar and mediastinal lymph nodes were dissected en bloc with surrounding adipose tissue. N1 station lymph nodes were removed on block with lung tissue and classified and separated by the pathologist into segmental (#13) and subsegmental (#14) stations. Systematic lymph node dissection was performed in the majority of cases (n = 1687). Lobe specific lymph node dissection was conducted in patients with ground-glass opacities that were clinically diagnosed as nodal stage I or II (n= 124) and in patients that did not qualify for systematic lymph node dissection due to other causes such as severe ischemic heart disease or high bleeding risk (n = 178). All sections were re-examined and classified based on the 8th addition of the TNM classification. Patients were followed at 3 month intervals for the first 2 years and at 6 month intervals thereafter. All the patients had at least 5 years of follow-up after surgery. Further CT or PET-CT imaging was performed with any signs of suspected recurrence.

Results:

- The mean number of resected lymph nodes was 15.5 (+/- 8.4).
- The majority of cases were small tumors with 57% of the cases in T1 categories, 27% in T2, 10% in T3 and 5% in T4
- 75% of cases were N0, 13% were N1, 12% of cases were N2 and 0% of cases were N3.
- In the multivariate analysis for overall survival age, male gender, invasive size>1.0 cm, pleural invasion, pulmonary metastasis, lymphovascular invasion, vascular invasion, N1 node positivity, N2 node positivity along with number of metastatic nodes (multiple-nodes versus single-nodes) and number of metastatic stations (multiple-station versus single-stations) were associated with reduced overall survival.
- Multiple nodal metastasis was an independent prognostic factor in pN1 disease (HR 1.41; p =0.04) but not pN2 disease (HR 1.29; p = 0.13).
- Increased numbers of positive nodes were independent prognostic factors in N1 disease (≥3 HR 1.57; p=0.01, ≥4 HR 1.82; p =<0.01, ≥5 HR 2.12; p=<0.01) as well as in N2 disease (≥3 HR 1.59; p=0.01, ≥4 HR 1.78; p =<0.01, ≥5 HR 1.87; p=<0.01)

Conclusion: Quantification of the number of positive metastatic lymph nodes had an impact on survival in addition to the current pN classification in the 8th edition of the TNM staging system.

Take home message: Assessment of the number of positive lymph nodes may provide additional prognostic information for patients with NSCLC.

In situ growth in early lung adenocarcinoma may represent precursor growth or invasive clone growth- A clinically relevant distinction.
Moore et al, Modern Pathol

Background: "Noguchi C" tumors are defined as lung adenocarcinomas showing areas of both invasive and in situ growth. The authors hypothesized that Noguchi C tumors actually represented a combination of adenocarcinoma in situ with progression to invasive adenocarcinoma (precursor lesion, i.e. true tumor progression from an in situ component to invasive component), as well as fundamentally "de novo" invasive adenocarcinoma in which the tumor cells are colonizing the adjacent alveoli in a lepidic fashion (tumor outgrowth, i.e. not a true adenocarcinoma in situ component, not a true tumor progression). The fact that metastases to the lung can sometimes show lepidic growth at the periphery would support this notion.

Methods: They included 110 Noguchi C tumors, and applied 3 morphologic characteristics to predict that a tumor had a true in situ component:
1. Obvious change in nuclear grade from in lepidic to invasive component
2. Invasive component located asymmetrically with regard to the center of the tumor ("architectural asymmetry")
3. Absence of a lepidic "penumbra", i.e. lepidic spread of uniform width at the periphery of the tumor.

Tumors with at least 2 of these 3 characteristics were considered "Noguchi C1", i.e. "real" AIS with invasion; while tumors with 1 or 0 were considered "Noguchi C2", i.e. lepidic colonization by invasive tumor. They created a tissue microarray in a subset of cases (44; 27 C1 and 17 C2), and tried to include lepidic and invasive components separately where possible. They did IHC for KI67, p53, and vimentin. They also employed laser capture microdissection to separately sample lepidic and invasive components of 18 C1 and 5 C2 tumors to collect DNA for NGS.

Results
- Their cohort included 42 C1 tumors (mostly lepidic predominant) and 68 C2 tumors (mostly acinar predominant).
- Rate of nodal mets was higher in C2 (26%) vs. C1 (8%) tumors (p=0.03), but there was no difference in patient outcome.
- KI67 labeling index was higher in C2 tumors overall; the C2 tumors did not show difference between the invasive and lepidic components, while the C1 tumors showed significantly higher KI67 in invasive compared to in lepidic components.
- About 20% of C1 tumors showed acquisition of mutant p53 staining in invasive areas, while all C2 tumors with mutant p53 pattern were concordant between invasive and lepidic areas.
- C1 tumors showed more vimentin expression in the invasive areas compared to lepidic areas, while there was no difference in vimentin expression between lepidic and invasive areas in C2 tumors.
- *KRAS*, *EGFR* and *BRAF* mutations were common and present in all components of all tumors tested, indicating they occur early in pathogenesis. Three of 18 C1 tumors had private

mutations in the invasive component (*TP53, PIK3CA, SMAD4*); none of the C1 tumors had any private mutations in the lepidic component. There were no private mutations in the C2 tumors, with all detected mutations being present in both invasive and lepidic components; the C2 tumors were much more likely to have *TP53* mutations (80% vs. 17%).

- They found that KI67 rate <10% was indicative of true in situ/precursor growth, and tumors with KI67<10 % in the lepidic component had a better prognosis than tumors with >10% KI67 in the lepidic component (please see K-M curves in figure 5).

Conclusions: They have presented morphologic, immunohistochemical and genetic evidence that tumors with a mix of lepidic and invasive growth represent a mix of tumors that are proceeding through the AIS-MIA-invasive adenocarcinoma progression, and de novo adenocarcinomas that are showing outgrowth along alveolar spaces but do not have a true AIS component. This could be important going forward when aiming to study invasive tumors that truly arise from precursor AIS.

Take home message: Not all lepidic pattern is created equal. Tumors with true in situ component can be recognized based on morphologic features, and should be considered separately from invasive tumors that show some focal outgrowth along the septa at the periphery.

Stage IV Lung Carcinoids: spectrum and evolution of proliferation rate, focusing on variants with elevated proliferation indices
Rekhtman et al, Modern Pathology

Background: Per WHO criteria, mitotic count <2 mitoses/2mm2 defines typical carcinoids and 2-10 mitoses defines atypical carcinoids. The application of these cut-offs to metastatic disease has not been well studied, and only 5-10% of pulmonary carcinoids are stage IV, so high stage tumors are not so easy to study. KI67 is not used in classification of pulmonary carcinoids, but the WHO suggests that pulmonary carcinoids should have KI67 <20%. Rare tumors fall in the "gray area" of this classification: they look like a carcinoid tumor and have >10 mitoses but less than usually seen in HGNEC

Methods: Manual mitotic count and KI67 were performed in 66 stage IV pulmonary carcinoids, including both primary and metastatic lesions when available. They included a mix of biopsy and resections. Immunostains for Rb (n=19) and targeted next-gen sequencing (n=9) was performed in cases with increased proliferation.

Results

- 70% of patients presented at stage IV, while 30% were resected at lower stage and developed mets later. 20 patients had resected primary tumors, and 80% of those were atypical carcinoids. About 40% of patients had one site sampled, while the rest had at least 2. Most common metastatic sites included liver, bone, skin, and brain.
- Mitotic counts tended to be higher at metastatic sites than in the primary tumor (mean mitotic count 5.6 vs. 3.0 per 2 mm^2; p = 0.06)
- Mitotic count >10 per 2 mm^2 was present in 19 of 81 (23%) metastases; 3 exceeded 20 mits. KI67 was higher in the mets than in the primary tumor. Hot-spot KI67 exceeded 20% in 13% of primary tumors and 27% of metastases.
- Elevated proliferation (either >10 mits or >20% KI67) was observed in 27% of all samples tested; 42% of patients had at least one sample with elevated proliferation. 90% of samples with increased proliferation were mets. 6 samples exceeded 20 mits (all metastatic lesions). KI67 labeling index was often heterogeneous, with hotspots in a background of lower level activity.
- Most cases with increased proliferation (28 of 35) had a "normal" carcinoid morphology. Some showed areas of more poorly differentiated morphology, including sheet-like growth, increased N:C ratio, crowding, and prominent nucleoli.
- Patients with increased proliferation had a slight predominance of females (64%) and 64% were never smokers. Median OS was 2.7 years, similar to those without increased proliferation.
- RB expression was retained by IHC and no mutations were identified in *TP53* or *RB1* in cases with increased proliferation. They did show mutations common in carcinoids, including *MEN1* and other chromatin modifiers, and had low mutation burden.
- Comparison of multiple samples from the same patient (n=48 pairs) showed marked heterogeneity of mitotic rate and KI67 though the course of disease. 35% showed increased in

mitotic rate or >10% increase in KI67 in mets; decreases also occurred but were less common (n=6). The rest had similar rates.

Conclusions: One quarter of stage IV carcinoids will exceed the current WHO mitotic rate or KI67 threshold, but do not show genetic features or clinical outcomes of HGNEC Mitotic rare can escalate at metastatic sites in 35%; therefore, the authors proposed that classification of pulmonary carcinoids as typical or atypical is not applicable to metastatic lesions.

Take home message: Mitotic rate can increase in carcinoid tumors when they metastasize, and this does not necessarily mean they are no longer carcinoid tumors and must be classified as HGNEC. The authors propose simply calling them metastatic carcinoid tumor with a comment regarding mitotic rate/grade. This data may be paving the way for a new category of neuroendocrine neoplasm, akin to the Grade 3 NET used in the GI/pancreas. I think it would be a great addition.

Molecular classification of neuroendocrine tumors of the thymus.
Dinter et al, JTO

Background: Thymic neuroendocrine tumors are rare and unusual. They are classified/ graded using the same system as pulmonary tumors (carcinoid, atypical carcinoid, LCNEC, SCC), even though there is a very different disease profile with a preponderance of atypical carcinoids and LCNEC in the thymus, contrasting to mainly typical carcinoids and SCC in the lung There is some evidence that they may be genetically different from pulmonary carcinoids as well.

Methods: 103 patients with thymic NETs were included, which encompassed both biopsy and resection specimens: 22 typical carcinoid (TC), 51 atypical carcinoid (AC), 28 LCNEC and 6 SCC. KI67 was performed and read by DIA. IHC was also performed for chromo, synapto, RB, p53, ATRX, and EZH2. Genetic testing included shallow whole-genome sequencing and chromosomal instability score (CNI). Next gen sequencing panel was performed in 11 cases.

Results
- Genetic analysis showed an increase in chromosomal instability (CNI) from TC to AC to HGNECs. A few outliers were noted, however- one LCNEC with very low CNI, and one atypical carcinoid with very high CNI. Copy number alterations had little in common between TC and AC, while AC had lot overlap with alteration seen in HGNEC. These chromosomal changes were different from what has been observed in pulmonary NETs.
- Unsupervised clustering of copy number alterations resulted in 3 clusters. CNI< 9 was cluster one (low), between 9-30 was cluster 2 (intermediate), and >30 was cluster 3 (high). All SCC were in the high CNI group, but interestingly, cases of LCNEC were divided into all 3 clusters. Similarly, AT also distributed into all 3 clusters. Most TCs distributed into clusters 1 and 2. Mitotic activity and necrosis were not able to predict the CNI cluster of any given tumor.
- LCNEC that clustered in the low-intermediate CNI group generally had carcinoid-like morphology, and IHC pattern suggestive of a lower grade tumor (chromogranin +, EZH2 -, preserved RB, wild-type p53); this would presumably be analogous to a NET grade 3 category. LCNEC that clustered in the high CNI group looked like a high-grade carcinoma in half of cases, and were mostly chromogranin -, EZH2+, and half showed loss of RB and overexpression of p53.
- A few cases had both primary and metastatic disease that could be tested, which generally showed progression in grade as well as in CNI score in the mets
- NGS unexpectedly revealed pathogenic *NF1* mutations in all 11 cases tested, which included NET grade 3 and LCNEC cases, with high allelic frequency (germline?). RAS/MAPK proteins were also frequently mutated.
- CNI predicted OS when dividing cases into the 3 groups mentioned. Therefore, the authors propose a modified three-tiered grading system for thymic NETs incorporating mitotic rate, KI67, CNI, and IHC for chromogranin and EZH2 (please see table 5).

Conclusions: Copy number variation scores can divide thymic neuroendocrine neoplasms into 3 prognostic groups, and the genetically determined risk groups contain a rather shocking

diversity of neuroendocrine neoplasms as determined by WHO criteria. Importantly, what we classify as LCNEC seems to subdivide into a grade 3 NET subgroup (chromo +, EZH2 -) and a "true" high grade LCNEC group (chromo -, EZH2 +).

Take Home Message: The current system used to classify thymic neuroendocrine neoplasms does not work very well, and has operated under the assumption that they are similar to lung tumors, which they are not. This proposed classification system may not catch traction because it requires molecular analysis- but this knowledge is a good place to start and may lead to refinement of our current morphology-based system with more study.

Articles for Notation

Neoplastic

Decoy receptor 3 expression is associated with wild-type EGFR status, poor differentiation of tumor, and unfavorable patient outcome
Chang et al, AJCP

Summary: IHC study performed on tissue microarrays containing 461 cases of lung adenocarcinoma. Decoy receptor 3 expression was associated with solid, micropapillary and acinar growth patterns, and tumors that were wild-type for EGFR. The expression rate varied quite widely with growth pattern, present in 2% of lepidic tumors up to 46% of solid tumors. It was also associated with poorer outcome in stage I patients.
Take home message: Decoy receptor 3 expression seems to be more common in growth patterns that are known to portend a worse prognosis.

Real-world prevalence of programmed death ligand 1 expression in locally advanced or metastatic non-small cell lung cancer: The global, multicenter EXPRESS study
Dietel et al, Lung Cancer

Summary: Large study of stage III/IV lung cancer patients worldwide with PD-L1 status known (clone 22C3), including 2,368 patients. 22% of patients had TPS >50%; 52% had TPS >1% and 48% had TPS <1%. 6% of tests were reported as insufficient tumor cells to determine PD-L1 status. Rates of PD-L1 positivity were very similar among different regions of the world. EGFR mutation rate was 19% and ALK mutation rate was 3%.
Take home message: PD-L1 positivity rate seems to have little variation based on ethnic/regional factors. When considering all NSCLC patients, about 22% will have TPS >50%, and about 6% of tests will be insufficient.

Characterization of lung adenocarcinoma with a cribriform component reveals its association with spread through air spaces and poor outcome
Ding et al, Lung Cancer

Summary: Study of 208 cases of lung adenocarcinoma, 67 of which had ≥5% cribriform growth. Patients who had tumors with a cribriform component had higher risk of both locoregional recurrence and distant metastasis. STAS was observed in 72% of cribriform tumors, and cribriform growth was an independent risk factor for STAS. When considering only tumors with a cribriform component, STAS was associated with a poorer outcome compared to cribriform tumors that did not have STAS.

Take home message: Tumors with a cribriform component have a high rate of STAS, and STAS is associated with poorer survival.

Cardiorespiratory fitness and incident lung and colorectal cancer in men and women: Results from the Henry Ford Exercise Testing (FIT) cohort
Handy Marshall et al, Cancer

Summary: Large data cohort of over 49,000 patients who underwent exercise stress testing. The patients in the highest fitness category had a 77% lower rate of lung cancer and 61% lower rate of colorectal cancer; this group also had the best outcome if they did get lung or colorectal cancer. Interestingly, for lung cancer, this held true even when adjusted for smoking status.
Take home message: High level of cardiorespiratory fitness seems to be protective against lung and colorectal cancer.

Heterogeneity of PD-L1 expression in non-small cell lung cancer: Implications for specimens sampling in predicting treatment response
Haragan et al, Lung Cancer.

Summary: Study of 107 resected NSCLC cases using SPT263 clone. Cases were assessed using digital analysis, and heterogeneity was determined on small scale (differences between 1 mm^2 regions of tumor), medium scale (differences between 1cm^2 areas of tumor), and large scale (between different tumor blocks). Nodal mets were also scored and compared to the primary. 78% of cases showed small scale heterogeneity, 50% had medium scale, and 46% large scale. 53% showed heterogeneity between primary tumor and nodal mets, and 17% had heterogeneity between N1 nodes and N2 nodes. Staining a second block lead to a clinically significant change in PD-L1 (i.e. across a treatment threshold value) in 8% of cases; a similar rate (12.5%) of cases would experience a clinically significant change based on medium scale heterogeneity (i.e. adding additional 1 cm^2 of tumor).
Take home message: PD-L1 staining is heterogeneous, and this heterogeneity can lead to clinically meaningful change in PD-L1 interpretation in a minority (about 10%) of cases.

Perioperative chemotherapy is not associated with improved survival in stage I pleomorphic lung cancer.
Henriksen et al. J Thorac Cardiovasc Surg

Summary: Pleomorphic carcinoma is a rare type of sarcomatoid NSCLC (0.1% of lung cancers) with a dismal prognosis. This is a population-based study to see if stage I patients with pleomorphic carcinoma might benefit from adjuvant chemo. They compared 1,408 patients

with pleomorphic carcinoma from the national cancer database to a control group of over 607,000 lung adenocarcinoma patients. Patients with pleomorphic carcinoma had a worse prognosis compared to adenocarcinoma for all stages, and were more likely to receive adjuvant chemotherapy. 253 stage I pleomorphic carcinoma patients were treated with surgery alone, while 57 received perioperative chemotherapy; there was no difference in outcome between these two groups.
Take home message: There does not seem to be a role for adjuvant chemo in stage I pleomorphic carcinoma based on this retrospective population-based study; the need for better therapies persists.

Prognostic significance of combining immunohistochemical markers for cancer-associated fibroblasts in lung adenocarcinoma tissue
Inoue et al, Virchows Archives

Summary: Study of 92 cases of pulmonary adenocarcinoma stained for SMA, podoplanin (D2-40) and periosin. These stains were scored on the cancer-associated fibroblasts. High SMA expression was weakly associated with high KI67 labeling, nodal mets, and poor 5 year OS. High podoplanin expression was weakly associated with high T stage, high KI67, distant mets, and poor 5 year OS. High periostin expression was weakly associated with high T stage and high KI67. They tried to risk stratify using a combination of these markers but did not come up with any statistically significant groups.
Take home message: While it seems that there are some differences in immunophenotype of cancer-associated fibroblasts between lung cancer cases that have some prognostic implications, it seems difficult to translate these into clinically meaningful groups.

TdT expression in germ cell tumors: a possible immunohistochemical cross-reaction and diagnostic pitfall.
Jaconi et al, J Clin Pathol.

Summary: Study of 22 cases of germ cell tumor, mostly (16) gonadal seminomas and the rest mixed germ cell tumors and other misc. germ cell tumors. They stained for TdT using a combination of monoclonal (clone EP266) and polyclonal antibodies. 13 of 14 were positive with the monoclonal antibody with moderate classical nuclear staining, and 6/17 were positive with the polyclonal antibody with focal and mild reactivity; the difference in staining between the monoclonal vs. polyclonal antibody was statistically significant. They were unable to confirm TdT protein expression in germ cell tumors using Western blot and mass spec.
Take home message: TdT can be expressed in germ cell tumors, which may be a diagnostic pitfall which seems most likely to be relevant in the mediastinum. It may be cross-reactivity in the IHC since protein expression cannot be confirmed by other methods.

Limited resection is associated with higher risk of locoregional recurrence than lobectomy in stage I lung adenocarcinoma with tumor spread through air spaces
Kadota et al, AJSP

Already discussed by Dr. Khoor during August Journal Club

PD-L1 expression in non-small cell lung cancer: Evaluation of the diagnostic accuracy of a laboratory developed test using clone E1L3N in comparison with 22C3 and SP263 assays.
Munari et al, Human Path.

Summary: PDL1 clone E1L3N is currently a "research use only" antibody from Cell Signaling, which is apparently an expensive compared to some of the other antibodies. They sought to validate clinical use of this antibody as a laboratory developed test by comparing it to the 2 commonly used clones 22C3 and SP263. They did this by staining tissue microarrays composed of 165 cases of lung cancer on the Ventana platform. E1L3N had very good concordance with SP263 at 1% and 50% cutoffs, and lower concordance with 22C3. However, they argue that since E1L3N had 100% sensitivity for the 50% cutoff observed with both other clones (i.e. they did not have any false negatives), it can be used for clinical testing.
Take home message: Another potential new PD-L1 clone on the block, which seems to be on the sensitive side.

Insulinoma-protein 1 is a robust nuclear immunostain for the diagnosis of small cell lung carcinoma in cytology smears
Nakra et al, Cancer Cytopathology

Summary: Study of INSM1 IHC on 37 small biopsies and 36 direct cytology smears previously diagnosed as small cell carcinoma. In 3 direct smears there was too much necrosis for interpretation. Sensitivity (with any staining in 1% or more of cells considered positive) in small biopsies was 97% compared to 91% in direct smears. 11 cases had both a small biopsy and a direct smear, and 91% of these cases had concordant results (one cytology smear was negative while the associated biopsy was diffusely positive, indicating a false negative stain in the smear). They tested 10 cases of NSCLC, all of which were negative.
Take home message: INSM1 seems to work pretty well on direct cytology smears (although false negatives can occur), and positive staining can be used to support the diagnosis of small cell carcinoma. I wonder what everyone's experience is with INSM1 so far? I have found the sensitivity to be a bit lower than I had hoped in real life "tough" cases…

A prospective cohort study to define the clinical features and outcome of lung cancers harboring Her2 aberration in Japan (HER2-CS study)
Ninomiya et al, Chest

Summary: Study of over 1100 lung cancers screened for HER2 abnormalities by IHC, FISH and direct sequencing. 3% of lung cancers had 3+ HER2 over-expression by IHC. An additional 3% had 2+ IHC staining with positive FISH. They sequenced HER2 in EGFR wild-type tumors (n=724), and about 3% of these tumors had *HER2* mutations detected by sequencing. None of these tumors with *HER2* mutation detected by sequencing had 3+ IHC. The mutant tumors were female predominant, while those with overexpression by IHC were more often male smokers. HER2 abnormalities were associated with worse prognosis when compared to *EGFR* and *ALK* mutations, and the small subset that were treated with HER2 targeted therapy did not seem to respond.
Take home message: HER2 overexpression and *HER2* mutations both occur at a low frequency in lung cancer, although they are pretty common (nearly 10%) when any type of abnormality is considered. Seems to have a poor prognosis. Treatment implications are yet to be elucidated, as early trials of currently available HER2 targeted therapy in NSCLC have been disappointing.

Molecular alterations in pulmonary adenocarcinoma of African Americans
Rodriguez et al, AJCP

Summary: Study of 113 African American patients with lung cancer, although molecular analysis was only performed in 91 (NGS hot-spot panel). Mutations were identified in 58%. KRAS was most common (45%) followed by EGFR (36%), BRAF (7.5%) and ALK (4%). Compared to Caucasians, AA patients presented at higher stages and had shorter survival.
Take home message: While the molecular profile of adenocarcinoma in AA patients is similar to Caucasians, they have poorer outcomes, an issue that needs to be addressed.

Impact of delayed and prolonged fixation on the evaluation of immunohistochemical staining on lung carcinoma resection specimen
Van Seijen et al, Virchows Arch

Summary: They obtained 10 sections from large tumors (>4 cm). 2 sections were fixed normally for 24 hours, 5 were delayed (6, 24, 48 and 96 hour delays) and 3 had prolonged fixation (2, 4 and 7 days). They then created TMAs, which were stained for 20 IHC antibodies. Delayed fixation was associated with loss of tissue from the slides, poor tissue quality, and significantly decreased expression of many antibodies including keratins, TTF1, napsin and PDL1. Prolonged fixation did not affect the IHC. This decreased intensity was observed starting at 24 hours of delay.

Tame home message: Delaying fixation 24 hours or more is bad for tissue quality and can lead to reduced staining of both diagnostic and prognostic markers.

Computed tomography-based score indicative of lung cancer aggression (SILA) predicts the degree of histologic tissue invasion and patient survival in lung adenocarcinoma spectrum.
Varghese et al, JTO

Summary: SILA is a computer algorithm that can use the CT scan characteristics of lung adenocarcinoma to assign a predicted risk of aggressive disease. This study includes 237 resected lung adenocarcinomas that were lovingly measured for maximum histopathologically determined invasive size (by me ☺). SILA assessment of preoperative CT was able to divide the group into indolent (AIS and MIA) tumors vs invasive adenocarcinomas, and SILA assessment correlated with size of maximal invasion as measured by a pathologist based on the resected tumors. SILA was able to stratify stage I lung cancers into indolent, intermediate and poor prognostic groups with 5-year OS rates of 100%, 79% and 58%, respectively.

Take home message: Computer algorithms like SILA based on preop CT are potentially powerful tools to determine which lung cancers are likely to behave aggressively preoperatively, which could help clinicians making decisions on which lesions might be safe to follow and which really need to come out. This would be especially helpful in patients that might not be ideal surgical candidates.

Heterogeneity analysis of PD-L1 expression and copy number status in EBUS-TBNA biopsy specimens of non-small cell lung cancer: Comparative assessment of primary metastatic sites.
Yoshimura et al, Lung Cancer

Summary: Study of 71 EBUS-TBNA samples tested for PD-L1 IHC as well as FISH for PD-L1 copy number alterations (which has been proposed by these authors as a potentially superior alternative to PD-L1 IHC), which were compared to corresponding specimens including 68 transbronchial biopsies, 13 surgical resections of the primary tumor, and 6 surgical resections of metastasis. The concordance of PD-L1 positivity >1% in EBUS-TBNA specimens compared to the other specimens was moderate for TBBx (kappa=0.63) and resection (kappa=0.68), and the kappa was 1 when compared to metastatic resection in that small cohort. Similar agreement rates were seen in the setting of FISH for copy number alteration, although some deeper analysis showed that copy number alteration seemed to be less heterogeneous than protein expression.

Take home message: PD-L1 copy number alterations may be less heterogeneous than protein expression. I think the therapeutic implications of copy number alterations in predicting response to immune checkpoint inhibitors needs more study.

Non-Neoplastic

Telomere length and use of immunosuppressive medications in idiopathic pulmonary fibrosis
Newton et al, AJRCCM

Summary: In large clinical trials, immunosuppression is known to be associated with increased adverse events (death, transplant, hospitalization, decreased FVC) in IPF patients. This is a complicated study with looked at telomere length in patients included in 2 existing IPF trials and 1 independent cohort. Around 55-60% of IPF patients had telomeres <10% of average. Exposure to prednisone, azathioprine, or NAC was associated with a higher adverse composite endpoint as defined above for patients with telomeres <10% of average.
Take home message: It seems that short telomeres may be a biomarker of IPF patients who will experience adverse events when treated with immunosuppression.

Pulmonary vascular density: Comparison of findings on computed tomography imaging with histology.
Rahaghi et al, Eur Resp J

Summary: Smoking leads to vascular remodeling and "vascular pruning" can be seen on CT. They did 3-D vascular reconstruction (i.e. vascular volume measurement) using CT imaging in 18 patients and 2-D reconstruction (i.e. vascular cross sectional area measurement) in 17. They compared this to manual measurement of cross-sectional vascular area obtained by histopathologic exam (yikes- that sounds like a lot of work!). They found they were able to detect decreased pulmonary vascular density in smokers by all 3 methods, which correlated with one another.
Take home message: Loss of small vessel volume by CT that is seen in smokers correlates with loss of cross sectional area of vessels assessed by histopathology.

Usefulness of gene expression profiling of bronchoalveolar lavage cells in acute lung allograft rejection
Weight et al, J Heart Lung Tanspl

Summary: Interesting idea to use gene expression profiling instead of transbronchial biopsy for monitoring of acute cellular rejection (ACR) after lung transplant, given the limitations and risk of biopsy. They performed RNA sequencing on cell pellets derived from bronchoalveolar lavage in 61 patients with ACR, 58 with lymphocytic bronchiolitis (their institutional method of B grading changed during the study period, thus they used this more generic term), 41 with infection, and 59 with no rejection or infection. They found 72 differentially expressed genes, mostly involved in T-cell receptor signally, NK cytotoxicity, and cytokine-receptor interactions. They chose 4 genes based on their training set, which demonstrated an accuracy in predicting

ACR of 76% with sensitivity of 60% and specificity of 82%. Cases designated as positive for ACR by the gene panel had a worse CLAD-free survival (hazard ration 2.4).
Take home message: While I think this has a way to go in terms of improving assay performance (particularly with regard to decreasing false positive tests and increasing sensitivity for ACR), I think it is a very attractive method given the less invasive procedure and shows real promise for diagnosis of ACR.

Reviews, Letters, and Case Reports

SMARCA4 loss is very rare in thoracic mesothelioma
Ahadi et al, AJSP

Take home point: Isolated SMARCA4 loss (without associated SMARCA2 loss) was present in only 2 of 296 mesotheliomas (0.7% of mesotheliomas; 1.1% of epithelioid mesotheliomas). Their diagnoses were "not unequivocal". This is lower than the 13% rate of loss recently reported by Perret et al.

In reply to "malignant mesothelioma and is non-asbestos causes"
Attanoos et al, Arch Pathol Lab Med

Take home point: Fiery commentary on the lack of evidence that talcum powder is a proven risk factor for non-asbestos associated mesotheliomas.

Rethinking bronchoalveolar lavage in acute cellular rejection: How golden is the standard of transbronchial biopsies?
Koutsokera, J Heart Lung Tanspl

Editorial covering the included paper by Weight et al

Commentary: Pleomorphic carcinoma: An aggressive type of non-small cell lung cancer that should be treated likely others.
Lui, J Thorac CV Surg

Editorial covering the included paper by Hendriksen et al.

Pulmonary lymphoepithelioma-like carcinoma
Sathirareuangchai et al, Archives

Nice review article on this rare subtype of non-small cell carcinoma associated with EBV.